

**QUANTIFICATION OF THE INFLUENCE OF *PLASMOPARA*
VITICOLA ON GROWTH, YIELD AND PLANT RESPONSE FOR THE
DEVELOPMENT OF AN INTEGRATED CONTROL STRATEGY ON
VITIS VINIFERA (CV. MERLOT)**

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Nulla si può tentare se non stabilire l'inizio e la direzione di una strada infinitamente lunga.

La pretesa di qualsiasi completezza sistematica e definitiva sarebbe, se non altro, un'illusione.

Qui il singolo ricercatore può ottenere la perfezione solo nel senso oggettivo che egli comunichi tutto ciò che è riuscito a vedere.

Georg Simmel

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È sempre la parte più difficile. Si corre il solito rischio di dimenticare qualcuno e dopo tutti questi anni (ormai sono un nonno per questo genere di lavori) si accentua ancora di più. Molti sono, infatti, coloro che hanno collaborato a questo progetto; il primo fra tutti è Ernesto Carrera. Ernesto è stato mio collaboratore diretto fino al 1999 e penso non abbia ancora digerito le migliaia e migliaia di foglie di vite controllate e i numerosi trattamenti eseguiti nelle parcelle. A lui vanno in primis i ringraziamenti per la pazienza (nel sopportare le mie seghe mentali), la costanza e la precisione del lavoro svolto. Un altro che sogna ancora adesso la peronospora è Reinhard Dietrich, il quale oltre ad aver sviluppato la parte modellistica nella sua tesi, si è pure sobbarcato con noi ore e ore di controlli di campo e con lui le mie due ragazze che mi hanno aiutato in questo lavoro, Marina e Natalia. Non da ultimo, vorrei ringraziare Vivian Zufferey e François Murisier per le loro sempre utili discussioni e commenti sugli aspetti di fisiologia della vite. Infine, vi sono due persone speciali, due amici, che sono gli ideatori del progetto, Philippe Blaise (anche Phil si è beccato una buona dose di foglie da controllare) e Cesare Gessler. Senza di loro tutto questo non sarebbe stato fatto. A Cesare va poi un pensiero particolare per la pazienza avuta nel sopportarmi e per la sua profonda e sincera amicizia.

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ABSTRACT

Scope of this work was primary to collect quantitative data under field conditions on the influence of the downy mildew epidemics, caused by the oomycete *Plasmopara viticola*, on the plant growth, yield quality and on plant response capacities, secondly, to use this data to develop a control strategy of downy mildew with a minimal input of pesticides, however able to avoid economic damages. The experimentation was separated in two parts. In the first, conducted during the period 1996-1998, the influence of downy mildew on grapevine response was analysed comparing three treatments: a) untreated canopy, b) reduced fungicide schedule and c) normally schedule applied in the vineyard. The measurements on the impact of the leaf infections have shown a drastic reduction of photosynthesis and gas exchange on the sporulating downy mildew leaf area. On the symptomless parts of an infected leaf we observed also a negative influence related to the disease severity. This negative effect was greater on lateral than on main leaves. A single fungicide application on clusters at the apparition of the first symptoms assured the same crop production as in the normally treated plots. The leaf infections in the untreated plots caused a decrease of the soluble solids in the must. However this reduction was not proportional to the decrease of the assimilation leaf area. The "Reduced fungicide schedule" resulted in a delay the epidemic progress and with consequently low differences at harvest in the must soluble content of the berries. The qualitative yield damaged was created during the first phase of the ripening process. The grapevine plant compensated the carbohydrates request of the cluster by mobilizing the starch reserves stored in the woody parts and particularly in the roots. The mobilisation follows a hierarchical pattern; first the mobilisation of the reserves stored in the roots, than those stored in the trunk, canes and shoots. The downy mildew could be considered as a stress factor for the grapevine. The impact of the decrease of the reserve content in the following growth season has negatively influenced the shoot elongation and the crop yield quantity of the "Untreated canopy" treatment. Nevertheless, a single recovery year was sufficient to rebuild the reserve pool. These results have permitted the elaboration of the "Minimal Fungicide Strategy" (MFS), with concept of delaying of the downy mildew epidemic exponential increase phase until veraison and exploiting the compensatory capacities of the plant. The MFS was based on a first fungicide/oomycetide application at the appearance of the first downy mildew sporulation in plot, followed by one or two applications whereas the timing depended from the epidemic progress (disease incidence increase), the time passed from the last fungicide application and from the rain pattern. The efficacy of the MFS was evaluated in the second part of the experimentation during the period 1999-2002 and compared with the normally schedule applied in the vineyard and an untreated plot. During the period 2003-2004 we analysed the recovery capacities of the plant. The MFS has

reduced the number of fungicide applications between 43% and 67%. The start of the exponential phase of the down mildew epidemic was delayed until after veraison, however in the MFS plots the yield quantity was reduced by of 15.69% and the must soluble content by 2.63% at harvest compared to the normal schedule. A significantly lower shoot growth and a reduction of the potential yield quantity were recorded starting from 2001 as consequence of the reserves decrease in the time. The recovering year 2003 has permitted to eliminate in 2004 the negative impact due to the four years of stress situation in the MFS plots. The conclusion is that the negative impact of the MFS over several years is minimal, but the reduction of pesticide application is relevant. However the rules determining the timing of the fungicide application are critical. For an optimal application of MFS, the rules need to consider quantitatively the role of primary and secondary infection in the epidemic development in relation to the yield formation. It desirable to integrate these interactions in a quantitative forecasting model of infection risk and epidemic development,

RIASSUNTO

Lo scopo del presente studio consisteva nel raccogliere dati quantitativi sull'impatto in campo delle infezioni di peronospora, il cui agente causale è l'oomicete *Plasmopara viticola*, sulla crescita vegetativa, la resa qualitativa, le capacità di compensazione e di recupero della pianta, sviluppando su questi dati una strategia di lotta che riducesse al minimo l'impegno di fungicidi evitando nel contempo danni economici. Lo studio è stato suddiviso in due parti. Nella prima parte, condotto nel corso del periodo 1996-1998, si è studiata la risposta della vite alle infezioni peronosporiche mettendo a confronto tre varianti: a) senza trattamenti fogliari (con un'applicazione specifica sul grappolo per prevenire danni quantitativi), b) lotta minimale (basata su degli interventi per frenare le prime fasi epidemiologiche), c) piano di trattamento preventivo normalmente applicato nel vigneto. Le misure dell'impatto delle infezioni fogliari sulla fotosintesi e gli scambi gassosi hanno evidenziato come sui tessuti fogliari ricoperti da sporulazioni vi sia una rilevante riduzione dell'attività fotosintetica e degli scambi gassosi, mentre su quelli sani circostanti la diminuzione dell'attività assimilatoria è correlabile con l'incremento del grado d'infezione fogliare. Tale fenomeno è stato maggiore sulle foglie dei tralci secondari rispetto a quelle del tralcio principale. Un solo intervento specifico sul grappolo all'apparizione delle prime sporulazioni di peronospora ha permesso una protezione sufficiente per garantire una resa uguale a quella ottenuta nella variante di lotta preventiva. Il danno fogliare si è espresso con una riduzione del tenore zuccherino delle uve, il quale non è correlato alla diminuzione della superficie assimilabile dovuta all'epidemia. La variante lotta minimale ha permesso di ritardare l'evoluzione dell'epidemia con una conseguente riduzione della perdita in zuccheri delle uve alla vendemmia. Il danno qualitativo si forma nel corso della prima fase di maturazione. Questo comportamento è dovuto alla capacità della vite di compensare il fabbisogno in carboidrati degli acini durante la fase di maturazione attraverso la mobilitazione delle riserve in amido delle sue parti legnose e in particolare dalle radici. La mobilitazione segue uno schema gerarchico, mobilitando prima di tutto le riserve delle radici, per poi passare a quelle del tronco, del capo a frutto e infine del tralcio dell'anno. La peronospora può quindi essere considerata per la vite alla stregua di qualsiasi altro fattore di stress. L'impatto della riduzione del tenore delle riserve sulla crescita nel corso della stagione successiva ha evidenziato una minore crescita del tralcio e una produzione inferiore nella variante senza trattamenti fogliari. Ciononostante, un solo anno senza stress ha permesso alla vite di ricostituire il suo pool di riserve. Questi risultati hanno permesso l'elaborazione di una strategia definita "Minimal Fungicide Strategy" (MFS) il cui scopo è ritardare l'evoluzione epidemica della peronospora nella sua fase esponenziale fino all'invaiaitura, per poi sfruttare le capacità di compensazione della vite. La MFS si basa su un primo trattamento al ritrovamento delle prime sporulazioni seguito da una

o due successive applicazioni in funzione dell'evoluzione dell'epidemia (crescita dell'incidenza), dell'intervallo tra le applicazioni eseguite e dall'evoluzione delle condizioni climatiche. L'efficacia della MSF è stata valutata nella seconda parte del lavoro mettendola a confronto con una variante di lotta preventiva normalmente applicata nel vigneto e a un testimone non trattato. Nel periodo 2003-2004 si sono invece analizzate le capacità di recupero della pianta. La MFS ha permesso di ridurre tra il 43% e il 67% il numero delle applicazioni antiperonosporiche. L'inizio della fase esponenziale dell'epidemia è stato ritardato fino a dopo l'invasiatura. La resa produttiva delle parcelle MFS è stata comunque mediamente minore del 15.69% rispetto alle parcelle normalmente trattate con una riduzione del tenore zuccherino del 2.63%. Una significativa diminuzione dell'accrescimento del tralcio è stato osservato dal 2001 quale conseguenza della riduzione nel tempo delle riserve. L'impatto maggiore si è comunque registrato nel potenziale produttivo. La stagione vegetativa di recupero 2003 è stata sufficiente per eliminare nel 2004 gli effetti negativi dovuti ai quattro anni di stress indotti dalla malattia nelle parcelle MFS. Si può pertanto affermare che l'impatto negativo dell'applicazione della MFS su diversi anni è minimo e rilevante la riduzione delle applicazioni di fungicidi. Comunque, la gestione delle basi decisionali per l'applicazione dei fungicidi è l'elemento critico. L'ottimale applicazione della MFS necessita la quantificazione del ruolo dell'infezione primaria e secondaria sull'evoluzione epidemica della peronospora in rapporto alla formazione della produzione. È auspicabile l'implementazione di queste interazioni in un modello previsionale quantitativo sui rischi d'infezione e di sviluppo della malattia.

Chapter 1

General introduction

Powdery mildew caused by the fungus *Erysiphe necator* was discovered in French vineyards in 1847. Disastrous epidemics over several years especially in the warm and drier south and the subsequent dispersal across all of Europe drastically changed grape growing and vineyard management. Controlling the disease was then the primary focus, that was achieved with sulphur application as a dust and later as a mixture of sulphur, lime and water. The phylloxera mite in the 1870s led to the praxis of grafting European vines (*Vitis vinifera*) on rootstock vines derived from American *Vitis* species. The third calamity, downy mildew caused by the oomycete *Plasmopara viticola*, appeared in 1878. This pathogen was probably introduced accidentally in Europe with American *Vitis* species used as breeding material for the development of rootstocks resistant to phylloxera. A year later it was found in Italy, in 1880 in Germany and in 1881 already in Greece. This surprisingly fast dispersal of the pathogen was ascribed to the dispersal of sporangia which were assumed to be carried over long distances with the wind (Koopman *et al.*, 2007). From our current knowledge the pathogen (Gobbin *et al.*, 2006) was more probably distributed in the form of oospores with soil and rootstock material. Even though soon afterwards lime-copper in the form of the Bordeaux mixture was revealed as an efficient and widely used fungicide, a relevant part of the vineyards were again changed from the highly susceptible *V. vinifera* cultivars to a large range of hybrids of *V. vinifera* with American species (Pearson and Goheen, 1988). However quality vines had to rely on the old well known *V. vinifera* cultivars such as Cabernet and Merlot. With the increasing request of quality vines in the 20th century hybrids again lost importance and only recently, thanks to a constant effort of breeding mostly in Germany, hybrids are again gaining ground. In the second half of the 20th century highly efficacious fungicides were developed and downy mildew control with fungicides is practiced in all vineyards with a frequency of application depending on the climatic conditions.

Plasmopara viticola (Berk. & Curt.) Berl. & de Toni (Order: Peronosporales, Family: Peronosporaceae) is a the heterothallic (Wong *et al.*, 2001) diploid obligate biotrophic Oomycete. The biology was studied in detail beginning in 1900 and it was immediately clear that the pathogen overwintered as oospore in the leaf or berries residues on the surface layer (Gregory, 1915). It was supposed that the oospores are able to germinate and, consequently, to infect the grapevine tissues in a short period of time from mid to end of May, losing their germination capacities from the middle of June (Cortesi and Zerbetto, 1994). Oospore germination is closely related to vapour pressure deficit and water activity of the leaf litter on soil, consequently there is sufficient water for the oospore development with a vapour pressure deficit lower than 2.13 hPa. Moreover, leaf litter moisture due to atmospheric water makes oospore development possible during non rainy periods (Rossi and Caffi, 2007). Oospore germination produces macrosporangia from which primary

infections (oosporic infection) on green organs of grapevine occur by rain-splash effect during a few hours of leaf wetness. After a latency period varying between five to ten days, (depending on leaf age, cultivar, temperature and humidity) sporangiophores and sporangia are produced during the night through the stomata on the abaxial side of the leaf or on the rachis and young berries and begin the secondary infections or asexual cycle. Berries are highly susceptible in their young phase when stomata are active. On these organs a whitish downy felt appears, clearly recognizable as visible “trees” up to about 0.10 mm long and in bundles. As the berries mature, the stomata necroses and with the disappearance of functional stomata they become less susceptible and sporulation is impeded. Already infected berries first turn pinkish red and drop easily and then a dry brown without sporulation. This symptom is named “peronospora larvata” in Italien (Pertot *et al.*, 2007) and brown rot (Lafon and Clerjeau, 1988). Infections of rachis may cause similar symptoms or just the drying of the distal part of the bunch. The climatic conditions for secondary infection are fairly similar to those required for the primary infection, e.g leaf wetness for a few hours (3-6) and any temperature above 10 °C (Blaeser and Weltzien, 1979; Lafon and Clerjeau, 1988; Schruft and Kassemeyer, 1999). It was assumed that primary infection played a starting role for the epidemic and only the secondary infections were quantitatively relevant for the disease progress in time (Blaise *et al.*, 1999).

In the last ten years, the use of molecular techniques has permitted reviewing the epidemiology of the downy mildew, clarifying the role of the primary and secondary infections. Contrary to the “traditional” epidemiological concept developed from the apparition of downy mildew in Europe, this approach has shown the principal role of the primary, or oosporic, infections and a great genetic diversity of the populations (Gobbin, 2004; Delmotte *et al.*, 2006). Oospore may continue to germinate, and to generate primary infections throughout the growing season (Gobbin, 2004; Jermini *et al.*, 2003; Kennelly *et al.*, 2007). A downy mildew population is therefore constituted mostly of single genotypes and of a single or few dominant genotypes which for unknown reasons were capable of creating large clonal offspring (Gobbin, 2004). The “traditional” concept of a clonal epidemic can be observed in dry areas such as the Greek islands (Rumbou and Gessler, 2006) or in Australia (Hug *et al.*, 2006), but it is an exceptional event in humid climates (Gobbin, 2004).

Downy mildew is considered, since its introduction in Europe, the most damaging disease of *V. vinifera* cultivars of the European humid regions. Today, the winegrower has at his disposal several different families of active ingredients as, strobilurin, cyazofamid, carbamate, phenylamid, dithiocarbamate, phthalimide or copper. As even short rain periods or even dew may lead to sufficiently long leaf wetness periods for infection, other factors contributing to a high number of infections being unknown, vinegrowers apply fungicides

according to a calendar schedule as insurance against the highly erratic appearance of the disease and of the potential damages it causes.

The necessity to develop a more ecological grapevine production, and therefore decreasing the use of chemical compounds for plant protection, has encouraged the development of downy mildew forecasting models in different viticulture areas as France (Strizyk, 1984; Tran Manh Sung *et al.*, 1990; Magnien *et al.*, 1991; Raynal *et al.*, 2006), Germany (Hill, 1993), Switzerland (Viret *et al.*, 2001) Italy (Rosa *et al.*, 1995; Orlandini *et al.*, 2008), Australia (Magarey *et al.*, 1991) and USA (Park *et al.*, 1997). These models give only a qualitative response on the achievement of the two most important events of the disease cycle of downy mildew, the infection and the sporulation. They have certainly improved a better management of the control strategies, but their aim is always to avoid any infection so not to incur in conditions favourable for the production of an infection by secondary inoculum to which the destructive potential of the disease was ascribed. Still the downy mildew control is targeted to have a fungicidal protection of the green plant tissue during infection condition or an eradication of the pathogen shortly after infection, however avoiding fungicide application when no infection conditions are expected.

It is difficult to predict downy mildew damages in time, because they are erratic and the annual losses vary. In northern Switzerland, for example, between 1960 and 1981, in only 4 years (62, 64, 69 and 76) the disease did not spread or not appear; in 9 years it appeared and spread slightly and belatedly without causing damage; in 7 years it was able to spread epidemically and cause light to middle damage and only in two years (60 and 81) a high disease incidence was recorded on untreated plots associated with severe yield losses (Bosshard, 1986). Similar situations are also reported in Italy (Goidanich, 1983). Even without specific data it can be guessed that half of the fungicide applications were unnecessary. Despite progress in the biologic and epidemic knowledge, the actual forecast models don't quantify the epidemic progress of the disease and its impact on plant growth and yield quantity and quality. Neither do they take into account the effect of the different fungicide applications on the epidemic progress and consequently they tend to overestimate the risk of infection. So, disease control has certainly improved mostly due to the quality of the modern fungicides, but always respecting the current grapevine production system, which requires a maximal quality-quantity yield production over a long period. In this crop system concept, the current pest or disease control strategies employed consider the plant as the growing substrate necessary for the fulfilment of the life cycle of the pathogens. This has led to a linear conception of the relationship between quantitative presence of the pest or diseases, time and final damage with a partial analysis of the potential real causes of the observed damages. However it is plausible and evident that this relationship is considerably altered by various host related factors, which in their turn are growth stage and

environmentally influenced. Consequently, our concept of pest and disease control strategies underestimates the interactions with the crop system, doesn't consider the plant compensation capacities and integrates only partially the interactions between vineyard and ecosystem. On this basis, the plant must be placed at the centre of the vineyard crop system (Delucchi, 1990), where diseases and pests can be considered as stress factors for the plant. Consequently, the analysis of the complete crop system and its interactions should be the basis for the elaboration of decision rules with the aim of optimising pest or disease control on an ecological (habitat management or biological control), cultural (alteration of some cultural practices) or chemical (definition of a damage threshold) basis. This analysis was mostly applied in the entomological field for different foliar pests (Boller *et al.*, 1989; Boller and Candolfi, 1990; Candolfi, 1991; Candolfi *et al.*, 1993; Jermini *et al.*, 2009; Linder and Jermini, 2001; Linder *et al.*, 2009; Martinson *et al.* 1997; McNally *et al.*, 1985), but never for diseases. A forecasting model for downy mildew has been developed, as a first approach to closing this gap, with the aim of evaluating the risks of quantitative and qualitative grapevine yield losses due to the downy mildew. It was composed of a disease model coupled with a dynamic crop growth model of grapevine (Blaise *et al.*, 1996).

The scope of this work was primarily to collect quantitative data under field conditions on the influence of the downy mildew epidemics, caused by the oomycete *Plasmopara viticola*, on the plant growth, yield quality and on plant response capacities, and secondly, to use this data to develop a control strategy of downy mildew with a minimal input of pesticides and minimal economic damages. In detail we investigated: 1) *the damage analysis*. This analysis consists of understanding the disease impact on leaf photosynthesis, as the primary source of carbohydrate production for the sink parts of the plant, and on the quantitative and qualitative yield losses. Quantity yield losses are generally a direct result of disease action on the clusters, specifically on the berries, and the damage level is related to the phenological and physiological stage of the berries and the epidemics. Quality yield losses are more related to the impact of disease on the leaf gas exchange, leaf area growth and therefore on the partitioning of the photosynthates assimilated during the season. The quantification of the epidemic progress determines the damage impact on the plant and consequently on the yield losses; 2) *the analysis of the compensation mechanisms*. The plant submitted to a stress situation reacts by inducing compensation mechanisms that depend on the physiological state of the plant during the season (Koblet *et al.*, 1996). These possible compensation mechanisms consist of a delay in the leaf senescence, an increased physiological response of leaves or of the number of lateral leaves/shoot and the mobilisation of the reserves contents in woody parts of the plant. At the cluster level it is possible to find an increase in the number of flowers or berries per cluster or an increase in the berry volume; 3) *the recovering capacity of the plant*. A stress situation can induce a lower growth or production

capacity of the plant the year after the stress and this situation has an important impact for the choice of a control strategy; 4) *the development of a control strategy*. The development and evaluation of a control strategy considers the results of the three points described above. Damage analysis is described in the second and third chapters, the analysis of the compensation mechanisms in the fourth, the recovery capacity in the fifth and the evaluation of the proposed control strategy in the sixth.

The value of this dissertation lies in the acquisition of quantitative data of the impact of downy mildew epidemics on the plant production and on the plant response to the disease, integrating for the first time a disease effect on the grapevine crop system. On the other hand, it permits us to provide a set of data for the development of a quantitative forecasting model and to propose a possible control strategy which recognizes the plant as the central element of the vineyard crop system and not the disease as has been done until now. Furthermore it proposes a new approach for evaluating disease impacts in the grapevine crop system.

Influence of *Plasmopara viticola* on gas exchange parameters on field-grown *Vitis vinifera* cv. "Merlot"

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Introduction

Downy mildew, caused by *Plasmopara viticola* Berk. & Curt. (Berl. and de Toni), is responsible for the most important disease on grapevine in Switzerland and the control strategies are based on preventive measures. The currently registered fungicides allow containing the yield quantity losses, but regular downy mildew epidemics are observed starting from July on leaf canopy with, depending on weather conditions, an important progress in August and September during the ripening phase and particularly on the lateral leaves. Grapevine is subjected to multiple stress situations during its whole growing season and it has a great potential for stress acclimation altering the assimilate allocation system (Koblet *et al.*, 1996). Edson *et al.* (1995) showed as grapevine has a balanced system of assimilate allocation based on a ranking of sink priority, that changes and localized photosynthetic rates may increase with sink stimulus (vegetative or reproductive) as the season progresses. The quantification of stress impact induced by pests or diseases calls for an analysis of the crop system. This is based on the analysis of the damage (impact of disease epidemic or pest population dynamic on plant growth, yield and plant physiology) of the existing interactions between damage and cultural practices and of the compensation mechanisms applied from the plant as response to a stress situation (Delucchi, 1990; Jermini *et al.*, 2006). This approach has been applied until now to analyse the plant response to injuries caused by some foliar pests (Candolfi, 1991; Candolfi *et al.*, 1993; Jermini *et al.*, 2009; Linder *et al.*, 2009). The first step in the damage analysis is the evaluation of the interactions between biotic stress factors and grapevine, which lead to changes in several physiological processes, where photosynthesis is one of the most sensible to different stress conditions. On grapevine, several studies focused on this aspect for foliar pests (Candolfi 1991; Candolfi *et al.*, 1993; Linder *et al.*, 2009; Mercader and Isaacs, 2003; Remund and Boller, 1995) and diseases (Beltrami *et al.*, 2004, Cabaleiro *et al.*, 1999; Goodwin *et al.*, 1988; Lakso *et al.*, 1982; Moriondo *et al.*, 2005; Nail and Howell, 2005; Shtienberg, 1992). Concerning downy mildew, studies had been carried out by Orlandini *et al.* (1998), Moriondo *et al.* (2005), Allegre *et al.* (2007) and Stoll *et al.* (2008). The first two authors indicated how the disease had a negative influence on gas exchange not only on the sporulating area, but also on the remaining symptomless tissues, determining a virtual lesion, but they didn't analyse the impact of the disease severity progress on the healthy part of infected main and lateral leaves. It is important to differentiate between these two types of leaves, because their role changes during the ripening phase. Main leaf photosynthesis has a limited importance during fruit maturation and, most likely, the lateral leaves assume the primary role (Candolfi-Vasconcelos, 1990). Following the concept of crop system analysis (Delucchi, 1990; Jermini *et al.*, 2006), we started a study during the period 1996-1998 with the aim of quantifying the

impact of downy mildew epidemics on the grapevine, considering the disease as a stress factor for the plant. This first work analyses under field conditions the impact of downy mildew on leaf assimilation capacity during the ripening phase by means of leaf gas exchange measurements to provide: 1) functions to describe the impact on main and lateral leaves of different disease severity levels on the gas exchange rate of symptomless portions of the same leaf in comparison with healthy leaves, 2) the gas exchange capacities of sporulating parts of the main and lateral leaves in comparison with healthy leaves.

Material and Methods

Plant material and experimental designs

The experiments were carried out during the period 1996-1998 in a vineyard of the Research station Agroscope Changins-Wädenswil ACW Centre of Cadenazzo planted with 'Merlot' grafted on 3309 rootstock. The vines were double cane pruned and vertical trained (double Guyot).

Three different treatments were compared: A = "Untreated canopy" (to prevent quantity losses, the clusters were treated once with a contact fungicide at the discovery of the first downy mildew sporulation); B = "Reduced fungicide schedule" (based on a first treatment at the appearance of the first symptoms, to avoid yield quantity losses followed by one or two additional fungicide applications during the early epidemic phase with the aim of delaying epidemic). C = "Standard schedule" (schedule normally applied in the vineyard). The experimental plot was moved each year in different but homogenous blocks of the vineyard to avoid stress influence due to a repetition of the trials on the same place. The 1997 trial was placed in a vineyard plot planted in 1991 with a vine spacing of 2.00 x 1.20 m between and within the rows. Each treatment consisted of a plot of 48 plants divided in 6 subplots of 8 contiguous plants. The number of shoots per plant, including the spurs, was regulated to 11 at the phenological stadium 53 BBCH (Baillod and Baggiolini, 1993). The yield regularization was made on August 22 so as to obtain a theoretical production per sub-plot of $1.1 \text{ kg}\cdot\text{m}^{-2}$, corresponding to the low potential yield estimated in the experiment. The first topping was done on June 23 and a second one on August 4. The 1998 trial was placed in a plot planted in 1974 with a vine spacing of 1.80 x 1.40 m between and within the rows. Each treatment consisted of a plot of 48 plants divided in 8 subplots of 6 contiguous plants. For this experiment the number of shoots per plant, including the spurs, was regulated to 10 in the same periods as for the other years. The yield regularization was made on July 30 with the aim of obtaining a theoretical production per subplot of $1.2 \text{ kg}\cdot\text{m}^{-2}$, corresponding to the low potential yield estimated in the experiment. The first topping was done on June 30 and a second one on July 30.

The downy mildew fungicide applications in the treatment A was made at the apparition of the first sporulation: at June 16 with Remiltine F pepite (37.5% folpet + 20% mancozeb + 6% cymoxanil) for 1997 and at June 29 with Phaltan 80 (80% folpet) for 1998. Three applications of Slick (250 g l⁻¹ difenoconazol) were made starting from bloom to prevent powdery mildew (*Uncinula necator*) and black rot (*Guignardia bidwellii*) infections and one with Switch (25% fludioxonil + 37.5 %cyprodinil) on clusters at the end of July to control grey mold (*Botrytis cinerea*) infections at the ha rate indicated by the manufacturer.

Diseased leaf area estimation

Disease severity was estimated in the field using a modified Horsfall scale (Horsfall and Cowling, 1978) in which a supplementary class for the lower disease level was introduced. In this way, the scale was divided into 12 classes: class 0 (0% damaged leaf area), class 1 (0* - 1% damaged leaf area), class 2 (1* - 3% damaged leaf area), class 3 (3* - 6% damaged leaf area), class 4 (6* - 12% damaged leaf area), class 5 (12* - 25% damaged leaf area), class 6 (25* - 50% damaged leaf area), class 7 (50* - 75% damaged leaf area), class 8 (75* - 88% damaged leaf area), class 9 (88* - 94% damaged leaf area), class 10 (94* - 97% damaged leaf area) and class 11 (97* - 100% damaged leaf area). The asterisk indicates a value slightly exceeding the indicated value.

Gas exchange measurement

Leaf gas exchange was assessed under field conditions during the ripening period with a portable open infrared gas analyzer system LCA-4 (Analytical Development Company, U.K) equipped with a Parkinson leaf assimilation chamber of 6.25 cm² (PLC-B for broad leaf).

All measurements were carried out under fully light saturated conditions (PAR >1'200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) between 1 pm and 3 pm with clear sky on plants that were never under water stress conditions because no dry period has been registered during the two year growing seasons considered for the measurements. During the period April - August 53 days of rainfall with an amount of 1'144 mm·m⁻² were measured in 1997 and 25 days with 1'108 mm·m⁻² in 1998. In June, July and August 1997 16, 11 and 9 days with rainfall were measured with a total of 466, 184 and 259 mm·m⁻², respectively. For the same months of 1998 11, 8 and 6 days with rainfall were measured with a total of 191, 116 and 78 mm·m⁻², respectively. The measurements were carried out only in the experimental years 1997 and 1998 and only in the treatments A and C to avoid influences of a partial downy mildew control on the measurements. The parameters were calculated using the equations proposed by Van Cammerer and Farquhar (1981). Mesophyll conductance and water use efficiency were defined in accordance with Candolfi-Vasconcelos (1990). Measurements on healthy leaves were carried out on plants in the subplots of treatment C and on diseased leaves in that of treatment A. The choice of the gas exchange assessment period was made in relation to the disease severity progress on the plant and on the leaves and the number of replicates

depended on the frequency of the occurrence of the different severity levels in the subplots of treatment A. To provide adequate sampling, no senescent main leaf was selected between the 6th and the 9th main negleaf from the shoot base. In the same way the leaves on lateral shoots were chosen from the middle part of lateral shoots, which had the same leaf number. The epidemic progress on main leaves in 1997 and 1998 was rapid and at veraison more than 68 % in 1997 and 73 % in 1998 presented a disease severity higher than class 5 of the modified Horsfall scale (Tab. 1).

Table 1. Distribution of the downy mildew severity on main leaves in the treatment A (Untreated canopy) at the phenological stage veraison in 1997 (n = 732) and 1998 (n = 1017) in according with the modified Horsfall scale: 0 = 0 % damaged leaf area, 1 = 0* - 1% damaged leaf area, 2 = 1* - 3%, 3 = 3* - 6%, 4 = 6* - 12%, 5 = 12* - 25%, 6 = 25* - 50%, 7 = 50* - 75%, 8 = 75* - 88%, 9 = 88* - 94%, 10 = 94* - 97% and 11 = 97* - 100%. The asterisk indicates a value slightly exceeding the indicated value.

Year	Classes of the modified Horsfall scale											
	0	1	2	3	4	5	6	7	8	9	10	11
1997	13%	4%	4%	7%	5%	7%	14%	26%	16%	5%	0%	0%
1998	11%	7%	5%	6%	9%	11%	18%	16%	20%	6%	2%	0%

This situation has limited the possibility of measuring the gas exchange on main leaves during the ripening phase considering an adequate number of leaves in accordance with the diseased level of the modified Horsfall scale. Therefore, the measurements on main leaves were carried out in 1997 between the phenological stage berry touch (July 19, 22 and 30) and the beginning of veraison (August 13); on leaves of the lateral shoots during the ripening phase (August 22 for 1997 and August 19, 22, 25 and 31 for 1998). Gas exchange measurements were made: on healthy leaves, chosen on plants of treatment C, on sporulating leaf area, greater than the leaf chamber area ($> 6.25 \text{ cm}^2$), and on the healthy area of a diseased leaf corresponding at the area without downy mildew symptoms (yellowing or presence of sporulation). Criteria to choose the leaves for the later measurement was that the area without downy mildew symptom to be measured was as far as possible from the diseased portion, however the position of the leaf on the trellis had to be equal to that of all other leaves measured. The diseased leaves were chosen on plants of the treatments A.

Statistical analysis

The paired t-test was used to compare the differences between healthy leaf and the sporulating area of an infected leaf. The effect of the disease severity on gas exchange in symptomless tissues of main and lateral infected leaves was quantified by means of regression analysis. Because gas exchange measurements at different disease severity levels were made at different times, the data were transformed to a relative rate (ratio

between the assimilation rate of healthy area of infected and healthy leaves). The dependent variable was the relative rate of the gas exchange parameters considered in the analysis. The independent variable was the disease severity defined as the midpoint of class of the modified Horsfall scale. Statistical analysis was performed utilising the Sigmastat (SSPS) statistical package.

Results

Gas exchange comparison between sporulating and healthy leaf area

A drastic reduction in the photosynthetic rate (A) was observed under field conditions at each date of measurement on the sporulating area of main and lateral leaf tissues (Tab. 2).

Table 2. Gas exchange parameters determined on sporulating area of an infected leaf of the cultivar Merlot with a damaged leaf area higher than class 5 of the modified Horsfall scale and on healthy leaf for the main and lateral leaves. Values are means \pm standard deviation from two different dates of measuring on a cloudless day at noon. At each date 10 leaves for each state were measured.

Date of measurement	Leaf measured	Gas exchange parameters								
			A	E	WUE _{ins}	g _s	r _s	g _m	C _i	Tleaf.
27.07.97	Main leaf	Healthy	10.27 \pm 1.25	4.98 \pm 0.51	2.06 \pm 0.22	0.199 \pm 0.057	5.37 \pm 1.45	0.052 \pm 0.009	199.94 \pm 24.46	37.41 \pm 1.19
		Sporulating area	1.81 \pm 1.19	2.19 \pm 0.87	0.77 \pm 0.37	0.042 \pm 0.023	33.04 \pm 25.36	0.008 \pm 0.006	237.03 \pm 35.06	41.68 \pm 1.12
		P value	< 0.001	< 0.001	< 0.001	< 0.001	0.002	< 0.001	< 0.001	< 0.001
30.07.97	Main leaf	Healthy	8.97 \pm 1.25	4.72 \pm 0.39	1.89 \pm 0.21	0.197 \pm 0.035	5.29 \pm 1.23	0.054 \pm 0.010	166.55 \pm 10.52	36.95 \pm 0.74
		Sporulating area	1.82 \pm 1.22	2.69 \pm 0.83	0.62 \pm 0.32	0.067 \pm 0.033	17.65 \pm 7.36	0.009 \pm 0.007	210.04 \pm 22.23	39.86 \pm 0.84
		P value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
25.08.98	Lateral leaf	Healthy	9.95 \pm 1.20	5.61 \pm 0.81	1.81 \pm 0.41	0.263 \pm 0.086	4.05 \pm 0.98	0.925 \pm 0.068	219.21 \pm 19.89	35.35 \pm 0.95
		Sporulating area	1.86 \pm 0.97	2.16 \pm 0.86	0.85 \pm 0.25	0.043 \pm 0.029	28.38 \pm 11.89	0.108 \pm 0.074	240.88 \pm 28.09	40.33 \pm 0.96
		P value	< 0.001	0.001	< 0.001	0.002	0.004	< 0.001	0.072	< 0.001
31.08.98	Lateral leaf	Healthy	9.87 \pm 1.05	3.90 \pm 0.38	2.54 \pm 0.26	0.172 \pm 0.029	5.99 \pm 1.10	1.110 \pm 0.093	198.05 \pm 10.36	33.43 \pm 1.26
		Sporulating area	1.27 \pm 1.23	1.45 \pm 0.65	0.72 \pm 0.48	0.032 \pm 0.020	39.93 \pm 21.21	0.072 \pm 0.079	261.33 \pm 33.74	38.06 \pm 0.73
		P value	< 0.001	< 0.001	< 0.001	< 0.001	0.012	< 0.001	0.003	0.002

A = Photosynthesis ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)

E = Transpiration ($\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)

g_m = Mesophyll conductance ($\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)

Tleaf = Leaf temperature ($^{\circ}\text{C}$)

g_s = Stomatal conductance ($\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)

r_s = Stomatal resistance ($\text{m}^2\cdot\text{s}\cdot\text{mol CO}_2^{-1}$)

C_i = Substomatal CO₂ concentration ($\mu\text{mol CO}_2\cdot\text{mol}^{-1}$)

WUE_{ins} = Water Use Efficiency instantaneous ($\mu\text{mol CO}_2$
 $\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)

The partial pressure of the CO₂ inside the leaf (C_i), although showing significant differences (Tab. 2), remains fairly constant because stomatal resistance (r_s) changed in proportion to A (Larcher, 1995) and an optimal concentration is maintained. The ratio C_i/C_a, where C_a is the CO₂ concentration of the air, remains constant, usually at about 0.6 - 0.7. Consequently, an

increase in C_i results from a decrease of A and is compensated for by a progressive stomata closure. On the sporulating leaf area, stomatal (g_s) and mesophyll (g_m) conductance decreased and r_s increased (Tab. 2), indicating the difficulty of CO_2 diffusing through the stomata into the mesophyll to the site of carboxylation. Transpiration (E) and A are closely related to g_s , which continuously regulates the balance between assimilation and water loss in the leaf. The decreased E in the leaf area with *P. viticola* sporulation was not important as decreased A and consequently water use efficiency (WUE_{ins}) was not negatively influenced (Tab. 2). The leaf temperature measured in the leaf assimilation chamber significantly increased on the sporulating area in comparison with the healthy leaf. For the main leaves the increase was of 4.27 °C and 2.91 °C and for the lateral leaf of 4.98 °C and 4.63 °C (Tab. 2).

Influence of the disease progress on the gas exchange of the green parts of a leaf with sporulation

Downy mildew affected more negatively the gas exchange parameters on the symptomless parts of a diseased lateral leaf than of a main leaf, indicating a greater susceptibility of lateral leaves. A decrease of g_s and consequently of the A , E and WUE_{ins} and an increase of r_s on the symptomless area of a lateral leaf was observed already at low severity level with increments of the disease severity on the leaf (Fig. 1). A and WUE_{ins} were reduced by 14% for a damage in class 2 of the modified Horsfall scale (middle value of the class 2% diseased leaf area) in comparison with the increase of 1% and respectively 8% measured on main leaves. E and g_s measurements on main and lateral leaves were similar until class 5 level of damage (middle value of the class 18.5% diseased leaf area), but, as severity increased they showed a greater decrease. At low disease severity, below class 5 of the modified Horsfall scale (middle value of the class is 18.5% diseased leaf area), downy mildew had little effect on the healthy leaf tissues of the main leaf (Fig. 1). At this infection level the mean reduction in A was 19% of the values measured on healthy leaves. With the increase of the severity the negative effect increased rapidly, 43% for class 7 (midpoint value of the class is 62.5% diseased leaf area) and 68% for the class 8 (midpoint value of class is 81.5% diseased leaf area). E and WUE_{ins} in the symptomless part of an infected main leaf were also affected, but to a lesser extent, e.g. a reduction of 29% and 28% respectively for class 7 and 45% and 43% respectively for class 8. At high disease severity levels, above class 6 (midpoint value of the class is 37.5% diseased leaf area) of the modified Horsfall scale, the rate of the gas exchange parameters measured on the symptomless area of lateral and main leaves were similar with the exception of r_s , which increased at a greater rate on lateral than on main leaves (Fi. 1).

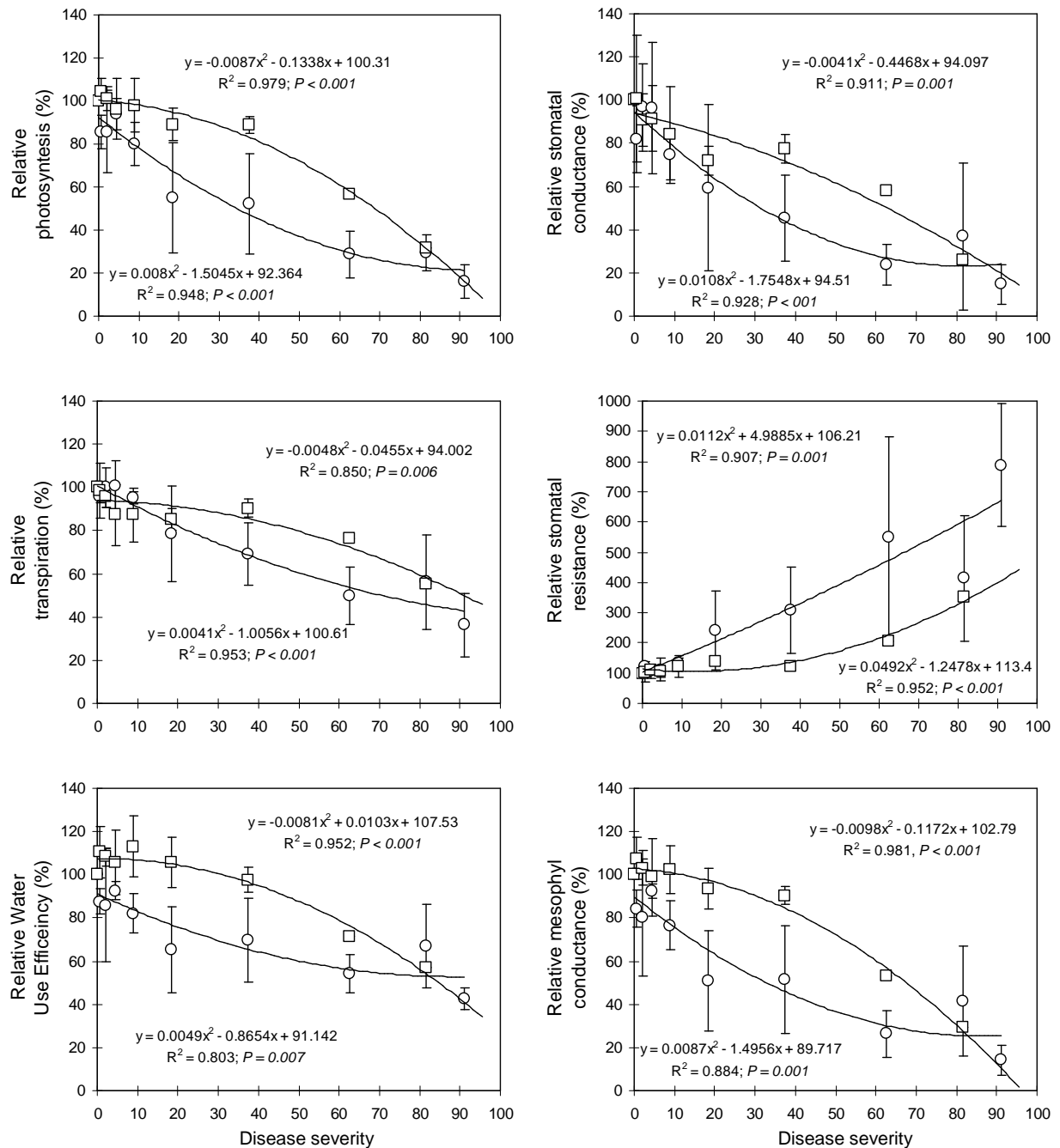


Figure 1. Relationship between disease severity, defined with the middle value of the modified Horsfall scale, and the photosynthesis, transpiration, water use efficiency, stomatal and mesophyll conductance and stomatal resistance rate expressed as ratio between values measured on symptomless area of infected and healthy leaves. Squares refer to main leaves and circles to lateral leaves. Bars indicate Std dev.

The measurements above class 6 for lateral and class 7 for main leaves could be an artefact. The “mosaic” is the typical downy mildew symptom in our canton on 'Merlot' and therefore we could not rule out the possibility that occasionally some sporulating leaf area may have been included in the leaf chamber of the LCA4 with an overestimation of the disease effect. This

possibility is higher on lateral leaves, which have a surface that is, on average, 20-30% less than that of main leaves.

Discussion

A drastic reduction in A observed on the sporulating area is in agreement with Orlandini and Giuntoli (1998), although they didn't make a distinction between main and lateral leaves. Contrary to our results, these authors indicated negative photosynthetic values on the sporulating oilspots. This difference could be due to the different type of lesions present on 'Merlot'. On this cultivar, the presence of oilspots, characterized by dense sporulation, is rare and the most common symptom is the "mosaic", where the sporulation is limited by the veins and sporangiophores are scarce (Goidanich, 1983). The downy mildew deregulates guard cell functioning of stomata causing significant water losses and a consequent collapse of the gas exchange (Allegre *et al.*, 2007). Non uniform stomatal patchiness closure is especially pronounced in environmental stress situations (Düring and Loveys, 1996), but also toxic excretions from the pathogen could induce the same type of stress (Turner and Graniti, 1969; Larcher, 1995) or, also, from the infected plant (Allegre *et al.*, 2007). Generally, the water diffusion from the leaf to the external environment was more rapid than the CO_2 diffusion from the air to the leaf tissues because g_m is very weak for water. This difference was even more pronounced in the sporulating tissues as the presence of the pathogen creates a further resistance for CO_2 rather than for water and explains the limited influence on E observed on the sporulating area. Stoll *et al.* (2008) have found that the pathogen development caused an increase in the leaf temperature on irrigated plants measured with a thermal imagery, at the inoculated point, but on plants subjected to severe drought stress the temperature was lower. Our results show a relevant increase of the leaf temperature in the sporulating area, on one hand confirming the finding of these authors on the other confirming that under our climatic and soil conditions the hydric supply was abundant. The impact of downy mildew infection on the gas exchange of symptomless tissues of an infected leaf is more important on lateral than on main leaves. A disease severity of class 1 of the modified Horsfall scale induces a decrease of 18% of the photosynthesis on lateral leaves, contrary to an increase of 14% at the same severity class on main leaves. Lateral leaves play a primary role in fruit maturation (Candolfi-Vasconcelos, 1990). Therefore, the greater reduction of A on symptomless areas of diseased lateral leaves could have an important negative impact on the fulfilment of the berry carbohydrate requirements with a consequently higher negative impact on yield quality. The photosynthetic rate on the symptomless tissues of main leaves wasn't negatively reduced until a disease severity of class 4 of the modified Horsfall scale (middle value of the class is 9% diseased leaf area) and this result is in accordance with the

observations of Moriondo *et al.* (2005), which didn't find differences in the photosynthesis between healthy leaves and symptomless parts of an infected main leaf with a severity of approximately 15%. Candolfi-Vasconcelos (1990) has shown how grapevine increased the assimilation rate of the main leaves as a plant response to lateral leaf defoliation and we cannot affirm that the increase observed on main leaves at low disease severity could be an attempt of the plant to react to a stress situation. We can not compare the plant response between abiotic and biotic stress situations induced by diseases. The fact that the negative effect of the disease on the remaining symptomless leaf area increased with the severity progress, indicates that the impact of the pathogen is not a mere direct physical impediment of cellular function, but a physiological processes outside the diseased tissues directly or indirectly influenced by the pathogen. Orlandini and Giuntoli (1998) suggest that the downy mildew pathogen is able to affect the leaf physiology without any outward visible symptoms and they assumed that the pathogen had a pathogenic influence beyond the visible diseased area, creating a virtual lesion as proposed for the first time by Bastiaans (1991) for the *Pyricularia oryzae*-rice pathosystem. Moriondo *et al.* (2005) confirmed this hypothesis showing a negative influence on the assimilation rate mainly around the sporulating area, whereas the green tissues away from the lesion were not affected. These results were confirmed in other pathosystems as in the rice-*Xanthomonas campestris* pv *oryzae*, where virtual lesion extended 1.1 cm beyond the true lesion for the gross CO₂-exchange rate at light saturation (Elings *et al.*, 1999), or in the bean *Uromyces phaseoli*, where Duniway and Durbin (1961) measured a significant reduction in stomatal aperture only up to 0.5 mm from the margin of isolated fungal colonies. Nevertheless, our results show a greater negative effect of downy mildew on gas exchange on the symptomless area of an infected leaf for disease severity above class 5 of the modified Horsfall scale. The explanation is probably due to the type of disease expression. Moriondo *et al.* (2005) described a lower concentration of photosynthetic pigments just around the lesion that lets us suppose that these authors have carried out their experiments considering only oilspots. Contrarily, the "mosaic" disease expression is more common on Merlot grapevine and the sporulating areas are distributed heterogeneously on the leaf surface and probably have a major border effect. Therefore, intrinsically small zones of such virtual lesion or latent lesion can coalesce and at high severity affect all the symptomless parts of the diseased leaf. Our results also indicate that stomatal conductance values of symptomless leaf area with sporulation are usually, as opposed to a healthy leaf, between 20 and 86% lower depending on the leaf disease severity, with a decrease in gas exchange activity and an increase in the stomatal dysfunction. Therefore, it is plausible that downy mildew infection induces a greater than normal response of the symptomless parts of an infected leaf to environmental stress factors and particularly to water stress. The effect may be due to the phytotoxic compounds

produced by the pathogen that diffuse to uncolonized portions of the leaf (Yarwood, 1967) or to several physiological changes in the leaf that stimulate the production of senescence phytohormones like ABA or jasmonic acid, which also stimulate the events leading to leaf abscission, or ethylene, that accelerates senescence. This effect has been reported for grapevines infected with the Pierce's disease bacterium (*Xylella fastidiosa*), where physiological changes in diseased leaves under relatively mild water stress induced a leaf senescence beginning at the leaf margin (Goodwin *et al.*, 1988). Shtienberg (1992) reported for some pathosystems a possible migration of carbohydrates from uncolonized portions of the leaf to the pathogen that was associated with an indirect influence on gas exchange. Powdery mildew of grapevine (*Erysiphe necator*) seems to induce an increase of sugar content in infected leaves, whereas downy mildew depletes leaf sucrose (Brem *et al.*, 1986). Orlandini and Giuntoli (1998) show also that the healthy parts of infected leaves (they have considered only leaves with a disease severity below 15%) are more susceptible to environmental stress factors. The results of our measurements, made during the early afternoon, support this hypothesis, because, on 'Merlot' grapevine, leaves began to abscise at disease severity levels between class 7 and 8 of the modified Horsfall scale. The visual assessment of the diseased leaf area doesn't reflect the actual part colonized by the pathogen and at least a portion of the leaf area determined as healthy has in fact a latent lesion. Therefore, the visual estimation of downy mildew infection may not give a good indication of the effect of the pathogen on host physiology, supporting other observations (Lakso *et al.*, 1982; Rabbinge *et al.*, 1985; Shtienberg, 1992). In these experiments we have considered only 'Merlot'. We cannot extend our conclusions to other cultivars, because the host response could be different. Lakso *et al.* (1982) demonstrated for powdery mildew of grapevine that visual infection estimation has been considered acceptable for "White Riesling" but not for "Concord". The results presented provide data sets for coupling disease severity of downy mildew with the effects on leaf physiology of main and lateral leaves, which are necessary for simulation models that quantitatively integrate the interactions between disease and crop growth (Dietrich *et al.*, 1997). The results also emphasize the important role of downy mildew as a stress element for the plant during ripening phase, a source element for carbohydrate production.

Quantitative effect of leaf damage caused by downy mildew (*Plasmopara viticola*) on growth and yield quality of grapevine “Merlot” (*Vitis vinifera*)

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Introduction

Downy mildew of grapevine, caused by *Plasmopara viticola* Berk. & Curt. (Berl. and de Toni), is one of the most important fungal diseases of European grapevine (*Vitis vinifera* L.). The causal agent attacks all green parts of the vine. Cluster infections are the most important factor for quantitative yield reduction. Leaf damage is, on the contrary, responsible for an indirect yield loss through a reduction of the carbohydrate production that negatively influences the grape quality, the reserve accumulation and the plant vigour in the next season (Goidanich, 1983). These are the reasons for which downy mildew, from its introduction in Europe, has been considered a disease with high destructive potential, which is still mostly controlled by chemical sprays without quantifying its real impact on the plant. Studies have been undertaken to compare the vine response at various levels of defoliation stress during the season (Candolfi-Vasconcelos, 1990; Candolfi-Vasconcelos *et al.*, 1994; Kliewer, 1970; Koblet *et al.*, 1994; Ollat and Gaudillere, 1998), but the dynamic character of disease epidemics and pest populations make it impossible to apply these results to estimate their influence on the grapevine. Progress in the actual application of the concept of Integrated Production (IP) requires knowledge about the quantitative interactions between pests or diseases and the crop system. In viticulture, this type of study has been undertaken for foliar pests with the aim of relating the population dynamics with vine growth, yield and fruit quality (Boller *et al.*, 1989; Boller and Candolfi, 1990; Candolfi, 1991; Candolfi *et al.*, 1993; Linder and Jermini 2001; Linder *et al.*, 2009; Martinson *et al.*, 1997; McNally *et al.*, 1985). In the pathological branch, some authors have compared differences of the yield quality parameters between healthy and infected plants for virus (Cabaleiro *et al.*, 1999; Credi and Babini, 1997; Guidoni *et al.*, 1997; Kliewer *et al.*, 1976; Reynolds *et al.*, 1997; Wolpert and Vilas, 1992), Esca disease (Chinnici *et al.*, 1999) and powdery mildew (*Erysiphe necator*) (Piva *et al.*, 1997) without considering the epidemic development. For Eutypa dieback (*Eutypa lata*) and Phomopsis cane (*Phomopsis viticola*) the disease progress has been related to yield quantity and vegetative growth (Kast, 1989; Munkvold *et al.*, 1994). Calonnec *et al.* (2004) have quantified the effect of different bunch infection levels of powdery mildew on grape yield, juice and wine quality, while Gadoury *et al.* (2004) have analysed the influence of powdery mildew on vine growth, yield and crop quality. Today it is exceptional to find important yield quantity losses caused by downy mildew epidemics in commercial vineyards. It is more common to observe epidemics causing different levels of leaf damage, which effect on plant growth and yield quality is generally unknown and therefore not quantified (Jermini *et al.*, 1997). Such knowledge is important for improving our IP strategies and in providing the basis for implementation of the plan in the new disease management systems which integrate the effect of disease on the plant.

Following the concept of crop system analysis (Delucchi, 1990; Jermini *et al.*, 2006), we conducted a study during the period 1996-1998 with the aim of quantifying the impact of downy mildew epidemics on the grapevine, considering the disease as a stress factor for the plant. The first step was to quantify the influence of the downy mildew infection on gas exchange capacity of the leaves (Jermini *et al.*, 2010a). This second work aims to analyze under field conditions the impact of downy mildew epidemics on the plant growth and yield quality in order to show a relationship between disease severity on leaves and yield quality losses.

Material and Methods

Plant material and experimental designs

The experiments were carried out during the period 1996-1998 in a vineyard of the Research station Agroscope Changins-Wädenswil ACW Centre of Cadenazzo planted with 'Merlot' grafted on 3309 rootstock. The vines were double cane pruned and vertical trained (double Guyot).

Three different treatments were compared: A = "Untreated canopy" (to prevent quantity losses, the clusters were treated once with a contact fungicide at the discovery of the first downy mildew sporulation); B = "Reduced fungicide schedule" (based on a first treatment at the appearance of the first symptoms, to avoid yield quantity losses followed by one or two additional fungicide applications during the early epidemic phase with the aim of delaying the epidemic). C = "Standard schedule" (schedule normally applied in the vineyard). The experimental plot was moved each year in different but homogenous blocks of the vineyard to avoid stress influence due to a repetition of the trials on the same place.

Three applications of Slick (250 g l⁻¹ difenoconazol) were made starting from bloom to prevent powdery mildew (*Erysiphe necator*) and black rot (*Guignardia bidwellii*) infections and one with Switch (25% fludioxonil +37.5% cyprodinil) on clusters at the end of July to control grey mold (*Botrytis cinerea*) infections. The fungicide applications for downy mildew control in the treatments are summarised in Tab. 1. On the canopy, the fungicides were applied with sprayer Fischer Mini-trac (Fischer Sarl, Collombey-le-Grand, Switzerland) with using a water volume 400 l ha⁻¹ and on the clusters with a motorized backpack sprayer Birchmeier M125 (Birchmeier Sprühtechnik AG, Sitten, Switzerland) using a water volume 1'100 l ha⁻¹ always at the ha rate indicated by the manufacturer.

Experiment 1996. This trial was placed in a plot planted in 1972 with a vine spacing of 1.80 x 1.40 m between and within the rows. Each treatment consisted of a plot of 48 plants divided in 6 sub-plots of 8 contiguous plants.

Table 1. Fungicide used, concentration and application date in the treatments “Untreated canopy”, “Reduced fungicide schedule” and “Standard schedule” for the experimental years 1996, 1997 and 1998. A fungicide application on clusters at the appearance of the first symptoms to avoid yield quantity losses was made on the plants of the treatment “Untreated canopy”, and at the first application of the experimental year 1996.

Year	treatments	Application date	Fungicide used	Active ingredients	Concentration use
1996	Reduced fungicide schedule	10.07	Curado D	6% cymoxanil + 40% folpet + 1.25% pyriphenox	4.0 kg ^{ha} ⁻¹
		20.08	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		29.08	Quadris	22.9% azoxystrobin	1.6 l ^{ha} ⁻¹
	Untreated canopy	10.07	Curado D	6% cymoxanil + 40% folpet + 1.25% pyriphenox	4.0 kg ^{ha} ⁻¹
	Standard schedule	24.05	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		05.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		18.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		03.07	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		17.08	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		02.08	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		20.08	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		29.08	Quadris	22.9% azoxystrobin	1.6 l ^{ha} ⁻¹
1997	Reduced fungicide schedule	16.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		16.07	Remiltine F pepite	37.5% folpet + 20% mancozeb + 6% cymoxanil	3.0 kg ^{ha} ⁻¹
	Untreated canopy	16.06	Remiltine F pepite	37.5% folpet + 20% mancozeb + 6% cymoxanil	3.0 kg ^{ha} ⁻¹
	Standard schedule	23.05	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		02.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		20.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		01.07	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		14.07	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		30.07	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		20.08	Cyrano	35% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
1998	Reduced fungicide schedule	29.06	Phaltan 80	80% folpet	2.0 kg ^{ha} ⁻¹
		10.07	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		04.08	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
	Untreated canopy	29.06	Phaltan 80	80% folpet	2.0 kg ^{ha} ⁻¹
	Standard schedule	02.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		09.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		24.06	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		10.07	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		23.07	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹
		04.08	Ridomil Viti	60% folpet + 7.5% methalaxyl	3.6 kg ^{ha} ⁻¹
		19.08	Cyrano	25% folpet + 4% cymoxanil + 50% Phosethyl-Al	3.2 kg ^{ha} ⁻¹

The number of shoots per plant, including the spurs, was regulated to 11 at the phenological stadium 53 BBCH (Baillod and Baggiolini, 1993) and the number of clusters was limited on August 8 (221 Julianday) to result in a homogeneous theoretical production for each sub-plot of 1.2 kg^m⁻², corresponding at the low potential yield estimated in the experiment. A first topping was done on June 18 (170 Julianday), a second one on July 16 (198 Julianday) and a last one on August third (226 Julianday).

Experiment 1997. This trial was placed in a plot planted in 1991 with a vine spacing of 2.00 x 1.20 m between and within the rows. The experimental design and the number of shoots per plant were the same as for the 1996 experiment. The yield regularisation was made on August 22 (234 Julianday) so as to obtain a theoretical production per subplot of 1.1 kg^m⁻²,

corresponding to the low potential yield estimated in the experiment. The first topping was done on June 23 (174 Julianday) and a second one on August 4 (216 Julianday).

Experiment 1998. This trial was placed in a plot planted in 1974 with a vine spacing of 1.80 x 1.40 m between and within the rows. Each treatment consisted of a plot of 48 plants divided in 8 sub-plots of 6 contiguous plants. For this experiment the number of shoots per plant, including the spurs, was regulated to 10 at the same periods as for the other years. The yield regularisation was made on July 30 (211 Julianday) with the aim of obtaining a theoretical production per subplot of 1.2 kg·m⁻², corresponding to the low potential yield estimated in the experiment. The first topping was done on June 30 (181 Julianday) and a second one on July 30 (211 Julianday).

Vegetative growth and disease assessment

One shoot per vine representing the middle vegetative growth of the plant was selected from each treatment replicate at the phenological stadium 53-55 BBCH (Baillod and Baggiolini, 1993). The number of main leaves, lateral shoots and leaves on lateral shoots was assessed weekly. Leaf area was measured on plant using the method proposed by Carbonneau (1976). Disease severity was estimated with the extended Horsfall scale (Horsfall and Cowling, 1978), in which a supplementary class for the lower disease level was introduced. In this way, the scale was divided into 12 classes: class 0 (0% damaged leaf area), class 1 (0* - 1% damaged leaf area), class 2 (1* - 3% damaged leaf area), class 3 (3* - 6% damaged leaf area), class 4 (6* - 12% damaged leaf area), class 5 (12* - 25% damaged leaf area), class 6 (25* - 50% damaged leaf area), class 7 (50* - 75% damaged leaf area), class 8 (75* - 88% damaged leaf area), class 9 (88* - 94% damaged leaf area), class 10 (94* - 97% damaged leaf area) and class 11 (97* - 100% damaged leaf area). The asterisk indicates a value slightly exceeding the indicated value.

Yield quality analysis

Samples of 25 berries were taken in each sub-plot choosing 2 berries on the upper, 2 in the middle and 1 on the lower part of the cluster. Each sample was crushed and soluble solids (°Brix at 20 °C), pH and titratable acidity (TA, as g·l⁻¹ tartaric acid) immediately measured. This control was carried out weekly from the beginning of veraison until the harvest (only for the 1996 experiment, the first control was made starting at full veraison). At vintage, each sub-plot was harvested individually and weighed. The crop was crushed to determine soluble solids, pH, TA, L-malic and tartaric acid. Soluble solids measurements were made with a refractometer (ERMA) with temperature correction. The pH was measured with a Metrohm 691 pH-meter (Metrohm AG Herisau, Switzerland) equipped with a microelectrode. TA was determined on 15 ml must by titration with 0.2 mol·l⁻¹ NaOH until pH 7.0. L-malic acid was analyzed by the enzymatic method (Boehringer Mannheim) and tartaric acid by the colorimetric method according to Rebelein (Lipka and Tanner, 1974).

Statistical analysis

Statistical analysis of the data was performed utilising the Sigmastat (SSPS) statistical package. Results were subjected to Anova and the Tuckey test was used to compare means.

Results

Disease progress

In 1996, 1997 and 1998 the first downy mildew sporulation appeared in the plots on June 25, 11 and, respectively, 24 corresponding to the phenological stages of full flowering for 1997 and fruit set for 1996 and 1998. The epidemic progress in the “Untreated canopy” treatment, expressed as disease severity (percentage of diseased leaf area/shoot), has shown the same tendency, independently of the late (1996 and 1998) or early (1997) apparition of the first sporulation in the field and increased starting from the beginning of the ripening phase (Fig. 1). At this phenological stage, the disease severity corresponded to 9.17%, 4.46% and 1.29% diseased leaf area/shoot for 1996, 1997 and, respectively, 1998 (Fig. 1). From veraison to harvest, the epidemic progressed rapidly and at the last control before harvest a disease severity of 37%, 34% and 48% of diseased leaf area/shoot was measured. The epidemic progress on main and lateral leaves followed a similar pattern and the final disease damage resulted higher on the lateral than on the main leaves, even though the disease increased 1-2 weeks before on the main leaves than on lateral leaves (data not shown). In 1996, the first fungicide application in the “Reduced fungicide schedule” treatment was made only on the clusters to avoid yield quantity losses and the first one on canopy was delayed with the aim to reduce a minimum the number of applications. The epidemic progress never increased until the beginning of veraison (August 6 = 221 Julian day) and at this phenological stage the disease severity was 1.5% and 1.3% in the “Reduced fungicide schedule” and, respectively, in the “Untreated canopy” treatments. Unfortunately, a very rapid increase was observed in the next fourteen days, so that the two later fungicide applications were inefficient in delaying the epidemic (Fig. 1). On the basis of this experience we changed the approach in 1997 and in 1998, applying the fungicides at the beginning of the epidemic and so achieve an important delay of the epidemic progress (Fig. 1). In the “Standard schedule” plots an increase of the disease was observed only after the end of the fungicide application, in Switzerland corresponding to the second half of August (Fig. 1).

Canopy development and vine vigour

The influence of downy mildew epidemics on the canopy development can be described by the amount of healthy leaf area per plant. This is an important parameter because it indicates

the amount of photosynthetic leaf area at the disposal to the vine for carbohydrate production.

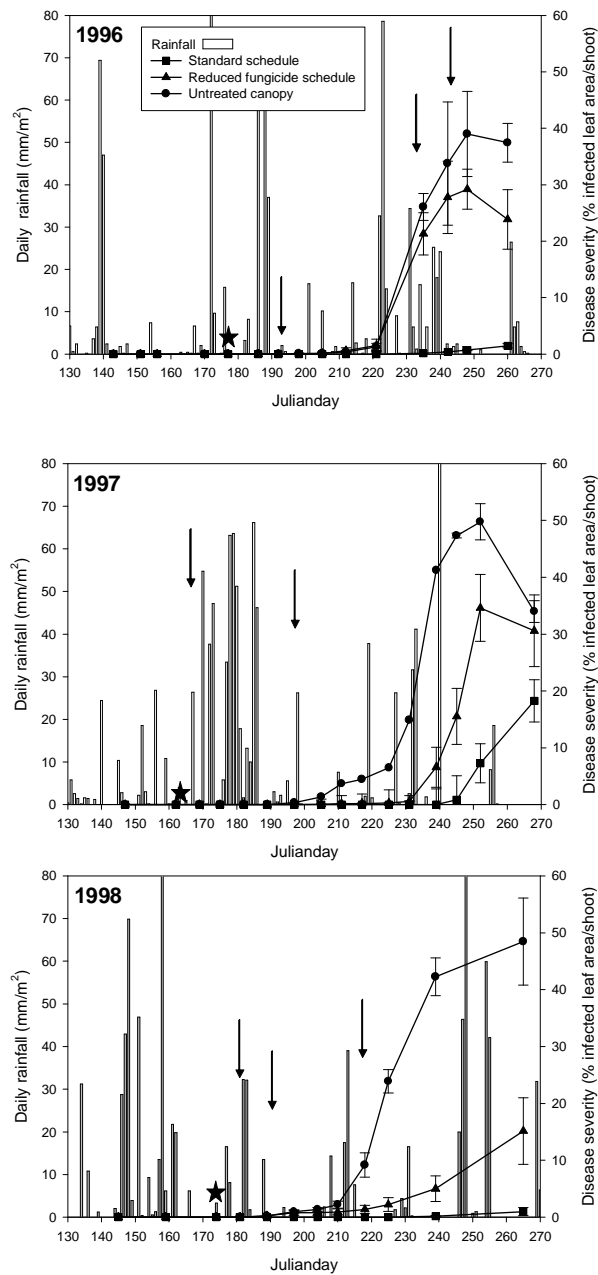


Figure 1. Daily rainfall (mm m⁻²) and disease severity progress, expressed as percentage of diseased leaf area per shoot on cultivar 'Merlot'. Three *P. viticola* control strategies are presented: "Untreated canopy", "Reduced fungicide schedule" and "Standard schedule" for the experimental years 1996, 1997 and 1998. Each point represents the average of 6 for 1996 and 1997 and 8 replications for 1998 with standard deviation. The star indicates the apparition of the first downy mildew sporulation in field and the arrow indicates the fungicide applications in the treatments "Untreated canopy" and "Reduced fungicide schedule". The fungicide applications in the "Untreated canopy" and the first made in 1996 in the treatment "Reduced fungicide schedule" were made only on clusters in correspondence to the first arrow.

During the three studied years, the disease did not influence the amount of total healthy leaf area per plant until veraison (Fig. 2A). From this phenological stage until harvest, the healthy leaf area per plant decreased rapidly at the same time as the epidemic increased (Fig. 2A). At the control before harvest, the average healthy leaf area/plant available for a vine in the “Untreated canopy” plots was 27.1% in 1996 of that of a plant in the “Standard schedule” plot and 32.7% and 22.9% for 1997 and, respectively, 1998. The application of a limited number of fungicides in the “Reduced fungicide schedule” plots has permitted keeping, on average, a healthy leaf area/plant of 56.6%, 54.8% and 88.1% for 1996, 1997 and, respectively, 1998 in relation to leaf area of a normally treated grapevine. During the ripeness phase, the decrease of healthy leaf area on the plant was attributed to the increase of the epidemic, which induced the leaf fall and a standstill of the new leaf formation (Fig. 2B). The leaf fall became important generally starting 3-4 weeks after veraison (Fig. 2B) and consequently, the plants in the “Untreated canopy” plots lost the 70.3% of the total leaves at harvest in 1996, 65.1% and 61.8% in 1997 and, respectively, 1998 in comparison with the “Standard schedule”. In the “Reduced fungicide schedule”, the effect of the fungicide application was more evident with a delay of the leaf fall, which had an intermediary dynamic with the exception of 1998 where the total leaf number remained stable until harvest (Fig. 2B). The plant vigour, expressed from the total pruning and one year shoot weight, did not show a clear effect following the important leaf area reduction due to downy mildew infection (Tab. 2).

Table 2. Effect of downy mildew epidemic on plant vigour of Merlot grapevine, expressed with the fresh weight of one year old cane (g) and the total pruning per vine ($\text{kg}\cdot\text{vine}^{-1}$), for the experimental years 1996, 1997 and 1998. Each value represents the average of 6 for 1996 and 1997 and 8 replications for 1998 with standard deviation. Means followed by same letter not significantly different at $p < 0.05$ (Tuckey test).

Attribute	Year	Treatment		
		Untreated canopy	Reduced fungicide schedule	Standard schedule
One year old cane fresh weight (g)	1996	45.36 \pm 2.86 a	44.42 \pm 3.57 a	42.51 \pm 2.67 a
	1997	57.46 \pm 4.28 a	60.50 \pm 9.74 a	48.74 \pm 5.55 a
	1998	56.40 \pm 8.51 b	72.40 \pm 8.31 a	63.00 \pm 8.15 ab
Total pruning fresh weight ($\text{kg}\cdot\text{vine}^{-1}$)	1996	0.613 \pm 0.024 a	0.613 \pm 0.046 a	0.576 \pm 0.053 a
	1997	0.497 \pm 0.042 ab	0.556 \pm 0.092 a	0.411 \pm 0.038 b
	1998	0.546 \pm 0.095 b	0.694 \pm 0.089 a	0.662 \pm 0.083 ab

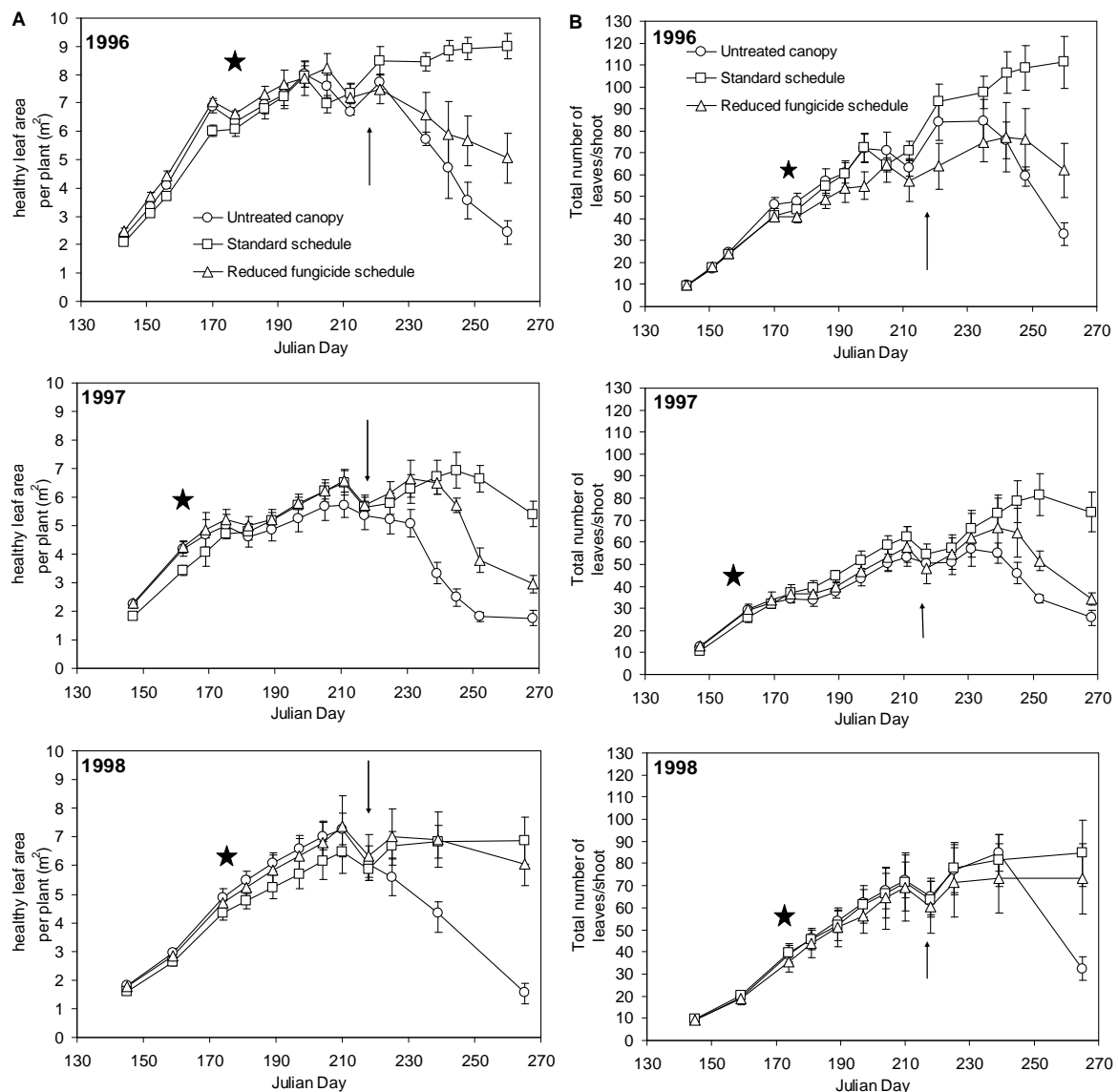


Figure 2. Evolution of the healthy leaf area/vine, expressed in m^2 per vine of the cultivar Merlot, where healthy leaf area = leaf area without downy mildew symptoms, yellowing or presence of sporulations (A) and of the total number of leaves/shoot (B) in the treatments “Untreated canopy”, “Reduced fungicide schedule” and “Standard schedule” for the experimental years 1996, 1997 and 1998. Each point represents the average of 6 for 1996 and 1997 and 8 replications for 1998 with standard deviation. The star indicates the finding of the first downy mildew sporulation in field and the arrow the beginning of the ripening phase (veraison).

Effect of downy mildew leaf infection on yield quantity and quality

The yield quantity didn't statistically differ between treatments for the experimental years 1996 and 1997 (Tab. 3). A significant difference in the production was found only in 1998 between the “Standard schedule” plot and the two other treatments probably due to an insufficient level of yield regulation in the “Standard schedule” plots and a greater berry weight (Tab. 3).

Table 3. Effect of downy mildew epidemic on yield quantity and juice quality of Merlot grapevine at harvest during the experimental years 1996-1998. The harvest was made on October 2 for 1996, September 23 for 1997 and September 29 for 1998. Each value represents the average of 6 for 1996 and 1997 and 8 replications for 1998 with standard deviation. Means followed by same letter are not significantly different at $p < 0.05$ (Tuckey test).

Attribute	Year	Treatment		
		Untreated canopy	Reduced fungicide schedule	Standard schedule
Yield ($\text{kg}\cdot\text{m}^{-2}$)	1996	1.150 ± 0.151 a	1.155 ± 0.149 a	1.013 ± 0.112 a
	1997	0.893 ± 0.130 a	1.044 ± 0.068 a	1.005 ± 0.050 a
	1998	1.022 ± 0.076 b	1.105 ± 0.123 b	1.357 ± 0.198 a
Berry weight (g)	1996	2.30 ± 0.28 a	2.24 ± 0.15 a	1.96 ± 0.03 a
	1997	1.67 ± 0.07 a	1.74 ± 0.11 a	1.64 ± 0.11 a
	1998	1.72 ± 0.06 b	1.91 ± 0.06 b	1.97 ± 0.05 a
Soluble solids (°Brix)	1996	17.8 ± 0.33 c	18.3 ± 0.15 b	19.20 ± 0.26 a
	1997	18.2 ± 0.11 b	18.8 ± 0.17 a	18.77 ± 0.10 a
	1998	17.4 ± 0.46 b	19.1 ± 0.36 a	19.46 ± 0.16 a
Juice pH	1996	3.37 ± 0.017 b	3.35 ± 0.014 b	3.39 ± 0.005 a
	1997	3.32 ± 0.015 a	3.27 ± 0.016 b	3.33 ± 0.004 a
	1998	3.58 ± 0.040 a	3.40 ± 0.024 b	3.44 ± 0.025 c
Titratable acidity ($\text{g}\cdot\text{l}^{-1}$)	1996	7.83 ± 0.17 a	7.70 ± 0.09 a	7.20 ± 0.14 b
	1997	7.15 ± 0.19 a	7.03 ± 0.16 a	6.67 ± 0.08 b
	1998	5.46 ± 0.19 b	5.92 ± 0.20 a	5.56 ± 0.20 b
Malic acid ($\text{g}\cdot\text{l}^{-1}$)	1996	4.98 ± 0.13 ab	5.08 ± 0.13 a	4.82 ± 0.09 b
	1997	3.37 ± 0.22 a	3.03 ± 0.19 b	3.12 ± 0.17 ab
	1998	3.47 ± 0.31 a	3.47 ± 0.17 a	3.22 ± 0.16 b
Tartaric acid ($\text{g}\cdot\text{l}^{-1}$)	1996	5.42 ± 0.20 a	5.13 ± 0.29 ab	4.95 ± 0.16 b
	1997	6.82 ± 0.09 ab	6.88 ± 0.23 b	6.50 ± 0.17 a
	1998	4.32 ± 0.11 a	4.45 ± 0.10 a	4.37 ± 0.08 a

Among the yield quality parameters, the sugar content, which is one of the most important, has been negatively influenced by the downy mildew leaf damage. The difference was particularly evident between the “Untreated canopy” and the “Standard schedule” plots with differences from 1.4 °Brix for 1996, 0.57 °Brix for 1997 and 2.04 °Brix for 1998. The “Reduced fungicide schedule” didn’t differ from the “Standard schedule” plot with the exception of 1996, where the result was intermediary between the two other treatments (Tab. 3). Fig. 3 shows the sugar accumulation dynamic during the three experimental years. The comparison between “Standard schedule” and “Untreated canopy” treatments emphasized, with exception of 1996 where the controls started later, how sugar accumulation in the berries begins to show a different dynamic 14 days (1997) and 7 days (1998) after the onset of ripening (Fig. 3). The increase in the difference of sugar content between these two treatments was generally regular and only in 1998 it remained constant between the end of August and the middle of September before decreasing in the last week before harvest. The sugar uptake dynamic of the crop in the “Reduced fungicide schedule” did not show, for 1997 and 1998, differences with the “Standard schedule” treatment. In 1997, a dynamic similar to that of the “Untreated canopy” treatment was observed (Fig. 3).

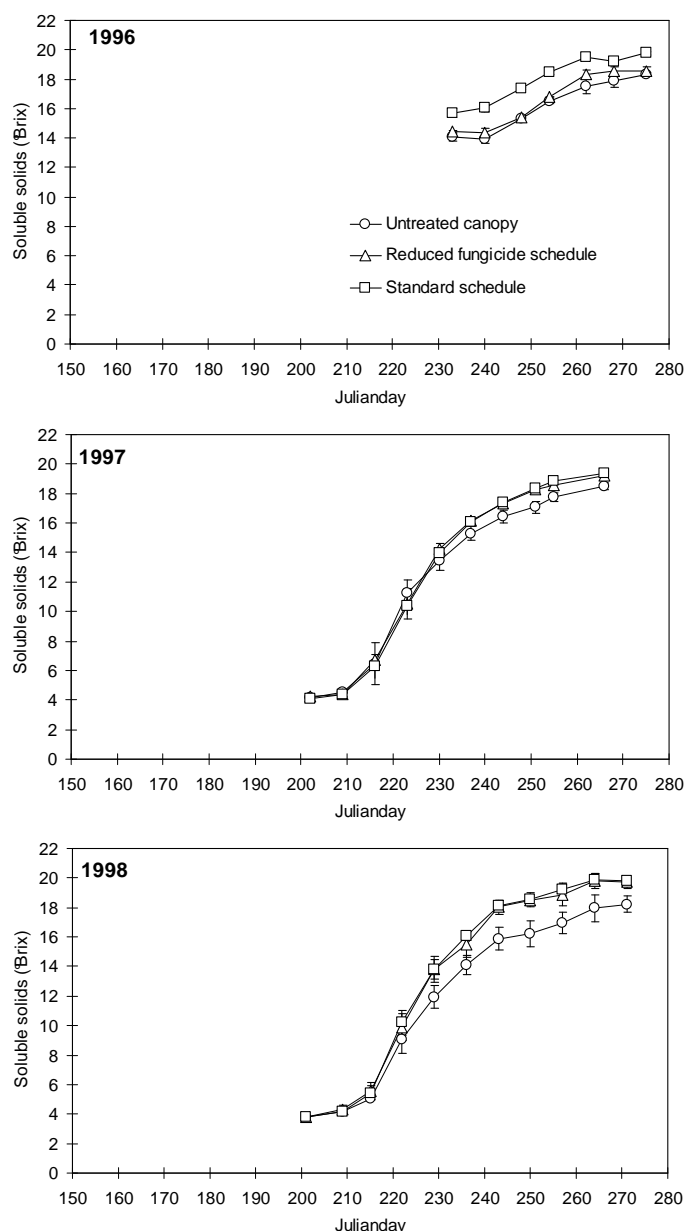


Figure 3. Accumulation of the must soluble solids (°Brix) in the berries of the cultivar Merlot during the ripening phase for the treatments “Untreated canopy”, “Reduced fungicide schedule” and “Standard schedule” for the experimental years 1996, 1997 and 1998. Each point represents the average of 6 for 1996 and 1997 and 8 replications for 1998 with standard deviation.

Contrary to expectations, no correlation between disease severity progress on the canopy and sugar accumulation in the berries from veraison until harvest was found in 1997 and 1998 (1996 not considered) (Fig. 4). For the other yield quality parameters measured at harvest (Tab. 3), only the titratable acidity showed a certain influence with values generally higher in the “Untreated canopy” and “Reduced fungicide schedule” treatments in comparison with the “Standard schedule”. The pH and the malic and tartaric acids didn’t show a clear effect due to the downy mildew leaf damages (Tab. 3).

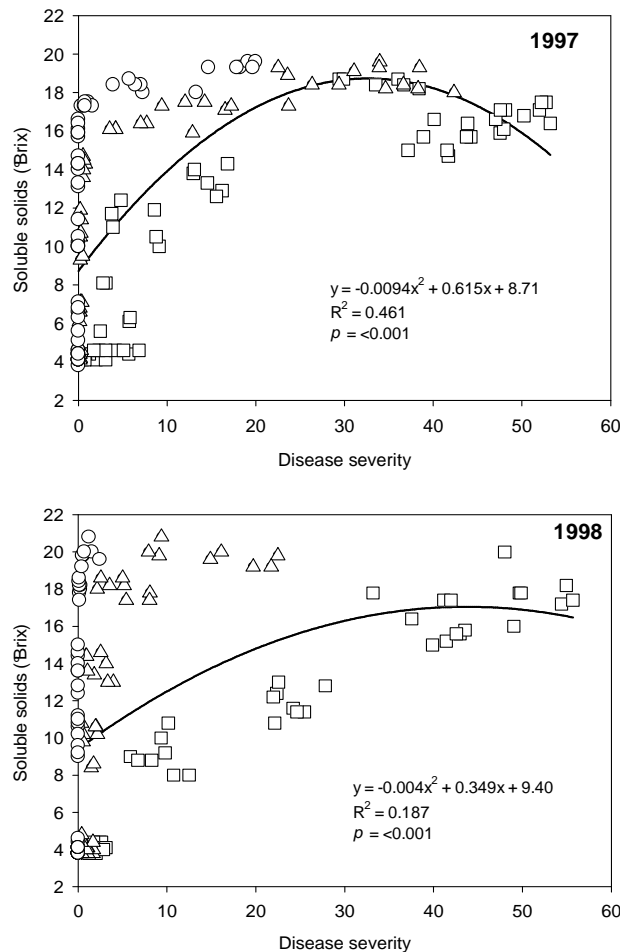


Figure 4. Relation between the must soluble solids and disease severity of *P. viticola* on the cultivar Merlot measured weekly from veraison to harvest for 1997 and 1998 experimental years. Squares correspond to “Untreated canopy”, triangles to “Reduced fungicide schedule” and circles to “Standard schedule” treatments. Each data point represents a single sample. For each of one replication, 6 in 1997 and 8 in 1998.

Discussion

Contrary to the expectations, the impact of downy mildew leaf damage on plant canopy on plant growth and on yield quality parameters were most pronounced only in the sugar content of berries. The plant growth and vigour wasn't affected by the important leaf area reduction due to downy mildew epidemics. In fact, Vasconcelos and Castagnoli (2000) indicate that vines in balance should have one year canes each weighing 30 to 40 g with 40 g fresh weight being preferred in cool climates. For each experimental year we always measured cane weight greater than 40 g fresh weight demonstrating the lower impact of the epidemics on this factor. The same observations have been made by Wolpert and Vilas (1992) and Cabaleiro *et al.* (1999), which have shown that grapevine leafroll didn't influence the plant vigour expressed by pruning weight. These results should be analysed considering our climatic conditions, which influenced the dynamic character of the downy mildew

epidemics, the cultural methods applied and the variety cultivated and consequently the plant response to a stress. The role of these elements has been reported by different authors. Credi and Babini (1997), for single and mixed virus infections, and Reynold *et al.* (1997), for the Rupestris stem pitting virus, have observed differences due to the type of virus combinations, their source and, respectively, to the variety. Kliwer and Fuller (1973) emphasise that with defoliation at veraison or later, the pruning weight of canes would not be a good indicator of reduced vine capacity due to loss in leaf area, because dry matter accumulation is more reduced in the trunk than in the canes. Under our climatic and growth conditions the exponential increase of the epidemics during the ripening can only partially influence the plant vigour of 'Merlot' cultivar. We have also applied each year a plot rotation and this choice could also have had an influence on the plant response, because, as demonstrated by Reynolds *et al.* (1989) in trials of different summer hedging levels, the effect of time and severity on the plant vigour is not substantially influenced by timing of hedging, but the severity consistently reduced vigour each season. An analysis of the real impact of the downy mildew epidemics on growth parameters needs a repetition in time.

One single application of a contact fungicide on clusters at the apparition of first sporulation has permitted preserving the crop production, with the exception of 1998, where a lower level of the crop limitation resulted in the differences observed. This result can not be generalised, because most early apparition of the downy mildew in the field requires certainly one more fungicide application. Nevertheless, the choice of a good timing for the cluster protection permits preserving yield quantity with a very limited number of fungicide applications. The leaf area at disposal to the plant also has a major role on the crop formations. Studies on artificial defoliation (Butterrose, 1966; Candolfi-Vasconcelos, 1990; Hunter *et al.*, 1995; Kliwer, 1970; Koblet *et al.*, 1994) have demonstrated that yield quality at harvest depends on the combination effect of time and defoliation severity. Main leaves appear to play a main role for the yield formation and sugar accumulation in the berries seems to depend on the available active leaf area of lateral leaves (Candolfi-Vasconcelos, 1990). Our results confirm these observations, but it is important to consider that a downy mildew epidemic has a dynamic character. The climatic conditions influence the oospores maturation during the season, the epidemic progress and, consequently, the time and severity of the leaf damage. It is therefore logical that the epidemic character of the downy mildew observed during these three experimental years, associated with the efficacy of the fungicide application on the clusters, has permitted preserving until the ripening an adequate main leaf area to supply the carbohydrates required by the cluster for the crop formation. Sugar accumulation is the main quality factor which was negatively influenced from downy mildew, but the content in the berries isn't proportional to the decrease of the leaf area. Hunter *et al.* (1995) show that a 33% defoliation level at veraison had no effect on soluble solid accumulation but increased

titratable acidity and reduced pH. Our results show a different situation. The factors influencing pH and total acidity are complex and the dynamic character of the epidemics could influence in time the chemical and enzymatic processes responsible for acid composition of the berries. Nevertheless, we could assume that the differences observed are probably due to the effect of a different K^+ content of the musts, because a different K^+ concentration influences the pH value. This behaviour of titratable acidity and pH is an example of the complex interaction between disease and plant. For the grapevine, some studies have been undertaken to compare the impact of disease on yield components (Chinnici *et al.*, 1999; Credi and Babini, 1997; Guidoni *et al.*, 1997; Kliewer *et al.*, 1976; Piva *et al.*, 1997; Reynolds *et al.*, 1997; Wolpert *et al.*, 1992), but the symptom expression and the impact of the epidemic development in relation to yield formation have not been taken into account. Other authors (Duso and Belvini, 1992) have tried to artificially simulate pest leaf damage by applying a progressive defoliation from veraison until harvest with different intensity. They have observed a negative influence on berry weight and fruit quality with defoliation levels between 25 and 50%, but the damage also seems to depend on the relationship between crop load and leaf area. Even though these experimentations partially confirm our results, it is impossible to extract indications explaining the grapevine behaviour, because they consider a fixed defoliation level made at a defined time. On the contrary, the healthy leaf area reduction caused by downy mildew has a dynamic evolution depending on the epidemic increase, which is modulated by weather.

Measurements of leaf gas exchange have furthermore indicated that healthy leaf parts of an infected leaf of the lateral shoot react more negatively than main leaves and that photosynthesis decreases with the increase of leaf damage severity (Jermini *et al.*, 2010). If we associate this impact factor with the major role of the lateral leaves during the berry ripening phase on the sugar accumulation (Candolfi-Vasconcelos, 1990), the low plant capacity of reconstructing the assimilating apparatus (Candolfi-Vasconcelos, 1990) at this time of the season and the rapid development of the epidemic with a colonisation of the new formed leaves, it is difficult to understand the low correlation between sugar accumulation and disease severity. This apparent contradiction is in accordance with the results of Koblet *et al.* (1994), which, in experiments made with different defoliation levels, have demonstrated that sugar accumulation is not proportional to the decrease in leaf area. They found that reserves might be exported from woody parts of the plant to the fruit under defoliation stress conditions to compensate for the carbohydrate requirements of the berries. The capacity of the vines to apply compensation mechanisms has also been demonstrated for abiotic (Buttrose, 1970; Candolfi-Vasconcelos, 1990; Candolfi-Vasconcelos *et al.*, 1994; Murisier, 1996) and biotic stress factors as the grape leafhopper *Empoasca vitis* (Candolfi *et al.*, 1993), the spider mite *Tetranychus urticae* (Candolfi, 1991) and eastern grape leafhopper

Erythroneura comes (Martinson *et al.*, 1997). In fact, during the ripening phase the berries represent the main sink for the plant and lateral leaves generally play the main role in supplying the fruit requirements (Candolfi-Vasconcelos, 1990). Our results emphasize the potential capacity of the grapevine to compensate for the stress induced by downy mildew and the importance of the timing of a fungicide application in delaying the epidemics. The comparison of the ripening dynamic between “Untreated canopy” and “Standard schedule” treatments indicates a constant increase of the sugar content of the berries until the end of the first ripening phase. Afterwards, the difference remains generally constant. In this case the stress situation is probably too high to permit the plant to compensate for the deficiency. The application of a reduced control schedule depends on the timing of the fungicide application. The 1996 trial stresses this importance, because during the two other experimental years a choice of the good timing delayed the epidemic, leaving the plant the possibility to supply the carbohydrate requirement of the berries. Fungicide applications at the first epidemic phase therefore contribute greatly in delaying the epidemic. Gadoury *et al.* (2001) have shown the same results with powdery mildew. Multiple fungicide applications during the peak period of fruit susceptibility give the most efficient control. It is therefore possible to assume that for downy mildew there exists a damage threshold during the ripening phase which limits the stress situation permitting the plant to enhance a compensatory mechanism. Our data show no significant differences in the soluble solids contents at harvest between the treatments “Reduced fungicide schedule” and “Standard schedule” if the disease severity is limited to between 1% at the beginning of ripening and 5% at the end of August, corresponding to the end of the first ripening phase. This hypothesis must be validated and its application should be supported by a simulation model that integrates, on a quantitative basis, the epidemic progression and the interactions between disease and crop growth (Dietrich *et al.*, 1997). It is also important to analyse this strategy on the same plot for a long period in order to better evaluate the disease impact on the plant growth.

Quantification of the influence of the downy mildew (*Plasmopara viticola*) epidemics on the compensatory capacities of *Vitis vinifera* cv “Merlot” to limit the qualitative yield damage

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Introduction

Grapevine is subjected in the field to a multitude of stress factors ranging from abiotic factors to pests or diseases. However grape has a great potential for stress acclimation (Koblet *et al.*, 1996). In the current grapevine production system, where a maximal quality-quantity yield production over a long period is requested, the assimilate allocation system of the plant is manipulated during the season to achieve this objective (Reynolds and Waedle, 1989). Canopy management practices are important tools in promoting suitable conditions for optimal quantitative and qualitative yield production. The effect of time and severity of defoliation and the crop load can negatively influence the plant, which applies strategies to compensate for these stress situations (Candolfi-Vasconcelos, 1990; Candolfi-Vasconcelos *et al.*, 1994; Hunter and Visser, 1988; Kliewer, 1970; Kliewer and Fuller, 1973; Koblet *et al.*, 1993; Koblet *et al.*, 1996; Koblet *et al.*, 1997; Murisier, 1996). Stress situations induced during the ripening period modify the sink priority of the vine and, consequently, the priority of the assimilate allocation (Candolfi-Vasconcelos *et al.*, 1994; Koblet *et al.*, 1993, Koblet *et al.*, 1996; Koblet *et al.*, 1997). Pests and diseases can be considered biotic stress factors, which are capable of inducing yield losses (Boller *et al.*, 1989; Boller and Candolfi, 1990; Cabaleiro *et al.*, 1999; Calonnec *et al.*, 2004; Candolfi, 1991; Candolfi *et al.*, 1993; Chinnici *et al.*, 1999; Credi and Babini, 1997; Gadoury *et al.*, 2004; Guidoni *et al.*, 1997; Kast, 1989; Kliewer and Linder, 1976; Linder and Jermini, 2001; Martinson *et al.*, 1997; McNelly *et al.*, 1985; Munkvold *et al.*, 1994; Piva *et al.*, 1997; Reynolds and Wardle, 1989; Wolpert and Vilas, 1992), but only for the spider mite *Tetranychus urticae* and the green leafhopper *Empoasca vitis* it was demonstrated that the plant was able to compensate for the leaf damages by increasing the leaf area of the lateral shoots (Candolfi, 1991; Candolfi *et al.*, 1993).

In two previous studies we have analysed the impact of down mildew (*Plasmopara viticola*) epidemics on leaf gas exchange (Jermini *et al.*, 2010a) and on the yield quality of grapevine, showing how the plant tried to compensate the stress induced by the leaf damage (Jermini *et al.*, 2010b). This third work aims to investigate the compensation system that the plants use to compensate for the stress situation induced by downy mildew and to quantify the plant response.

Material and Methods

Plant material and experimental designs

The experiments were carried out during the period 1996-1998 in a vineyard of the Research station Agroscope Changins-Wädenswil ACW Centre of Cadenazzo (South part of Switzerland) planted with the cv. Merlot grafted on 3309 rootstock. The vines were double cane pruned and vertical trained (double Guyot). Three different *P. viticola* control strategies

were compared (Jermini *et al.*, 2010b): A = “Untreated canopy” (to prevent quantity losses, the clusters were treated once with a contact fungicide at the discovery of the first downy mildew sporulations); B = “Reduced fungicide schedule” (based on a first treatment at the appearance of the first symptoms, to avoid yield quantity losses followed by one or two additional fungicide applications during the early epidemic phase with the aim to delay epidemic). C = “Standard schedule” (normally schedule applied in the vineyard). The experimental plot was moved each year in different but homogenous blocks of the vineyard to avoid stress influence due to a repetition of the trials on the same place.

Experiment 1996. This trial was placed in a plot planted in 1972 with a vine spacing of 1.80 x 1.40 m between and within the rows. Each treatment consisted of a plot of 48 plants divided in 6 sub-plots of 8 contiguous plants. The number of shoots per plant, including the spurs, was regulated to 11 at the phenological stadium 53 BBCH (Baillod and Baggiolini, 1993) and the number of clusters was limited on August 8 to result in a homogeneous theoretical production for each subplot of $1.2 \text{ kg}\cdot\text{m}^{-2}$, corresponding to the low potential yield estimated in the experiment. A first topping was done on June 18, a second one on July 16 and a last one on August 13.

Experiment 1997. This trial was placed in a plot planted in 1991 with a vine spacing of 2.00 x 1.20 m between and within the rows. The experimental design and the number of shoots per plant were the same as for the 1996 experiment. The yield regularisation was made on August 22 so as to obtain a theoretical production per subplot of $1.1 \text{ kg}\cdot\text{m}^{-2}$, corresponding to the low potential yield estimated in the experiment. The first topping was done on June 23 and a second one on August 4.

Experiment 1998. This trial was placed in a plot planted in 1974 with a vine spacing of 1.80 x 1.40 m between and within the rows. Each treatment consisted of a plot of 32 plants divided in 8 sub-plots of 6 contiguous plants. For this experiment the number of shoots per plant, including the spurs, was regulated to 10 at the same periods as for the other years. The yield regularisation was made on July 30 with the aim of obtaining a theoretical production per subplot of $1.2 \text{ kg}\cdot\text{m}^{-2}$, corresponding to the low potential yield estimated in the experiment. The first topping was done on June 30 and a second one on July 30.

Vegetative growth and disease assessment

One main shoot per vine representing the middle vegetative growth of the plant was selected from each treatment replicate at the phenological stadium 53-55 BBCH (Baillod and Baggiolini, 1993). The number of main leaves, lateral shoots and leaves on lateral shoots was assessed weekly. Disease severity was estimated with the extended Horsfall scale (Horsfall and Cowling, 1978), in which a supplementary class for the lower disease level was introduced. In this way, the scale was divided into 12 classes: class 0 (0% damaged leaf area), class 1 (0*-1% damaged leaf area), class 2 (1* - 3% damaged leaf area), class 3 (3* -

6% damaged leaf area), class 4 (6* - 12% damaged leaf area), class 5 (12* - 25% damaged leaf area), class 6 (25* - 50% damaged leaf area), class 7 (50* - 75% damaged leaf area), class 8 (75* - 88% damaged leaf area), class 9 (88* - 94% damaged leaf area), class 10 (94* - 97% damaged leaf area) and class 11 (97* - 100% damaged leaf area). The asterisk indicates a value slightly exceeding the indicated value.

Wood samples harvesting and reserve analysis

Samples were taken for each subplot and treatment during pruning in the month of February of the year following the experiment. One-year-old wood samples were chosen between the 3rd and 6th internode taking one shoot per plant and, where it was possible, generally the shoot considered for the disease assessment was selected. At the same time, the two-year-wood (cane) samples included 2 parts of each plant taken between the 3rd and 4th node and the 6th and 7th node. The trunk sample consisted of 3-5 g of sawdust produced by perforation at different heights of the trunk with an electric drill fitted with a 3 mm bit. The roots were collected after to having dug a profile along the plot of 80-100 cm depth. Roots of fine and middle diameter (0.5-5 mm) were collected from the plants of the plot and washed. All samples were cut and oven-dried at 65 °C and then crushed in a hammer mill. The obtained powder was dried during at least 2 weeks over di-phosphor pentoxide (P_2O_5) before extraction. Glucose, fructose and sucrose were extracted by a hydroalcoholic solution (70 vol.% ethanol) at 80 °C and then analysed by the enzymatic method (Boehringer Mannheim). The solid residue of the extraction was dried over di-phosphor pentoxide (P_2O_5) and starch was extracted with dimethyl sulfoxide in a boiling water bath. Starch was analysed in this second extract by the enzymatic method (Boehringer Mannheim). All results are given in mg sugar per g of dry matter.

Statistical analysis

Statistical analysis of the data was performed utilising the Sigmastat (SSPS) statistical package. Results were subjected to Anova and the Tuckey test was used to compare means.

Results

Compensation through increase of leaf area

The first downy mildew sporulation in the field was found on June 25, June 11 and 24 in 1996, 1997 and, respectively, 1998 and the epidemics remained at low level until the beginning of the veraison (first week of august), where we had a severity from 1.3%, 4.5% 9.1% and for 1996, 1997 and, respectively 1998 in the “Untreated canopy” treatment (Jermini *et al.*, 2010b). The “Reduced fungicide schedule” delayed, with the exception of 1996, the epidemic progress and the severity was, at the same periods, from 1.5%, 0.2% and 1.4% for

1996, 1997 and, respectively 1997 in the “Reduced fungicide schedule” treatment (Jermini *et al.*, 2010b). The total number of leaves per shoot and the total number of leaves per lateral shoot did not differ significantly between “Untreated canopy” and “Standard schedule” plots from the first sporulation apparition until ripening (Fig. 1).

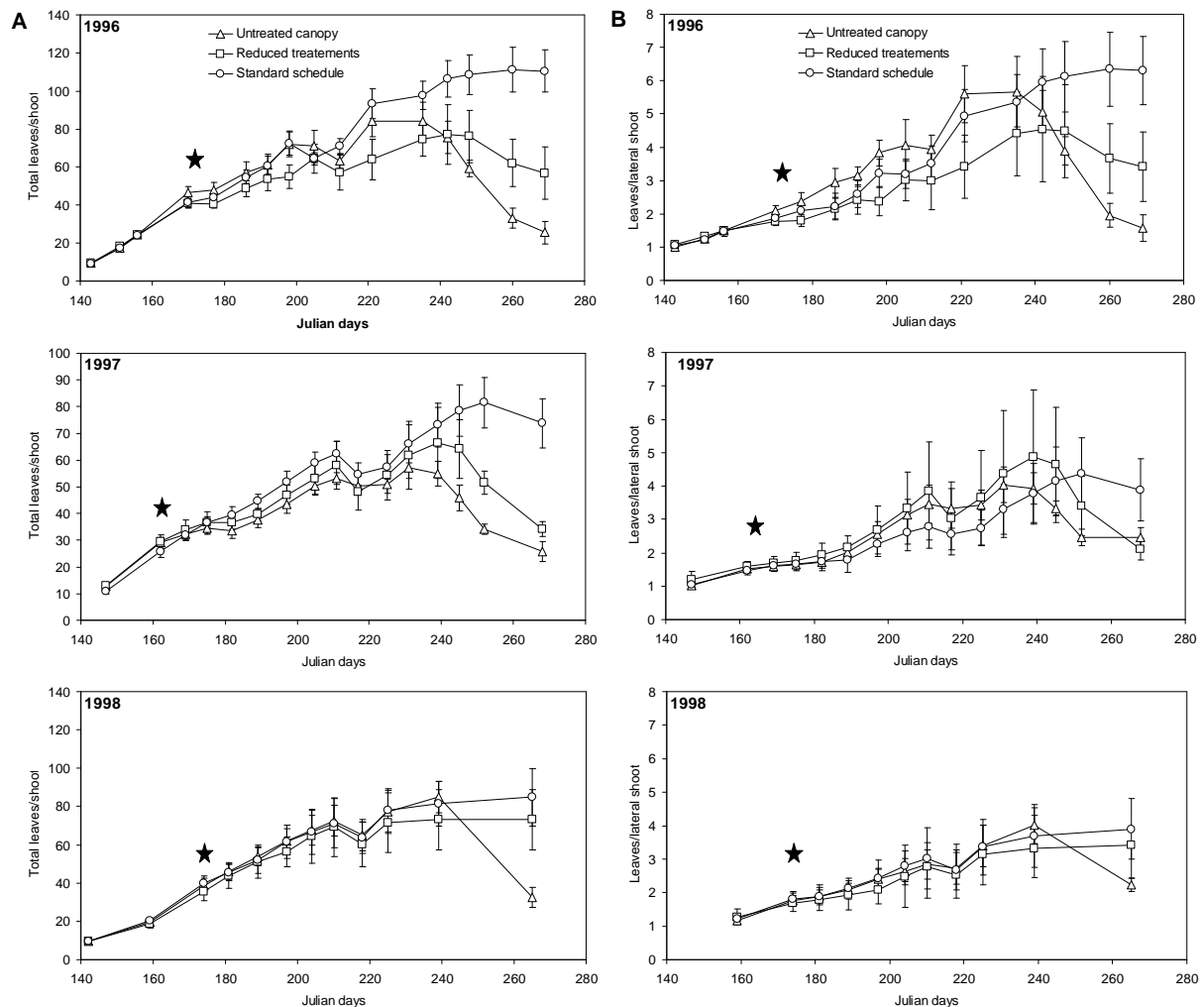


Figure 1. Effect of the downy mildew epidemics 1996, 1997 and 1998 on A) the total number of leaves per shoot and B) the total number of leaves per lateral shoot of plants of the cultivar Merlot. Each point represents the average of 6 for 1996 and 1997 and 8 replications for 1998 with standard deviation. The star indicates the finding of the first downy mildew sporulation in the field.

Low disease level did not induce lateral shoot growth. In August of 1996 and 1997 the epidemic progress (Jermini *et al.*, 2010b) reduced the total number of leaves per shoot as a consequence of the high disease severity on the leaves (Fig. 1). The good efficacy obtained in the “Reduced fungicide schedule” plot in 1998 resulted in a total number of leaves/shoot similar to that in the “Standard schedule” plot. In this year leaf abscission was not observed until the last period of ripening and consequently no differences between treatments were found (Fig. 1). Nevertheless, the disease incidence on August 13 was 76.3% in the “Untreated canopy” plot and 50.1% in the “Reduced fungicide schedule”.

Compensation through mobilisation of the starch reserves

To compensate for the impact of the downy mildew epidemics the grapevine of the “Untreated canopy” plots reduced between 37% and 58% the total reserve content of the roots (Tab. 1).

Table 1. Effect of the downy mildew epidemics on the starch and sugar (glucose, fructose and saccharose) content expressed as $\text{mg}\cdot\text{g}^{-1}$ dry matter (DM) of the roots of rootstock plant 3309 grafted with the cultivar Merlot. Data represent the average of 6 replications for 1996 and 1997 and 8 for 1998. Means followed by same letter are not significantly different at $p < 0.05$ (Tuckey test).

Attribute	Year	Untreated canopy	Reduced fungicide schedule	Standard schedule
Glucose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	6.00 ± 0.35 a	5.25 ± 1.33 a	2.60 ± 0.19 b
	1997	7.36 ± 0.83 a	5.37 ± 0.37 b	4.00 ± 0.31 c
	1998	11.60 ± 1.64 a	3.95 ± 0.62 b	2.95 ± 0.47 b
Fructose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	7.85 ± 0.46 a	6.85 ± 1.69 a	3.35 ± 0.24 b
	1997	10.89 ± 1.04 a	8.22 ± 0.49 b	6.09 ± 0.45 c
	1998	14.45 ± 1.50 a	6.10 ± 0.87 b	4.07 ± 0.77 c
Saccharose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	15.40 ± 2.18 a	17.07 ± 2.18 a	20.50 ± 0.74 b
	1997	21.50 ± 1.56 a	25.62 ± 1.94 ab	25.25 ± 1.35 b
	1998	18.16 ± 1.56 a	20.45 ± 1.62 b	21.85 ± 1.52 b
Total sugars ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	29.25 ± 2.17 a	29.17 ± 3.81 a	26.45 ± 0.54 a
	1997	39.75 ± 2.85 a	39.21 ± 2.33 a	35.34 ± 1.53 b
	1998	44.21 ± 2.54 a	30.50 ± 2.09 b	28.87 ± 1.32 b
Starch ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	28.53 ± 4.57 a	46.25 ± 7.32 b	112.20 ± 14.66 c
	1997	52.44 ± 8.99 a	91.49 ± 8.48 b	112.06 ± 9.68 c
	1998	27.67 ± 11.24 a	94.54 ± 12.77 b	129.79 ± 18.81 c
Total reserves ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	57.78 ± 4.34 a	75.42 ± 8.34 b	138.65 ± 14.50 c
	1997	92.19 ± 9.34 a	130.70 ± 9.59 b	147.40 ± 10.12 c
	1998	71.88 ± 9.93 a	125.04 ± 11.77 b	158.66 ± 18.56 c

For the three experimental years a reduced spray schedule has permitted limiting the amount of the mobilised reserves, while leaving the total reserves stable, although significantly lower than the “Standard schedule” plot. The mobilisation effect was particularly evident for the starch, which is the main assimilate storage form of the plant (Hunter *et al.*, 1995). In comparison with the “Standard schedule” plot, we observed a significant reduction of the starch contents in the “Untreated canopy” plot varying between 53.2% for 1997 and 78.5% for 1998. The application of a reduced number of fungicides has resulted in an intermediary situation but is insufficient in avoiding an important reserve mobilisation. The same situation has been found for the total sugar content of the roots. Between the different sugars, which constitute the most important constituents of the reserves, glucose and fructose content was significantly higher in the plants submitted to the downy mildew epidemics and saccharose was significantly lower. Similarly as for the starch content, the effect of a partial fungicide protection induced an intermediary situation, with in some cases no significant differences in the glucose content. Despite saccharose constituting the most important sugar of the root’s reserves, the impact of the epidemics of the “Reduced treatments schedule” plot was less

important than on glucose and fructose and significant differences have been found only in 1997. The total sugar content in the roots was generally significantly higher in the “Untreated canopy” and “Reduced treatments schedule” plot with the exception of 1997 where no differences were observed.

In the trunk (Tab. 2) a significant reduction of the total amount of reserve components was observed only for 1998 with a difference of 5.3% between the “Standard schedule” and the “Untreated canopy” plot.

Table 2. Effect of the downy mildew epidemics on the starch and sugar (glucose, fructose and saccharose) contents expressed as $\text{mg}\cdot\text{g}^{-1}$ dry matter (DM) of the trunk of the vine of cultivar Merlot. Data represent the average of 6 replications for 1996 and 1997 and 8 for 1998. Means followed by same letter are not significantly different at $p < 0.05$ (Tuckey test).

Attribute	Year	Untreated canopy	Reduced fungicide schedule	Standard schedule
Glucose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	27.63 ± 1.32 a	25.39 ± 1.59 b	21.05 ± 1.06 c
	1997	37.19 ± 2.86 a	32.00 ± 2.28 b	26.43 ± 1.70 c
	1998	32.32 ± 1.95 a	25.32 ± 1.35 b	23.49 ± 1.38 b
Fructose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	23.70 ± 1.36 a	22.64 ± 1.54 a	18.56 ± 0.78 b
	1997	34.21 ± 2.78 a	29.09 ± 2.03 b	24.69 ± 1.44 c
	1998	30.06 ± 1.48 a	24.31 ± 0.99 b	23.54 ± 1.03 b
Saccharose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	27.83 ± 2.50 a	30.32 ± 1.59 a	26.51 ± 3.49 a
	1997	23.94 ± 2.53 a	28.32 ± 4.26 a	33.52 ± 1.78 b
	1998	38.34 ± 2.22 a	45.01 ± 1.54 b	44.21 ± 2.16 b
Total sugars ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	79.16 ± 3.18 a	78.35 ± 3.85 a	66.12 ± 4.61 b
	1997	95.34 ± 5.48 a	89.41 ± 6.09 ab	84.64 ± 3.07 b
	1998	100.72 ± 3.00 a	94.64 ± 2.95 b	91.24 ± 2.83 b
Starch ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	12.69 ± 1.03 a	15.38 ± 2.71 a	19.98 ± 2.45 b
	1997	34.43 ± 3.61 a	41.51 ± 4.67 ab	43.71 ± 7.24 b
	1998	13.50 ± 2.33 a	29.64 ± 2.48 b	29.39 ± 2.84 b
Total reserves ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	91.85 ± 3.47 a	93.73 ± 6.11 a	86.10 ± 6.77 a
	1997	129.77 ± 8.55 a	130.92 ± 9.97 a	128.35 ± 10.04 a
	1998	114.22 ± 3.02 a	124.28 ± 3.29 b	120.63 ± 3.02 b

No significant differences have been observed between the “Standard schedule” and the “Reduced fungicide schedule” plots. The trunk did not constitute an important storage point for starch, but, as in the roots, a significant decrease of reserve elements in the plants of the “Untreated canopy” plot was observed. The reduction was of 36.6% for 1996, 21.2% and 54% for 1997 and, respectively, 1998. The starch content in the plants of “Reduced treatments schedule” plot was significantly lower than the “Standard schedule” plot only in 1996. The total sugar content was more important than starch and in the “Standard schedule” plot constituted 77% of the total reserves for 1996, 66% and 76% for 1997 and, respectively, 1998, but showed the same tendencies observed for the starch with a significantly higher content in the plants of the “Untreated canopy” plot as response to a stress situation. The saccharose content was variable between the treatments and years. Fructose and glucose content was generally higher in the “Untreated canopy” in comparison

with the “Standard schedule” plot. The application of a reduced fungicide schedule induced, as in the roots, an intermediary situation.

The total reserves content of the cane (Tab. 3) did not diverge significantly between the three treatments for each experimental year. This was due, as observed for roots and trunk, to a transformation of the starch, which decreased 47% in 1996, 21% and 63% for 1997 and, respectively, 1998, indicating for this last experimental year a marked stressed situation.

Table 3. Effect of the downy mildew epidemics on the starch and sugar (glucose, fructose and saccharose) contents expressed as $\text{mg}\cdot\text{g}^{-1}$ dry matter (DM) of the cane (two year old wood) of the vine of the cultivar Merlot. Data represent the average of 6 replications for 1996 and 1997 and 8 for 1998. Means followed by same letter are not significantly different at $p < 0.05$ (Tuckey test).

Attribute	Year	Untreated canopy	Reduced fungicide schedule	Standard schedule
Glucose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	24.51 ± 1.87 a	22.76 ± 1.11 a	19.33 ± 1.60 b
	1997	28.26 ± 0.91 a	26.05 ± 0.98 b	22.08 ± 1.88 c
	1998	30.14 ± 1.62 a	25.08 ± 1.87 ab	23.38 ± 0.68 b
Fructose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	24.70 ± 2.97 a	23.89 ± 1.28 a	20.15 ± 1.61 b
	1997	28.16 ± 0.69 a	26.81 ± 0.84 a	24.73 ± 1.67 b
	1998	29.30 ± 1.34 a	25.05 ± 1.60 ab	23.75 ± 0.99 b
Saccharose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	22.54 ± 4.63 a	23.43 ± 5.33 a	20.62 ± 2.97 a
	1997	35.63 ± 2.18 a	35.35 ± 2.72 a	38.32 ± 1.66 a
	1998	46.28 ± 2.89 a	48.79 ± 2.21 ab	50.00 ± 2.37 b
Total sugars ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	71.75 ± 7.76 a	70.06 ± 5.73 a	60.10 ± 2.78 b
	1997	92.05 ± 2.92 a	88.21 ± 3.09 ab	85.13 ± 4.85 b
	1998	105.72 ± 3.44 a	98.92 ± 4.81 b	97.13 ± 3.22 b
Starch ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	9.31 ± 1.27 a	13.00 ± 0.91 b	17.68 ± 2.65 c
	1997	20.03 ± 2.29 a	25.19 ± 2.40 b	25.35 ± 2.13 b
	1998	6.56 ± 2.44 a	15.25 ± 1.25 b	17.98 ± 2.04 c
Total reserves ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	81.06 ± 8.57 a	83.06 ± 6.50 a	77.78 ± 4.50 a
	1997	112.08 ± 4.55 a	113.40 ± 4.44 a	110.48 ± 6.51 a
	1998	112.28 ± 3.44 a	114.17 ± 4.62 a	115.11 ± 2.39 a

The starch degradation corresponded generally with an increase of the sugar content and particularly of glucose and fructose, which was significantly higher in the “Untreated canopy” plot in comparison with the “Standard schedule”. The reduced fungicide schedule had an intermediary behaviour, but more closely related to the “Untreated canopy” plot. Only saccharose content, with the exception of 1998, did not show differences between the treatments.

In the shoot (Tab. 4), the total reserves contents showed, with the exception of 1998, the same tendencies as observed for the cane. The starch mobilisation is generally higher, as observed for the other woody parts, in 1998 within the “Untreated canopy” plot a decrease of 63% of the content was noted. With the exception of 1996, the sugar (glucose, fructose and saccharose) content didn’t show significant differences between the three treatments. In 1997 and 1998 glucose and fructose content increased in the “Untreated canopy” plot in comparison with the “Standard schedule” plot, and the “Reduced fungicide schedule” showed an intermediary behaviour.

Table 4. Effect of the downy mildew epidemics on the starch and sugar (glucose, fructose and saccharose) contents expressed as $\text{mg}\cdot\text{g}^{-1}$ dry matter (DM) of the shoot (one year old wood) of the vineof cultivar merlot. Data represent the average of 6 replications for 1996 and 1997 and 8 for 1998. Means followed by same letter are not significantly different at $p < 0.05$ (Tuckey test).

Attribute	Year	Untreated canopy	Reduced fungicide schedule	Standard schedule
Glucose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	24.02 ± 1.66 a	23.52 ± 1.80 a	21.70 ± 1.33 a
	1997	30.23 ± 1.10 a	26.80 ± 1.24 b	23.71 ± 1.65 c
	1998	22.76 ± 1.49 a	20.25 ± 1.73 b	18.69 ± 1.23 b
Fructose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	26.06 ± 2.52 a	26.40 ± 2.08 a	23.45 ± 2.25 a
	1997	31.12 ± 0.90 a	26.88 ± 1.07 b	25.05 ± 0.96 c
	1998	28.26 ± 1.13 a	24.38 ± 1.61 b	23.09 ± 1.36 b
Saccharose ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	15.13 ± 3.83 a	13.30 ± 2.74 a	13.41 ± 3.06 a
	1997	32.88 ± 2.16 a	33.79 ± 2.89 a	35.03 ± 0.86 a
	1998	47.25 ± 3.22 a	48.72 ± 3.26 a	49.77 ± 2.89 a
Total sugars ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	65.21 ± 7.29 a	63.22 ± 4.04 a	58.56 ± 5.45 a
	1997	94.23 ± 1.97 a	87.47 ± 2.80 a	83.79 ± 2.52 b
	1998	98.27 ± 3.11 a	93.35 ± 2.97 b	91.55 ± 3.94 b
Starch ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	10.57 ± 1.25 a	12.68 ± 1.02 b	17.21 ± 1.73 c
	1997	13.41 ± 0.90 a	17.98 ± 1.96 a	19.94 ± 1.63 b
	1998	7.82 ± 3.45 a	17.20 ± 1.25 b	21.21 ± 2.04 c
Total reserves ($\text{mg}\cdot\text{g}^{-1}$ DM)	1996	75.78 ± 8.40 a	75.90 ± 4.85 a	75.77 ± 6.99 a
	1997	107.64 ± 1.80 a	105.45 ± 3.25 a	103.73 ± 3.15 a
	1998	106.09 ± 5.28 a	110.55 ± 3.05 ab	112.76 ± 4.19 b

Discussion

Koblet *et al.* (1996) report that leaf physiology modifications, enhancement of the number of new leaves and lateral shoots and reserve mobilization are the most important compensation mechanisms involved in a defoliation stress situation. Considering the possible leaf physiology modifications as a compensation mechanism, different authors (Hunter and Visser, 1988; Candolfi-Vasconcelos, 1990; Hunter *et al.*, 1995; Koblet *et al.*, 1996) have shown that grapevine is able to face a stress situation with a photosynthesis or chlorophyll increment or delaying the leaf senescence and abscission. Our preview results (Jermini *et al.*, 2010a) reject this possibility. Moreover, the epidemics increase rapidly starting from beginning of the veraison (Jermini *et al.*, 2010b) and consequently the grapevine does not induce physiological changes during ripening to compensate for leaf damage as also shown by Koblet *et al.* (1993). The enhancement of the number of new leaves and lateral shoots is a compensation mechanism offsetting the leaf area loss as shown in some artificial defoliation stress experiments where the main leaves have been removed (Candolfi-Vasconcelos, 1990; Kliewer, 1970; Klieser and Fuller, 1973; Reynolds and Wardle, 1989). The same compensation mechanism has been observed on Merlot grapevine submitted to a defoliation stress caused by the green leafhopper (*Empoasca vitis*) (Candolfi *et al.*, 1993). Kliewer and Fuller (1973) showed that defoliation at veraison or later had little or no effect on lateral elongation. The high epidemic level of downy mildew in 1996 and 1997 (Jermini *et al.*,

2010b) has induced leaf abscission that could be more important than the lateral shoot growth. Moreover, the new formed leaves are more susceptible to the pathogen and they are rapidly colonised with an inhibition of their development, which could negatively influence the shoot elongation. Nevertheless, in 1998 the disease epidemic did not cause leaf abscission in the reduced fungicide plot and the plant did not react by increasing the leaf area of lateral shoots. Our results clearly indicate that a stress induced by a regular increase of defoliation during the ripening period causes an important mobilisation of the reserve contents in the woody parts and particularly in the roots. This mobilisation is probably correlated with the carbohydrate requirement of the berries during this period. This is in accordance with Coombe (1992), which indicates that veraison varies between vines within a vineyard and more particularly between berries within each cluster, because each berry appears to develop independently, an independence that may derive from as early as anthesis, maybe earlier. On this basis, Candolfi-Vasconcelos *et al.* (1994) also hypothesize that defoliation when 50% of the berries are entering the sugar-accumulation phase could block or delay the onset of ripening of the other 50%. In this way, the sink strength of the whole cluster would be reduced. Our results support this hypothesis. Therefore, a vineyard is composed of an age structured berry population with its own ripening dynamic depending on the berry distribution in age classes and stress is the element capable of transforming a cluster into a more powerful sink. The defoliation level therefore has a central role. Consequently the "Reduced fungicide schedule" provides an intermediary response for the carbohydrate requirement of the berries and this is in agreement with experiments considering different artificial defoliations or crop load levels (Candolfi-Vasconcelos, 1990; Candolfi-Vasconcelos *et al.*, 1994; Hunter *et al.*, 1995; Koblet *et al.*, 1993; Koblet *et al.*, 1997; Murisier, 1996). Candolfi-Vasconcelos *et al.* (1994) have shown that grapevine responds to a defoliation stress by altering the natural translocation pattern and directing carbon stored in the lower parts of the plant to the fruit in order to supply the assimilate requirements of the berries, which represent a very powerful sink during ripening. The reserve mobilisation is also the compensatory mechanism applied by the vine in limiting the stress caused by downy mildew epidemic on leaf canopy and it explains very well why sugar accumulation in the fruit is not proportionally reduced with the increase of the assimilating leaf area loss during the ripening phase (Jermini *et al.*, 2010b). Roots are the most important site of carbohydrate accumulation irrespective of root size (Hunter *et al.*, 1995; Yang *et al.*, 1980a) and the comparison between the total reserve contents of the different woody parts clearly indicates a retranslocation of the stored assimilates. Our results stressed these observations, but they also let us suppose that the grapevine could make some mobilization priorities: first roots followed by the trunk, cane and one year old wood without completely exhausting the reserves of each woody part. This hypothesis is evident in 1998. Mullins *et al.* (1992)

demonstrated that the reserve content of root and trunk is 2 times lower in the 10 than in 25 year old plants. The same observations have been made by Williams (1986) comparing 5 and 20 year old vines. Even reserve contents in the woody parts have a seasonal fluctuation (Mullins *et al.*, 1992; Winkler and Williams, 1945) and also depend on cultivars, yield, climatic conditions and plant growth (Murisier, 1996). Despite the fact that the comparison is not made during the same growing years, our results do not confirm these observations. The 6 year old vines of 1997 present in the “Standard schedule” plot reveal reserve contents of the roots similar to that measured in 1996 and 1998 on 24 old year plants without differences in plant growth and production (Jermini *et al.*, 2010b). Consequently, the mobilization strategy applied by the vine and the amount of stored compounds retranslocated from the woody parts to fruits depends mainly on defoliation thresholds that induce changes in the source/sink relationship. These defoliation thresholds vary in time as a result of the interactions between disease epidemic and the ripening dynamic of the berry population. Although this work provides a range of data sets on the plant compensation response to downy mildew epidemics, its implementation in a simulation model (Dietrich *et al.*, 1997) clashes with the lack of quantitative data on the berry population structure that prevents a quantification of the ripening process and consequently the determination of defoliation thresholds in function of the fruit ripening progress. Nevertheless, these results support the control strategy based on delaying the disease epidemic and limiting a stress situation during the first weeks after veraison, where the fruit represents an important sink in function of damage thresholds. This type of study needs repeating with other varieties and growing conditions to test the generality of the conclusions, because the influence of the production system is never sufficiently considered and could be greater than expected (Koblet *et al.*, 1993; Murisier, 1996).

Response of Merlot (*Vitis vinifera*) grapevine to defoliation caused by downy mildew (*Plasmopara viticola*) during the following growing season

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Introduction

Grapevine has a great potential for stress acclimation (Koblet *et al.*, 1996), but it needs, as for all the perennial woody plants, to maintain available annual resources in order to mature both reproductive and vegetative tissues. Therefore, each growing season has to be considered in relation to the one prior and the stress level to which the plant is submitted and its impact on the compensatory capacities will influence the vine during the following growing season. A few studies (Candolfi-Vasconcelos, 1990; Howell *et al.*, 1994; Koblet *et al.*, 1993; Murisier and Aerny, 1994; Murisier, 1996) have been carried out in this field considering only the stress impacts caused by cultural practices. Amongst the compensation mechanisms, the mobilization of the carbohydrate reserves, particularly those stored in the roots is generally employed by the plant to compensate for the strong sink requirements of the berries during the ripening period (Candolfi-Vasconcelos *et al.*, 1994; Jermini *et al.*, 2010c; Koblet *et al.*, 1997). The roots are the most important sites of carbon accumulation, which is retranslocated for the early shoot growth in the spring of the following season (Koblet *et al.*, 1996; Yang and Hori, 1979; Yang *et al.*, 1980b). Consequently, the reduced availability of the carbohydrate from the reserves could negatively influence the plant in the following growing season as observed by Murisier and Aerny (1994).

Our studies have shown that downy mildew (*Plasmopara viticola*) leaf epidemics reduce the assimilating leaf area during the ripening phase (Jermini *et al.*, 2010a; 2010b) and, consequently, the plant compensates for the carbohydrate requirement of berries principally by mobilising the reserves stored in the roots (Jermini *et al.*, 2010c). This fourth work aims to investigate and quantify the possible negative influences of the reserve mobilisation on the recovery capacity of grapevine submitted to a downy mildew canopy epidemic during the previous growing season.

Material and Methods

Plant material and experimental designs

Field-grown grapevine, cv. Merlot grafted on 3309 rootstock, double cane pruned and vertical trained (double Guyot) were used for the investigations. Two trials were made during the years 1995-1996 and 1997-1998 in two different plots of the experimental vineyard of Cugnasco of the Research station Agroscope Changins-Wädenswil ACW Centre of Cadenazzo. For each trial, in the first year plants were subjected to *P. viticola* stress at various intensity, in the second year (recovery phase) all plots were treated in accordance with the normally spray schedule applied in the vineyard so to avoid *P. viticola* stress. The 1995-1996 experiment was placed in a plot of 298 vines planted in 1972, with a vine spacing of 1.80 x 1.40 m between and within the rows, which was divided in the two subplots

corresponding to two treatments: A = “Untreated canopy” (to prevent quantity losses, the clusters were treated once with a contact fungicide at the discovery of the first downy mildew sporulation) and B = “Standard schedule” (normally schedule applied in the vineyard), where 12 grapevines were chosen for treatment A and 10 for B. Controls were carried out for the stress and recovery years on all shoots of the grapevines chosen in 1995 at the phenological stadium 51-53 of the BBCH scale (Baillod and Baggiolini, 1993). The 1997-1998 experiment was placed in a plot planted in 1991 with a vine spacing of 2.00 x 1.20 m between and within the rows and each treatment included 48 plants divided in 6 replications of 8 contiguous plants (Jermini *et al.*, 2010b) where, only treatments A and B were considered in the recovery year. For the stress years (1995 and 1997), the number of shoots per plant and the yield quantity were regulated at the same level on each subplot. For 1995, the number of shoots/plant was limited at 11 (minimum 8 and maximum of 15 shoots/plant) and 12 (minimum 8 and maximum 15 shoots/plant) for 1997. In the recovery years (1996 and 1998) the number of shoots/plant was regulated in accordance with the normally practices applied in the vineyard and no yield quantity regulation was carried out.

Disease severity

On each main and lateral leaf of the selected shoot, the disease severity was estimated during the stress year using a modified Horsfall scale (Horsfall and Cowling, 1978), in which a supplementary class for the lower disease level was introduced. In this way, the scale was divided into 12 classes: class 0 (0% damaged leaf area), class 1 (0* - 1% damaged leaf area), class 2 (1* - 3% damaged leaf area), class 3 (3* - 6% damaged leaf area), class 4 (6* - 12% damaged leaf area), class 5 (12* - 25% damaged leaf area), class 6 (25* - 50% damaged leaf area), class 7 (50* - 75% damaged leaf area), class 8 (75* - 88% damaged leaf area), class 9 (88* - 94% damaged leaf area), class 10 (94* - 97% damaged leaf area) and class 11 (97* - 100% damaged leaf area). The asterisk indicates a value slightly exceeding the indicated value.

Plant growth and leaf area

From the phenological stadium 51 BBCH (Baillod and Baggiolini, 1993) shoot length was measured weekly until the first topping from the base to the beginning of the apex. The number of main leaves, lateral shoots and leaves on lateral shoots was assessed the first time at the phenological stadium 51 BBCH for main leaves and at the phenological stadium 55 for lateral shoots (Baillod and Baggiolini, 1993), at the shoot topping and at the veraison. Leaf area of main and lateral leaves was estimated on all unfolded leaves of 10 randomly selected shoots using the method described by Cabonneau (1976).

Shoot fertility and number of flowers/cluster

The number of shoots/plant and clusters/shoot was assessed at the phenological stadium 55 BBCH (Baillod and Baggiolini, 1993) counting, for 1996, the total number of shoots and clusters present on the plants chosen and for 1998 on a series of 10 consecutive plants. The number of flowers/cluster was calculated with the method described by Casteran *et al.* (1981) on all clusters of the selected plants of 1997 and on 20 consecutive clusters for the 1998 replication.

Yield quantity and quality analysis

At vintage, each plot/plant was harvested individually. After weighing, the crop was crushed to determine must soluble solids, pH, titratable acidity and content of malic and tartaric acids. Must soluble solids, expressed by Brix, was evaluated with a refractometer ERMA with temperature correction, and pH was measured with a Metrohm 691 pH-meter (Metrohm AG Herisau, Switzerland) equipped with a microelectrode. Total acidity was determined on a 15 ml must sample by titration with 0.2 mol/l NaOH until pH 7.0.

Reserve analysis

The reserve analysis was carried out only for the 1997-1998 experiment. For each plot, the one-year-old wood sample was taken, during pruning in the February of the year following the experiment, between the 3rd and 6th internode taking one shoot per plant. Samples of the two-year-old wood (cane) included 2 parts of each cane taken between the 3rd and 4th node and the 6th and 7th node. The trunk sample consisted of 3-5 g of sawdust produced by perforation at different heights of the trunk with an electric drill fitted with a 3 mm bit. After having dug a profile along the plots from 80-100 cm depth, root samples of fine and middle diameter (0.5-5 mm) were collected from the plants and washed. All samples were cut, oven-dried at 65 °C and then crushed in a hammer mill, obtaining a powder, which was dried at least during 2 weeks over di-phosphor pentoxide (P₂O₅) before extraction. Glucose, fructose and sucrose were extracted by a hydroalcoholic solution (70 vol. % ethanol) at 80°C and then analysed by the enzymatic method (Boehringer Mannheim). The solid residue of the extraction was dried over di-phosphor pentoxide (P₂O₅) and starch was extracted with dimethyl sulfoxide in a boiling water bath. Starch was analysed in this second extract by the enzymatic method (Boehringer Mannheim). All results were given in mg·g⁻¹ of dry matter.

Statistical analysis

Statistical analysis of the data was performed utilising the Sigmastat (SSPS) statistical package. The t-test was used to compare the differences between the two treatments.

Results

The impact of the stress years 1995 and 1997 on the plant

During the stress years, the first downy mildew symptoms were found on June 16 in 1995 and June 11 in 1997. The disease development of the stress year 1997 was more rapid and greater than that of 1995, but their progress was similar with the logit phase during the first ripening period and the terminal phase from the beginning of September until vintage, where a decrease of severity was due to the defoliation (Fig. 1).

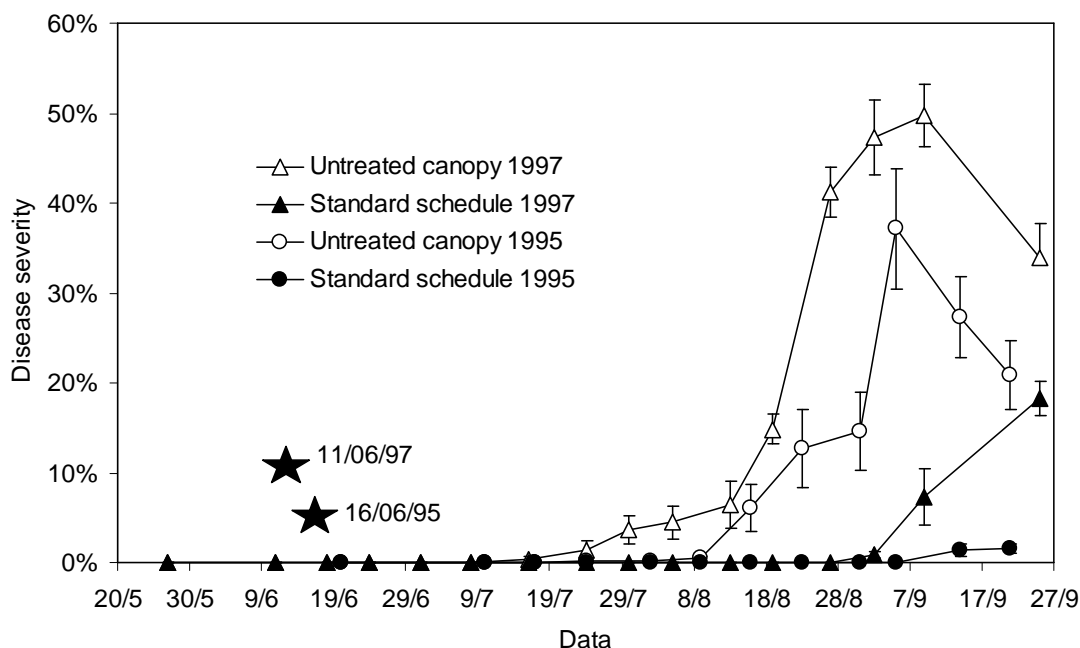


Figure 1. Disease severity expressed as percentage of diseased leaf area per shoot during the stress years 1995 and 1997 in the “Untreated canopy” plot and “Standard schedule” plot on cultivar Merlot. The star and the date indicate the finding of the first downy mildew sporulation in field.

The consequence of the stress year was a negative influence on yield quality with a significant decrease of the must soluble solids of 1.39 °Brix for 1995 and respectively 0.57 °Brix for 1997 in the “Untreated canopy” plots (Tab. 1). The difference observed in yield production in 1995 (Tab. 1) was due to the difficulty in regulating the crop production on the single plants.

Table 1. Effect of the downy mildew epidemics on quantity and quality yield parameters of cv. Merlot during the stress years 1995 and 1997 and the recovering seasons 1996 and 1998. Data represent the average \pm standard deviation.

		Treatment		<i>t</i>	<i>P</i>
		Untreated canopy	Standard schedule		
Stress year 1995	Crop yield (kg·m ⁻²)	1.009 \pm 0.151	1.198 \pm 0.206	-2.488	0.022
	Must soluble solids (°Brix)	16.97 \pm 0.603	18.36 \pm 0.460	-5.952	< 0.001
	pH	3.24 \pm 0.077	3.23 \pm 0.034	0.453	0.655
	Total acidity (g·l ⁻¹)	7.53 \pm 0.381	7.47 \pm 0.466	0.326	0.748
Recovering year 1996	Number shoots/plant	11.5 \pm 1.624	11.5 \pm 1.509	0.000	1.000
	Crop yield (kg·m ⁻²)	1.202 \pm 0.103	1.791 \pm 0.029	-9.468	< 0.001
	Must soluble solids (°Brix)	19.45 \pm 0.173	18.67 \pm 0.208	5.456	0.003
	pH	3.30 \pm 0.022	3.38 \pm 0.001	-3.912	0.011
	Total acidity (g·l ⁻¹)	7.95 \pm 0.173	7.83 \pm 0.115	1.000	0.363
Stress year 1997	Crop yield (kg·m ⁻²)	0.893 \pm 0.130	1.005 \pm 0.500	-1.586	0.174
	Must soluble solids (°Brix)	18.20 \pm 0.110	18.77 \pm 0.103	-11.461	< 0.001
	pH	3.32 \pm 0.015	3.33 \pm 0.004	-1.387	0.224
	Total acidity (g·l ⁻¹)	7.15 \pm 0.187	6.67 \pm 0.082	6.100	0.002
Recovering year 1998	Number shoots/plant	11.90 \pm 0.626	11.85 \pm 0.545	0.130	0.900
	Crop yield (kg·m ⁻²)	1.438 \pm 0.081	1.815 \pm 0.260	-3.400	0.005
	Must soluble solids (°Brix)	19.02 \pm 0.232	18.67 \pm 0.282	2.415	0.033
	pH	3.47 \pm 0.027	3.47 \pm 0.041	0.367	0.720
	Total acidity (g·l ⁻¹)	5.13 \pm 0.111	5.54 \pm 0.273	-3.395	0.005

Vine growth

The most important impact during the recovery season was a significant reduction of the shoot elongation from phenological stadium 55 of the BBCH scale (Baillod and Baggiolini, 1993) until topping (Tab. 2).

Table 2. Effect of the downy mildew epidemics 1995 and 1997 on shoot length (cm) of cv. Merlot during the recovery season 1996 and 1998. Data represent the average \pm standard deviation.

Year	Control data	Treatment		<i>t</i>	<i>P</i>
		Untreated canopy	Standard schedule		
1996	May 13	12.30 \pm 3.650	14.89 \pm 1.840	-2.032	0.056
	May 21	24.20 \pm 6.146	28.68 \pm 3.205	-2.076	0.051
	May 28	38.78 \pm 7.603	46.41 \pm 5.771	-2.604	0.017
	June 04	55.60 \pm 9.202	68.58 \pm 9.250	-3.287	0.004
	June 13	81.99 \pm 12.910	104.02 \pm 17.391	-3.409	0.003
1998	May 19	25.91 \pm 1.864	27.91 \pm 1.710	-1.713	0.125
	June 02	46.14 \pm 5.405	58.99 \pm 7.183	-3.246	0.012
	June 08	63.01 \pm 8.019	77.46 \pm 11.136	-2.396	0.043
	June 17	78.59 \pm 10.920	100.97 \pm 15.823	-2.671	0.028

The shoot growth difference between the two treatments increased regularly and it was significant starting on May 28 for 1996 and June 2 for 1998 with, at the last control before the topping corresponding to the end of flowering stage, a difference of 22.03 cm in 1996 and 22.4 cm in 1998 (Tab. 2). The slow shoot growth did not always correspond to a delay of main leaves, lateral shoots and leaf apparition.

Table 3. Effect of the downy mildew epidemics 1995 and 1997 on number of main leaves per shoot, lateral shoot per shoot, leaves per lateral shoot and total leaves per shoot of cv. Merlot during the recovery seasons 1996 and 1998. Data represent the average with standard deviation.

Year	Attribute	Control data	Treatment		<i>t</i>	<i>P</i>
			Untreated canopy	Standard schedule		
1996	Main leaves/shoot	May 13	4.11 ± 0.506	4.74 ± 0.276	-3.550	0.002
		June 13	15.32 ± 1.526	16.54 ± 1.041	-2.134	0.045
		August 05	20.91 ± 2.033	19.11 ± 2.128	1.966	0.064
	Lateral shoot/shoot	May 28	1.93 ± 0.775	2.43 ± 0.661	-1.584	0.130
		June 13	7.27 ± 1.290	8.47 ± 1.117	-2.306	0.032
		August 05	13.18 ± 0.956	11.75 ± 1.728	2.427	0.025
	Leaves/lateral shoot	May 28	1.03 ± 0.068	1.10 ± 0.145	-1.415	0.173
		June 13	1.61 ± 0.156	1.76 ± 0.331	-1.384	0.182
		August 05	3.04 ± 0.675	4.35 ± 1.927	-2.188	0.041
	Total leaves/shoot	May 13	4.11 ± 0.506	4.74 ± 0.276	-3.550	0.002
		June 13	10.71 ± 1.598	12.66 ± 1.489	-2.947	0.008
		August 05	25.14 ± 3.865	26.65 ± 2.980	-0.978	0.340
1998	Main leaves/shoot	May 19	6.92 ± 0.259	6.87 ± 0.433	0.208	0.840
		June 17	16.29 ± 0.932	16.81 ± 0.554	-0.991	0.351
		August 05	19.82 ± 1.483	17.50 ± 3.323	1.528	0.165
	Lateral shoot/shoot	June 02	2.42 ± 0.744	2.37 ± 0.854	0.082	0.937
		June 17	6.90 ± 0.903	7.56 ± 1.908	-0.754	0.472
		August 05	10.71 ± 1.575	11.62 ± 2.487	-0.722	0.491
	Leaves/lateral shoot	June 02	1.02 ± 0.031	1.04 ± 0.048	-0.880	0.404
		June 17	1.53 ± 0.181	1.65 ± 0.295	-0.859	0.416
		August 05	1.82 ± 0.351	2.44 ± 1.020	-1.420	0.193
	Total leaves/shoot	May 19	6.92 ± 0.258	6.87 ± 0.433	0.193	0.852
		June 17	27.44 ± 3.709	30.69 ± 5.060	-1.180	0.272
		August 05	70.73 ± 7.095	50.31 ± 14.751	-1.396	0.200

A significant influence on these growth parameters was observed only in the recovery year 1996, where the difference in the shoot elongation corresponded to a decrease in the number of main leaves for the two first controls and, consequently, a delay in the lateral shoot development (Tab. 3).

Table 4. Effect of the downy mildew epidemics 1995 and 1997 on main and lateral leaf area expressed of cv. Merlot as cm² during the recovery seasons 1996 and 1998. Data represent the average with standard deviation.

Year		Treatment		<i>t</i>	<i>P</i>
		Untreated canopy	Standard schedule		
1996	Main leaf	143.76 ± 40.353	170.19 ± 49.935	-1.164	0.264
	Lateral leaf	35.05 ± 14.153	39.55 ± 4.474	-0.670	0.513
1998	Main leaf	102.49 ± 12.182	128.02 ± 6.736	-3.775	0.005
	Lateral leaf	30.44 ± 3.339	37.71 ± 6.666	-2.316	0.049

Nevertheless, at the veraison there were no significant differences in the total number of leaves per shoot (Tab. 3). No effects were observed in the recovery year 1998 (Tab. 3). On the contrary, we found a significant reduction of the main and lateral leaf area in 1998 and no differences in 1996 (Tab. 4).

Yield components and fruit composition

The effect of downy mildew defoliation did not influence the number of clusters per shoot and the number of flowers per cluster during the recovery year (Tab. 5). Nevertheless, at vintage, the untreated canopy plots presented a significant yield reduction of $0.589 \text{ kg}\cdot\text{m}^{-2}$ and $0.377 \text{ kg}\cdot\text{m}^{-2}$ for the recovery years 1996 and, respectively, 1998 (Tab. 1). The final number of berries per cluster always differs from the number of flowers per cluster before bloom, because badly fertilised berries and non fertilised flowers fall off and this drop period is normal in all grapevine cultivars. At vintage, the yield quality showed a significant lower must soluble solid contents of 0.78 and 0.35 °Brix in the unstressed plots (Tab. 1). This result is essentially due to the difference in the crop level between the two plots, which negatively influenced the carbohydrate accumulation in the berries (Murisier, 1996).

Table 5. Effect of the downy mildew epidemics 1995 and 1997 on the number of flowers per cluster and the number of clusters per shoot of cv. Merlot during the recovery seasons 1996 and 1998. Data represent the average with standard deviation.

Year		Treatment		<i>t</i>	<i>P</i>
		Untreated canopy	Standard schedule		
1996	n°flowers/cluster	351.70 ± 25.541	371.00 ± 56.074	-1.071	0.297
	n°clusters/shoot	1.69 ± 0.217	1.81 ± 0.191	-1.367	0.187
1998	n°flowers/cluster	365.31 ± 61.737	408.35 ± 37.278	-1.238	0.251
	n°clusters/shoot	1.53 ± 0.106	1.66 ± 0.075	-1.961	0.086

Reaccumulation of the assimilate in woody tissue

The impact of the epidemic on the plant reserves during the stress year 1997 showed a significant decrease of 57% of the starch and of 37% of the total reserve content of the roots (Fig. 2) as response of the grapevine to a stress situation during the ripening period (Jermini *et al.*, 2010c). In the other woody parts, the starch decrease was lower than in the roots, but always 33% for the shoots (one year wood) and 21% for the cane (two year wood) and trunk, but the total reserve content did not change significantly between the treatments (Fig. 2). A single recovery year was enough for the grapevine to reconstitute the reserve pool (Fig. 2) and particularly those of the roots, where no differences have been found in the starch and sugar content and, consequently, in the total reserves. The same results have been observed for the cane (two year old wood), but not for the shoot (one year old wood) and the trunk, where a significant lower content of 5.32% and, respectively, 5.95% of the total reserves (Fig. 2) due to significant lower sugar content of 6.1% and 7.1% was observed. No differences have been found in the starch content of these woody parts.

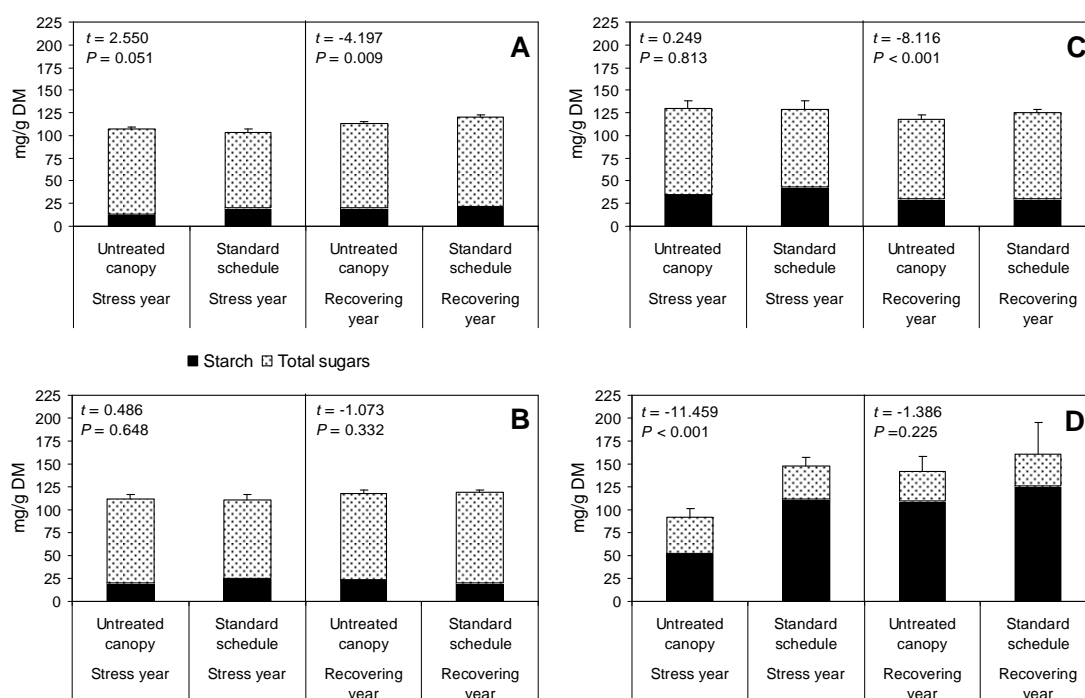


Figure 2. Comparison of the reserve content (total sugars and starch) in the woody parts of the grapevine Merlot grafted on 3309 for the stress year 1997 and the recovery year 1998. A = Shoots (one year old wood), B = cane (two year old wood), C = trunk and D = roots. The t test and significant values refer to total content of the reserves.

Discussion

The stress induced by downy mildew in the “Untreated canopy” plot has negatively influenced the shoot elongation in the recovery years. These results are confirmed by Murisier (1996) in his experimentations on the effect of different crop loads during the recovering year. Yang and Hori (1979) and Yang *et al.* (1980b) also showed that the new shoot growth in the spring depends on carbohydrate reserves stored in the perennial parts of the vine during the previous growing season. This retranslocation reaches a maximum at about the 8-leaf stage and ceases at about the flowering stage, because the carbohydrate requirement is gradually supported by assimilates produced in the leaves. Our previous results indicated clearly that the plant mobilises the carbohydrate reserves in the stress year to compensate for the requirements of the berries with a consequently strong reduction of their content in the perennial parts of the plant and particularly in the roots (Jermini *et al.*, 2010c). The moderate shoot growth during the recovery years is probably the result of a limited amount of assimilates from budbreak available in the plant after the stress years. Nevertheless, the dynamic of leaf formation does not seem to follow that observed for the shoot growth, because in the recovery year 1998, contrary to 1996, it doesn't show significant differences in the leaf number between treatments. This result is in contrast to the

epidemic dynamic observed in the stress year, because 1997, in comparison with 1995, was characterised by a more important disease severity and consequently by a probably more important stress situation. On the other hand 1998 was characterised, contrary to 1996, by a significant reduction of the leaf area of the main and lateral leaves. Murisier (1996) observed the same tendencies in his experimentation on the chlorosis apparition as an effect of a stress situation due to a high crop load. Howell *et al.* (1994), however did not observe at vintage the influences of six levels of defoliation, occurring six weeks after blooming (berry pea-size), on the vine growth during the recovery year. Nevertheless, it is not possible to exclude for the experiment 1995-1996 that the important difference in the crop load between the treatments could have amplified the stress effect of the downy mildew and consequently the grapevine response in the recovery year. The number of clusters per shoot and flowers per cluster in the recovery years is not influenced by the downy mildew stress. The cluster initiation in the bud takes place generally in June and July (Huglin, 1986) and the low disease severity during this period in the stressed years did not influence this physiological process. Nevertheless, differences on yield production at vintage have been found and probably caused by an important berry drop at fruit set induced by a reduced leaf area of the plant or an insufficient carbohydrate uptake of the berries (Huglin, 1986). As for the growth parameters, we obtained different outcomes which support or reject these results. Murisier (1996) observed a significant decrease of the yield components of the plants submitted to a high crop load level contrary to Howel *et al.* (1994), which found no differences between these components as in the final crop yield. Our results support the hypothesis proposed by these last authors, which lead us to speculate on the importance of the stress type and duration on the plant response in the recovery year. Independently of the differences observed in the growth and yield parameters, the recover year permits the plant to reconstitute the reserve pool in the woody parts and particularly in the roots. Candolfi-Vasconcelos (1990) and Koblet *et al.* (1993) have also shown that one year of post-defoliation recovery seems to be sufficient to reconstitute the reserve pool in the woody parts of the plant above the ground without considering the roots, which are the important source for carbohydrate mobilisation in compensating for a stress situation during the ripening phase of the grapevine (Candolfi-Vasconcelos, *et al.*, 1994; Koblet *et al.*, 1993; Murisier, 1996). Our results are limited to the first recovery year after the stress which plays a central role because it gives the plant the possibility to reconstitute the reserve pool. Consequently it is only in the second following year that we have a complete recovery as shown by Candolfi-Vasconcelos (1990) in their defoliation experiments.

Application of the Minimal Fungicide Strategy for the control of the downy mildew (*Plasmopara viticola*): effect on epidemics, yield parameters and on the plant recovery capacities

Introduction

The downy mildew, caused by *Plasmopara viticola* Berk & Curt. (Berl and de Toni), is the major disease in the vineyards of the southern part of Switzerland. Recurrent regular leaf damage makes the downy mildew the key pathogen in the spray schedule of winegrowers. For its control, a preventive strategy consisting of 7-9 fungicide applications, based on systemic and preventive fungicides, is employed from May until the middle of August, the last deadline for the use of organic active ingredients. The previous studies (Jermini *et al.*, 2010a-d) have permitted quantifying the interactions between downy mildew and the grapevine analysing the damage and the compensation mechanisms applied by the plant. We have also emphasized the possibility of reducing the number of fungicide applications, delaying the epidemic in time without negative influences on the yield. Grapevine is therefore a perennial woody plant that has evolved survival mechanisms of reproduction, vegetative maturation and acclimation to environmental stresses. Such a strategy requires that the vine effectively allocates available annual resources in order to fully develop both reproductive and vegetative tissues (Howell *et al.*, 1994). Consequently, the amount of reserve contents in the woody parts of the plant is an important element in securing the early shoot growth in the spring. The experimentations have shown that the reserve mobilisation depends on the stress level without having significant effects on plant growth and productivity the year following the stress season. If the influence of a single stress year on the following season is limited, it is possible to expect a negative effect if the stress is repeated on the same plant for more than a single season.

The previous results (Jermini *et al.*, 2010a-d) have permitted the elaboration of a control strategy, called Minimal Fungicide Strategy (MFS), which was applied during the period 1999-2002 on the same plot and compared with the standard control used in the vineyard. The aims of this experimentation were: 1) to verify the effective possibility in delaying the epidemic by 1 to 5% during the first ripening phase, as proposed by Jermini *et al.* (2010b), 2) to analyse the strategy's impact on the yield quantity and quality, 3) to evaluate its possible negative influences over the years on the plant growth and productivity, 4) to evaluate, during the period 2003-2004, if a recovery year (2003) without stress is enough to eliminate the stress situation in the following year (2004).

Material and methods

Plant material and experimental design

The experiment was conducted in southern Switzerland in a 10 year old Merlot grapevine plot grafted on 3309 rootstock, trained to a double Guyot and with a vine spacing of 2.00 x 1.20 m between and within the rows. The experimental design consisted of the comparison

between two treatments: the minimal fungicide application strategy (MFS) and the standard schedule (SS), corresponding to the normal preventive downy mildew control applied in the vineyard. An untreated control served as verification of the damage potential of the downy mildew. Each treatment consisted of a plot of 4 rows with 61 plants/row for an amount of 244 plants divided in 8 subplots. This experimental design was maintained for the period 1999-2004. The impact of the MFS vs SS was measured during the period 1999-2002 and the recovery capacity of the grapevines of the MFS during the years 2003 and 2004.

Application of the Minimal Fungicide Strategy

The MFS was based on:

- A first fungicide application after the discovery of the first downy mildew sporulation in the plot and considering the weather forecast;
- One or more fungicide applications depending on: 1) epidemic progress, defined through practical visual controls of the disease incidence in the plot, considering the appearance of new symptoms and the increase of infected leaves in the plot area where the first sporulation was found; 2) the time elapsed since the last fungicide application and the disease incidence increase observed during this period 3) weather (past and forecast);
- A possible last fungicide application in the middle of August (last term in Switzerland allowed for the application of organic fungicides).

The date of application and fungicide used in the MFS and SS treatments are reported in Tab. 1. Three applications of Slick (250 g l⁻¹ difenoconazol) were made starting from bloom to prevent powdery mildew (*Erysiphe necator*) and black rot (*Guignardia bidwellii*) infections and one with Switch (25% fludioxonil +37.5% cyprodinil) on clusters at the end of July to control grey mold (*Botrytis cinerea*) infections.

Disease assessment

Between 13 and 16 representative shoots (1 shoot every 2 plants) per each subplot were selected at the phenological stadium E (BBCH 11-13) (Baillod and Baggiolini, 1993) before the beginning of the downy mildew epidemic. Disease severity was assessed on these shoots upon observing the first sporulation, after the following treatments and at the veraison using an extended Horsfall scale (Horsfall and Cowling, 1978), in which a supplementary class for the lower disease level was introduced. In this way, the scale was divided into 12 classes: class 0 (0% damaged leaf area), class 1 (0* - 1% damaged leaf area), class 2 (1* - 3% damaged leaf area), class 3 (3* - 6% damaged leaf area), class 4 (6* - 12% damaged leaf area), class 5 (12* - 25% damaged leaf area), class 6 (25* - 50% damaged leaf area), class 7 (50* - 75% damaged leaf area), class 8 (75* - 88% damaged leaf area), class 9 (88* -

94% damaged leaf area), class 10 (94* - 97% damaged leaf area) and class 11 (97* - 100% damaged leaf area). The asterisk indicates a value slightly exceeding the indicated value.

Table 1. Dates of the fungicide applications, with the active ingredients (a.i.), in the Minimal Fungicide Strategy (MFS) plot and in the Standard Schedule plot (SS).

Year	MFS		SS	
	Date	Commercial product and concentration use	Date	Commercial product and concentration use
1999	04.06	Quadris 0.1%	26.05	Cyrano 0.2%
	30.06	Cyrano 0.2%	08.06	Cyrano 0.2%
	29.07	Cyrano 0.2%	17.06	Cyrano 0.2%
	17.08	Cyrano 0.2%	30.06	Cyrano 0.2%
			15.07	Ridomil viti 0.15%
			27.07	Ridomil viti 0.15%
			13.08	Cyrano 0.2%+Turbofal 0.25%
2000	26.05	Cyrano 0.2%	15.05	Quadris 0.1%
	05.07	Cyrano 0.2%	26.05	Cyrano 0.2%
	11.08	Cyrano 0.2%	07.06	Cyrano 0.2%
			16.06	Cyrano 0.2%
			27.06	Quadris 0.1%
			05.07	Cyrano 0.2%
			27.07	Cyrano 0.2%
			11.08	Cyrano 0.2%
			25.08	Cuprofix 0.3%
2001	07.06	Cyrano 0.2%	22.05	Cyrano 0.2%
	26.06	Cyrano 0.2%	05.06	Cyrano 0.2%
	30.07	Cyrano 0.2%	18.06	Cyrano 0.2%
	14.08	Cyrano 0.2%	02.07	Cyrano 0.2%
			17.07	Quadris 0.1%
			02.08	Ridomil viti 0.15%
			14.08	Cyrano 0.2%+Turbofal 0.25%
			30.08	Cuprofix 0.3%
2002	07.06	Cyrano 0.2%	24.05	Cyrano 0.2%
	04.07	Cabrio 0.04% + Phaltocid 0.125%	07.06	Cyrano 0.2%
	17.07	Cabrio 0.04% + Phaltocid 0.125%	20.06	Cyrano 0.2%
	02.08	Cyrano 0.2%	04.07	Cyrano 0.2%
			17.07	Cabrio 0.04%
			02.08	Cyrano 0.2%
			19.08	Cyrano 0.2%+Turbofal 0.25%

The composition in active ingredients of the fungicides used are: Cabrio (250 g l⁻¹ pyraclostrobin), Cuprofix (50% Cu as oxyclorid), Cyrano (25% folpet + 4% cymoxanil + 50% phosethyl-Al), Ridomil vino (60% folpet + 7.5% methalaxyl), Phaltocid (80% folpet), Quadris (22.9% azoxystrobin), Turbotal (280 g l⁻¹ folpet + 146.7 g l⁻¹ copper).

Phenology, plant growth and leaf area assessment

From the year 2000, the first stress year, until 2004, 20 plants per treatment distributed between the sub-plots were selected at the beginning of April and the phenological evolution of the buds and shoots were followed weekly using the scale proposed from Baillod and Baggiolini (1993). Shoot growth (shoot length from the base to the apex), number of main

leaves, lateral shoots, leaves on lateral shoots and leaf area was assessed by non-destructive methods, choosing 13-16 representative shoots (1 shoot every 2 plants) per each sub-plot at the phenological stadium E (BBCH 11-13). The area of main and lateral leaves was estimated on 10 shoots per treatment with the method described by Carbonneau (1976) during the period 2000-2003. During the period 1999-2002, these controls were carried out until the beginning of the new epidemic (apparition of the first sporulation) and for 2003-2004 until the first topping of the vines.

Yield parameters

Number of shoots per plant, including the spurs, was regulated at 12 shoots per plant before bloom. Shoot fertility (number of clusters per shoot) was assessed on 10 plants in each subplot. The expected yield quantity was estimated at the end of July without considering the eventual downy mildew damage on clusters. The expected yield quantity was calculated on the basis of the number of berries per cluster (determined by the average of the number of berries of 10 representative clusters per plot), the number of clusters per plant (determined as the average of the number of clusters per plant of the subplot), the berry weight assuming an average weight of 1.7 g (10 year average of the final berry weight at harvest of the vineyard) and the plant density of 2.4 plants·m⁻². The crop load of each subplot was adjusted at the end of July to a theoretical production of 1.0 kg·m⁻², corresponding to the production limit allowed in Ticino. At vintage each plot was harvested individually and yield components analysed by the laboratory of the viticulture and oenology department of ACW following the methods described in the *Manuel suisse des denrée alimentaires*.

Statistical analysis

The statistical comparison between the 2 plots was performed with a paired t-test, utilising the Sigmastat (SSPS) statistical package.

Results

Impact of the minimal fungicide strategy on epidemics

Downy mildew sporulations appeared on June 2 in 1999. The first fungicide application in the MFS plot was made only on clusters on June 4 with a disease severity of 0.0013% (Tab. 2). This first fungicide application was followed by a period of 17 days of rainfall for a total of 321.4 mm·m⁻². This unfavourable climatic condition induced a rapid increase of the epidemic. Consequently, the second treatment was applied on June 30 with a disease severity of 1.48%. A third and fourth treatments, made on July 29 and respectively on August 17, resulted in an insufficient efficacy and at the beginning of veraison the disease severity was 5.07% (Tab. 2). This situation caused an important cluster infection with a severity of 34.7% at the beginning of August (Table 3).

Table 2. Disease severity of the downy mildew epidemics in the MFS (minimal fungicide strategy) plot for the years 1999-2002 expressed as % of damaged leaf area per shoot evaluated at the apparition of the first sporulation and at the period of the fungicide application. Each value is the average of 8 repetitions with the standard deviation.

	Date	Severity
1999	June 9	0.0013 + 0.001
	July 1	1.48 + 0.82
	July 30	5.072.71
	August 13	13.07 + 6.04
2000	June 2	0.038 + 0.016
	July 17	0.057 + 0.048
	August 10	2.04 + 1.222
2001	May 30	0.00040 + 0.0005
	June 27	0.00031 + 0.0008
	August 13	13.03 + 4.76
2002	June 3	7.46E-8 + 2.2E-7
	June 26	0.00093 + 0.0020
	July 9	0.049 + 0.063
	September 9 ²	13.62 + 2.98

² Control made at the end of the first ripening period

In 2000, the first sporulation was discovered on May 24. Three fungicide applications were made in the MFS plot against the 9 of the SS plot (Tab. 1) thereby reducing the treatments by 67%. Contrary to 1999, the first treatment was applied on the whole plant immediately after the finding of the first sporulation, permitting a delay in the epidemic, which at veraison was measured at 2.04% (Tab. 2). Damage of 0.6% was observed on clusters in comparison with the 37.7% in the untreated plot (Tab. 3).

In 2001, the first sporulation appeared in the field on May 22. Four fungicide applications were made in the MFS and 8 in the SS plot (Tab. 1). The epidemic was maintained under control until the end of June and no damage was found on clusters (Tab. 3). From the end of July the disease increased rapidly and at the middle of August the disease severity was 13% (Tab. 2).

In 2002, the first sporulation appeared on June 3.

Table 3. Severity of damage from the downy mildew on clusters expressed as % of damaged cluster in the SS (standard schedule), MFS (minimal fungicide strategy) and untreated plot. Each value for the MFS and SS is the average of 8 repetitions with the standard deviation. The results reported for the untreated plots correspond to a single plot for 1999 and the average of 4 replications for the other years.

Year	1999	2000	2001	2002
Date of control	July 19	July 12	July 14	July 26
SS	0.0%	0.0%	0.0%	0.2% ± 0.27
MFS	34.7% ± 4.20	0.6% ± 0.47	0.0%	17.5% ± 8.03
Untreated plot	94.7%	30.2% ± 8.25	48.5% ± 19.40	59.1% ± 19.10

Only 4 treatments were applied in the MFS plot in comparison with the 7 applications for the SS plot. The MFS permitted an important delay of the epidemic which increased only during

the ripening period (Tab. 2). On clusters, the damage was limited to 17.5% in comparison with the 59.1% of the untreated plot (Tab. 3).

Impact of the minimal fungicide strategy on yield quantity and quality

During the first experimental year (1999), despite the difficulty in downy mildew epidemic management, the disease severity of 34.7% on clusters was evaluated as acceptable, because the estimated yield quantity production, considering the yield loss due to downy mildew infection, was of $1.424 \text{ kg}\cdot\text{m}^{-2}$, $0.424 \text{ kg}\cdot\text{m}^{-2}$ higher than the production limit of $1.0 \text{ kg}\cdot\text{m}^{-2}$ allowed in Ticino. For the same reason the 17.5% damage rate of 2002 did not directly influence the yield quantity because the potential yield production was $0.245 \text{ kg}\cdot\text{m}^{-2}$ higher than the production limit. In all experimental years a crop load regulation was applied to attain the production limit of $1.0 \text{ kg}\cdot\text{m}^{-2}$.

At harvest, the yield quantity in the MFS plot differs significantly from that of the “Standard schedule” plot for the years 1999 and 2001 with a lower production of 37.9% and 14.4% for 1999 and, respectively, 2001 (Tab. 4). Berry weight between the MFS and SS didn't differ for the years 1999, 2000 and 2002 and it was, in average, higher in the MFS. Only in 2001 it was significantly lower than $0.1 \text{ g}\cdot\text{berry}^{-1}$. During the experimental years 1999-2002 no yield was harvested in the untreated plots.

Table 4. Results of the most important yield components at harvest (date of harvest: September 29 for 1999, September 22, September 26 and October 2, September 2 and September 29 for 2000, 2001, 2002, 2003 and respectively 2004) from minimal strategy (MFS) and standard schedule (SS) plots. Each value is the average of 8 repetitions with the standard deviation.

Year	Plot	Berry weight (g)	Yield ($\text{kg}\cdot\text{m}^{-2}$)	Must soluble contents (°Brix)	Total acidity ($\text{g}\cdot\text{l}^{-1}$)
1999	MFS	1.87 ± 0.066	0.820 ± 0.077	18.08 ± 0.191	6.01 ± 0.203
	SS	1.82 ± 0.075 $P = 0.304$	1.320 ± 0.067 $P = < 0.001$	19.04 ± 0.663 $P = 0.03$	5.71 ± 0.146 $P = 0.04$
2000	MFS	1.78 ± 0.090	1.160 ± 0.198	19.29 ± 0.136	5.58 ± 0.116
	SS	1.84 ± 0.081 $P = 0.290$	1.112 ± 0.262 $P = 0.44$	19.79 ± 0.259 $P = < 0.001$	5.21 ± 0.210 $P = 0.04$
2001	MFS	1.64 ± 0.059	0.793 ± 0.212	19.44 ± 0.207	7.35 ± 0.233
	SS	1.74 ± 0.054 $P = 0.013$	0.926 ± 0.257 $P = 0.030$	19.94 ± 0.169 $P = < 0.001$	7.29 ± 0.083 $P = 0.39$
2002	MFS	2.07 ± 0.109	0.865 ± 0.101	19.70 ± 0.120	7.11 ± 0.185
	SS	1.99 ± 0.072 $P = 0.114$	1.016 ± 0.196 $P = 0.067$	19.79 ± 0.125 $P = 0.155$	6.94 ± 0.223 $P = 0.04$
2003	MFS	1.25 ± 0.058	0.740 ± 0.090	21.05 ± 0.207	3.62 ± 0.070
	SS	1.41 ± 0.052 $P = < 0.001$	0.626 ± 0.050 $P = 0.011$	20.66 ± 0.239 $P = 0.015$	3.80 ± 0.160 $P = 0.006$
2004	MFS	1.46 ± 0.076	0.775 ± 0.109	20.52 ± 0.149	7.02 ± 0.191
	SS	1.48 ± 0.072 $P = 0.316$	0.960 ± 0.049 $P = 0.005$	20.55 ± 0.120 $P = 0.563$	7.21 ± 0.125 $P = 0.030$

The must soluble solids content of the MFS crop was significantly lower for the years 1999 - 2001 in comparison with the SS plot. A difference of 0.96°Brix was found in 1999, 0.5°Brix in

2000 and 2001 (Tab. 4), corresponding to a reduction of 5% and respectively 2.5%. The total acidity was generally significantly higher in the MFS plot with exception of 2001 (Tab. 4). In the recovery year 2003, corresponding to the 4th stress year, the MFS resulted in, contrary to the tendency observed until 2002, a higher production and berry weight with a significant difference for the crop production of 0.113 kg·m⁻² and 0.16 g for berry weight.

A higher must soluble content of 0.39 °Brix and a lower total acidity was also found (Tab. 4). In 2004, the year following the recovery season, a significantly higher yield quantity was measured in the SS plot without noticeable differences in berry weight and must soluble content as compared to the MFS plot. Only the total acidity was significantly lower in the MFS plot (Tab. 4).

Influence of cumulated stress years on plant phenology, plant growth, leaf area and potential crop load

The cumulated stress from 2000 (first stress year) to 2003 (fourth stress year, in which the MFS plot was normally treated to induce a recovery season) led to no differences on bud burst date and on bud and shoot phenology (data not shown). The most important growth parameter measured was a significantly lower shoot length on the vines in the MFS starting from the 3rd stress year (2002), with a difference of 3.72 cm at the first control and 7.09 cm at the second control; in the 4th stress year with a significant difference of 8.39 cm at the last control before topping (Tab. 5).

Table 5. Shoot growth, expressed in cm, measured in the MFS (minimal fungicide strategy) and SS (standard schedule) plots during the 4 stress years following 1999 (first year of the application of the MFS) and 2004 (year after the recovery year 2003). Each value is the average of 8 repetitions with the standard deviation.

	Shoot length (cm)		Total number of leaves per shoot	
2000 (1 st stress year)	May 19	June 2	May 19	June 2
MFS	40.33 ± 4.85	76.19 ± 6.58	8.22 ± 0.31	17.40 ± 1.47
SS	40.55 ± 3.40	76.24 ± 6.63	8.15 ± 0.36	17.44 ± 0.65
	<i>P</i> = 0.861	<i>P</i> = 0.766	<i>P</i> = 0.608	<i>P</i> = 0.939
2001 (2 nd stress year)	May 21	May 30	May 21	May 30
MFS	39.96 ± 3.95	68.44 ± 4.76	7.13 ± 0.39	13.09 ± 1.02
SS	40.45 ± 2.91	67.79 ± 6.63	7.26 ± 0.38	13.68 ± 1.44
	<i>P</i> = 0.743	<i>P</i> = 0.773	<i>P</i> = 0.463	<i>P</i> = 0.418
2002 (3 rd stress year)	May 29	June 11	May 29	June 11
MFS	47.24 ± 3.31	80.23 ± 5.99	10.53 ± 0.49	21.98 ± 1.61
SS	50.96 ± 1.69	87.32 ± 4.76	10.28 ± 0.39	22.52 ± 1.22
	<i>P</i> = 0.022	<i>P</i> = 0.031	<i>P</i> = 0.258	<i>P</i> = 0.435
2003 (4 th stress year)	May 22	June 20	May 22	June 20
MFS	40.78 ± 3.26	98.95 ± 7.92	8.02 ± 0.43	26.74 ± 2.69
SS	38.17 ± 2.45	107.54 ± 7.87	8.04 ± 0.23	31.42 ± 2.73
	<i>P</i> = 0.052	<i>P</i> = 0.023	<i>P</i> = 0.912	<i>P</i> = 0.003
2004 (after recovering)	May 27	June 17	May 27	June 17
MFS	40.54 ± 2.56	79.44 ± 3.23	11.38 ± 0.93	25.79 ± 1.31
SS	39.24 ± 2.65	82.72 ± 5.95	10.92 ± 0.67	27.32 ± 2.39
	<i>P</i> = 0.298	<i>P</i> = 0.192	<i>P</i> = 0.310	<i>P</i> = 0.235

An influence on the total number of leaves per shoot has been found only at the second control of June 20 during the 4th stress year (recovery year) as a result of four previous stress years (Tab. 5). The difference was noticed in a greater number of principal leaves: 19.6 main leaves per shoot for SS vines in comparison to the 18.4 main leaves per shoot of MFS vines and in a greater number of lateral shoots per main shoot, which was, in average, of 8.6 and 6.7 for SS and, respectively, MFS vines. In 2004, after the recovery year, no difference was found for these influenced parameters confirming the recovery capacity of the plant. Leaf area of main and lateral leaves, measured only in the stress period 1999-2003, showed a tendency to decrease from the 2nd stress year (Tab. 6) but these differences were not statistically significant.

The estimation of the crop potential expected at harvest was quantified in late July, because before onset of ripening the crop load was thinned to a potential of 1.0 kg·m⁻² in all plots according to the production limit.

Table 6. Leaf area expressed as cm² of the main and lateral leaves in the MFS (minimal fungicide strategy) and SS (standard schedule) plots during the 3 stress years. Measurements made on June 5 for 2000, June 15 and 27 for 2001 and respectively 2002 and July 2 for 2003. In the MFS plot plants without downy mildew symptoms were chosen. Each value is the average of 8 repetitions with the standard deviation.

Year	Treatment	Main leaf area (cm ²)	Lateral leaf area (cm ²)
2000	MFS	118.84 ± 16.02	19.82 ± 7.65
	SS	114.53 ± 16.58 <i>P</i> = 0.490	17.73 ± 3.29 <i>P</i> = 0.310
2001	MFS	126.12 ± 15.86	22.19 ± 5.70
	SS	138.19 ± 28.12 <i>P</i> = 0.156	28.88 ± 6.60 <i>P</i> = 0.183
2002	MFS	155.57 ± 25.92	39.34 ± 3.69
	SS	170.51 ± 27.19 <i>P</i> = 0.127	41.97 ± 7.21 <i>P</i> = 0.290
2003	MFS	127.07 ± 28.12	33.58 ± 5.72
	SS	119.65 ± 27.19 <i>P</i> = 0.156	33.57 ± 5.36 <i>P</i> = 0.990

Differences have been observed on plant fertility, expressed as number of clusters per shoot, only in 2001. The estimated crop potential has shown a significant decrease in the MFS plot with a difference of 0.290 kg·m⁻² in the 2nd stress year and 0.392 kg·m⁻² in the 3rd stress year (Tab. 7).

Contrary to our expectations, no difference was found at harvest in the 4th stress year, but in 2004 after the recovery season (Tab. 7). Despite these differences the potential productivity in the MFS plot was still 0.500 kg·m⁻² higher than the production limit of 1.0 kg·m⁻².

Table 7. Shoot fertility expressed as number of clusters per shoot, and potential yield quantity estimated at the end of July in the MFS (minimal fungicide strategy) and SS (standard schedule) plots for the stress years 2000-2003 and 2004. Each value is the average of 8 repetitions with the standard deviation.

Year	Treatment	Fertility (clusters per shoot)	Potential yield quantity (kg m ⁻²)
2000	MFS	1.64 ± 0.094	1.572 ± 0.149
	SS	1.60 ± 0.126 <i>P</i> = 0.170	1.610 ± 0.152 <i>P</i> = 0.412
2001	MFS	1.46 ± 0.264	1.542 ± 0.289
	SS	1.57 ± 0.158 <i>P</i> = 0.043	1.832 ± 0.188 <i>P</i> = 0.003
2002	MFS	1.79 ± 0.079	1.510 ± 0.039
	SS	1.73 ± 0.110 <i>P</i> = 0.549	1.902 ± 0.218 <i>P</i> = 0.001
2003	MFS	1.75 ± 0.044	1.987 ± 0.204
	SS	1.73 ± 0.047 <i>P</i> = 0.495	2.164 ± 0.168 <i>P</i> = 0.167
2004	MFS	1.65 ± 0.084	1.812 ± 0.072
	SS	1.59 ± 0.075 <i>P</i> = 0.021	1.964 ± 0.091 <i>P</i> = 0.003

Discussion

The application of the MFS has permitted reducing, on average, by 50% the number of fungicide applications with a generally low impact on the yield. The total number of applications made depended on the yearly epidemic progress, so that the number of fungicide applications was reduced by a maximum of 66% in 2000 and a minimum of 43% in 2002 and 1999 (considering for this year the fungicide application on clusters). Although this limited number of fungicide applications has delayed all the same the epidemic progress of downy mildew, the impossibility of estimating in time the disease progress remains an important limit. Consequently we were not able to contain the epidemic each year within the expected range of 1% to 5% at the beginning of the ripening phase. This difficulty was particularly evident in 1999, where the choice to begin leaf protection at a severity of 1.48% demonstrated the impossibility of always managing the epidemic at low levels.

The two first fungicide applications upon observance of the first sporulation play a central role because they limit the apparition in the field of fit genotypes, which are generally responsible for the disease spread in the plot (Gobbin *et al.*, 2003; Gobbin, 2004). Downy mildew populations are characterised by one or two most important genotypes which appear generally at the beginning of the epidemic with the capacity in time to generate an important number of asexual cycles (Gobbin, 2004). The genetic analysis made in 2001 in the MFS and in the untreated plots has indicated that the epidemic progress observed in the MFS was probably influenced by an important migration of fit genotypes from the untreated plot (Gobbin *et al.*, 2003). The genetic analyses have permitted estimating that the presence of an untreated plot was probably able to reduce by 17% the efficacy of the MFS in July

(Gobbin *et al.*, 2003). This is a speculation, because we do not have any proof. However untreated vines neighboring the MFS certainly had an effect on the epidemic progress observed, but it is impossible, with the exception of 2001, to evaluate the impact of the possible migration of fit genotype or its selection in the plot. We can not distinguish if genotypes were killed by fungicide or by natural selection because the high mortality of genotypes, or restricted asexual reproduction, seems to be an intrinsic natural characteristic of *P. viticola* (Gobbin, 2004). Another difficulty in the fungicide application decision is the evaluation of the necessity of the last application at the middle of August for the control of downy mildew infection on the lateral shoots, which are responsible for carbohydrate production for berry ripening (Candolfi-Vasconcelos, 1990). We have always made this last application because the epidemic progress on the lateral shoots is generally rapid and consequently this spray serves only as a prevention against a reduction of the leaf area of the lateral shoots. At harvest we have obtained a yield quantity which was, in average, 18% lower in the MFS. The yield quantity formation depends on the leaf area during the period between blooming and some weeks later (Candolfi-Vasconcelos and Koblet, 1990). The low disease severity level observed on the canopy in June does not justify the possible negative influence of downy mildew on the crop formation. Cluster infections, with the exception of 1999, were never so high as to explain an important role of the disease. The berry weight was also generally higher in the MFS than in the SS. We can only suppose that these differences are due to the difficulty of regulating the crop load to a maximum of $1.0 \text{ kg}\cdot\text{m}^{-2}$ by eliminating the appropriate number of clusters per plant which in the MFS plot leads to an overly severe reduction. Nevertheless, the yield quantity obtained in the MFS plot can be considered appropriate because it respects the production limit applied in Ticino. The same discussion is valid for the yield quality, because the must soluble solids are generally lower at harvest in the MFS than in the SS, but the reduction was only 2.6% on average, with the exception of 1999, where the downy mildew stress was probably amplified by late topping of the plants in August with a consequent elimination of a significant healthy leaf area of the lateral shoots. The delay of the epidemic was enough to induce plant compensation without creating a high stress level. These results can be considered positive and they confirm our results in the previous experimentation.

Each growing season is independent of the previous one, because the reconstitution or depletion of the reserve pool depends on the plant stress during the ripening period (e.g. years with a high downy mildew severity versus years without relevant disease). In this type of experiment it is also very difficult to obtain an annual control of the reserve content of the roots, the main source of carbohydrate reserves, without damaging the plant. Contrary to our expectations, the impact of a repeated stress does not have important consequences on the plant growth. Only shoot elongation, from the third stress year and the total number of leaves

per shoot in the fourth stress year has been influenced. A single recovery year without a stress situation, as expected, has been enough to eliminate the stress symptoms on the plant growth induced by the stress situation. It is therefore logical to suppose that a single recovery year permits the vine to return the carbohydrate reserves to a normal level. The potential yield quantity, estimated before crop regulation, is the second parameter negatively influenced by repeated stress situations. However its consequences are negligible because the estimated production was always higher than the limit generally applied in Ticino. If the results demonstrate this effect on the crop formation during the MFS application period, it becomes difficult to explain the grapevine behaviour during the recovery season 2003 and the following one. We can only speculate that the increased berry weight registered in the MFS plot is responsible for the major crop production, or else there are other physiological elements (Candolfi-Valsconcelos, 1990), beyond the use of the reserves, that play a role in berry formation. Nevertheless, our results confirm the recovery capacity of the plant shown by Koblet *et al.* (1993) with artificial defoliation. The delay in time of downy mildew epidemics, as a consequence of the MFS application, is therefore sufficient to reduce the reserve mobilisation to a level that does not induce important depletion of the carbohydrate reserves. The impact of the downy mildew stress caused by the incomplete control of *P. viticola* on plant growth and yield quantity and quality is small and the risk due to the application of the MFS over several years minimal. The decision role for the fungicide application is the critical point observed. The optimal application of MFS necessitates quantifying the role of primary and secondary infection in the epidemic development and their implementation in a quantitative forecasting model (Rossi *et al.*, 2009) that integrates, in addition to the epidemic development, the yield formation and their interactions with the disease. MFS is a good basis for the full application of the IP concept. However, the reduction of yield to the imposed maximum quantity still appears as a difficult task under the MFS, as it is difficult if not impossible to account for the reduction caused by downy mildew in these plots.

Chapter 7

General discussion

The grapevine response to downy mildew

In viticulture fungal diseases (including, systematically erroneously, the Oomycetes) have been considered an inevitable constraint which must be impeded during the growing season. Therefore, control strategies are targeted at avoiding any infections or, if occurring, at eradicating them. In this Thesis I have chosen to follow the teaching of the entomologists (Delucchi, 1990), which consider in their control strategies the interaction of the host with the pest establishing damage levels and intervention levels often dependent on the host phenology and growth stage in addition to quantitative presence of the pest. This study has permitted quantifying the impact of downy mildew foliar epidemics on the plant. The crop system analysis (Jermini *et al.*, 2006) applied has improved our knowledge of the grapevine response to *Plasmopara viticola* epidemics. The current downy mildew control strategy, (avoiding the infections) does not consider the interactions between disease and crop system, where the plant is the central element. During the growing season, the plant prioritizes various sinks (reserves, shoot growth, berries) with the carbohydrate produced. Therefore, the knowledge of the plant's response to a stress situation induced by downy mildew epidemics on the canopy is the basis for understanding the disease threshold over which yield quality damage is detected. Before ripening onset, the downy mildew epidemics, observed during the experimental years, don't induce the plant to apply a compensation mechanism, neither with an increase of the leaf assimilation capacities nor of the leaf area of lateral shoots. The reduction of the assimilation leaf area caused by the epidemic was certainly too low to create an imbalance in the carbohydrate allocation system of the plant. The important increase of the downy mildew epidemics coincides with the berries ripening phase, during which berries represent an important sink and the lateral leaves play a major role in fulfilling their carbohydrate request (Candolfi-Vasconcelos, 1990). During the berry ripening phase the increased reduction of the assimilating leaf area, the inhibition of the assimilation capacities of the leaves, and, particularly, of the lateral leaves, induce the mobilisation of the reserves stored in the woody parts and particularly in the roots. The mobilised assimilates will primarily serve the berries; therefore, up to a certain level of loss of assimilates, no negative effects are observed on the berries but the trunk and roots will demonstrate a reduced level of reserves in the fall. As a result, no immediate effect of moderate leaf damage on the product was measured. Depletion or reduced storage of reserves over a longer period led to retarded early season growth. However, a single season of undamaged leaf canopy fully restored the reserves and therefore the early season growth. Photosynthesis measurements on main and lateral leaves have allowed us to understand the influence of the disease on the symptomless tissues of an infected leaf in relation to the disease severity. In the "mosaic sporulation type", as observed on Merlot, the impact of these virtual lesions could effectively play an important role and let us presuppose a possible

systemic behaviour of downy mildew in the leaves, but this hypothesis isn't until now demonstrated. The presence of "virtual lesions" far from the borders of the sporulating area, and especially for the mosaic symptoms, doesn't reflect the actual part colonized by the pathogen and at least a portion of the leaf area determined as healthy has in fact a latent lesion. Therefore, the visual estimation of downy mildew infection may not give a good indication of the effect of the pathogen on host physiology, supporting other observations (Lakso *et al.*, 1982; Rabbinge *et al.*, 1985; Shtienberg, 1992). Consequently, the use of visual estimation of disease severity is unsatisfactory for modelling the carbohydrate production of leaves in a quantitative model in which the grapevine physiological growth is coupled with the disease epidemic impact (Blaise *et al.*, 1999). The use of the whole plant photosynthesis measurement, which represents the mean activity of the entire continuum of the leaves forming the canopy and, contrary to the single leaf measurement, is linearly related to vine dry mass (Edson *et al.*, 1993; Miller *et al.*, 1997) could probably be a more adequate analysis because it includes the potential role of the "virtual lesions".

In the "Untreated canopy" treatment, the grapevine has never totally compensated for the carbohydrate request of the berries. At the beginning of the ripening the disease severity was higher than in the other treatments but the must soluble solids in the berries, with exception of 1996 where we started later with the controls, was similar between the treatments. It is only during this time that we have an increase in the sugar accumulation. It seems that grapevine needs a certain reaction time before applying a compensation mechanism. The stimulus is certainly connected to a leaf damage threshold, which depends more probably on the canopy assimilation capacity's response to the sink requests. The important finding of this work is the different priority level applied to the mobilisation process of the reserves. Grapevine doesn't exhaust the starch reserves stored in the roots, but it applies a priority between the different woody parts beginning with roots and in succession the trunk, cane and one year old wood. A specific study needs to determine this differentiation because this response depends certainly on the leaf damage, which is related to the physiology of the berries population. In fact, Coombe (1992) indicates that veraison varies between vines within a vineyard and more particularly between berries within each cluster, because each berry appears to develop independently, an independence that may derive from as early as anthesis, maybe earlier. Therefore, a vineyard is composed of a berry population classed in physiological age cohorts. If a large part of the berries have the same physiological age, their carbohydrate request is high and consequently the plant, applies the observed reserves mobilisation priority in order to compensate for the high level stress situation. The berries allocation in physiological age cohorts also has an important implication not only in determining the carbohydrate request during ripening, but also in determining the control strategies of cluster diseases such as downy mildew and black rot (*Guignarida bidwellii*),

because for each age cohort can be related to a different susceptibility to the infection (Hoffman *et al.*, 2002).

The damage threshold applied in the Minimal Fungicide Strategy (MFS) application was theoretic because of the lack of knowledge of the compensation induction mechanisms and the quantification in time of the carbohydrate request of the berries as constraining factors. Despite undergoing stress, grapevine also demonstrated a great recovery capacity in the following growing season, indicating that downy mildew does not negatively influence the yield formation in the following season. This is an important factor, which has never been considered in most of the disease control strategies because their aim is to avoid all possible damages.

Downy mildew remains an important disease with a high destructive potential, but I demonstrate that a partial control doesn't have negative influences and it is therefore possible to change our concept of a control strategy, which we have incorporated in the elaboration of the MFS. This work has also permitted us to demonstrate on a quantitative basis the impact of this disease on yield formation for the implementation of these results in a model which couples the grapevine's physiological growth with the disease epidemic impact to give therefore a different approach in a decision support system (Blaise *et al.*, 1999). The development of this decision support tool requires more knowledge of the mechanisms which enhance compensation capacities of the plant and of the quantification of primary inoculum, which plays a primary role in the epidemic progress during the whole season (Gobbin, 2004). These results can not be generalised. It is important at this moment to have evidenced the plant response, which must be verified in other climatic areas and particularly with other cultivars and training systems.

Application of the Minimal Fungicide Strategy (MFS)

The results of the impact of downy mildew foliar epidemics on the plant have permitted the elaboration of the Minimal Fungicide Strategy (MFS), which is based on a partial control of the disease with the aim of delaying the epidemics' progress so as to limit the disease severity between 1% and 5% during the first ripening phase and to capitalize on the plant compensation capacities to avoid yield quality losses. The results must be considered greatly positive because I have reduced between 43% and 66% the number of fungicide applications without significant negative influences on the yield and plant growth in time. Nevertheless, the MFS presents a series of elements that limit its application in the practice:

- The substantial investment necessary for the monitoring and the adeptness of finding the symptoms in the field. Efficient downy mildew monitoring plays an important role in the spray decision and this fundamental aspect determines the efficacy of the control. The appearance of downy mildew is erratic and the distribution in the field of

the first infected plants is random. Moreover, the primary infections occur during the whole season (Gobbin, 2004; Jermini *et al.*, 2003; Kennely *et al.*, 2007). Consequently, a field control requires a large investment of time and costs for the controls. The lack of a monitoring pattern for the detection of low symptoms densities in the field is a constraint. After reviewing the downy mildew biological cycle (Gobbin, 2004), it is evident that these elements play a fundamental role. Therefore, we need to improve our knowledge of the oospores ripening dynamic. A development of accurate models for the oospores ripening and primary infection (Rossi *et al.*, 2008) is an important step in this direction. A second factor that limits the accuracy of the field control is the difficulty, at least on Merlot, of detecting the symptoms during the monitoring (Dietrich *et al.*, 1999).

- The need of a quantitative model for the epidemiological progress. The genetic analysis of the downy mildew population has shown the primary role of the primary infections, but also the complexity of the population, which is composed of a multitude of genotypes. Only a small part of these, the dominant genotypes, are able to make a great number of secondary asexual cycles (Gobbin, 2004). Downy mildew dispersion follows four strategies (Gobbin, 2004), but we don't know if these strategies are typical for a vineyard or if the pathogen selects a strategy in function of the climatic conditions of the year. On this basis a downy mildew quantitative epidemiological model must quantify the epidemic progress during the season integrating, beyond the dynamics of the primary infection, the dispersion capacities of downy mildew in the field.
- Efficacy of fungicides. The MFS is based on a partial control of the pathogen and therefore the fungicide application aims to delay the epidemic progress. The type of fungicide used, its persistence and efficacy play an important role. The model should also, as proposed by Blaise *et al.*, (1999), consider this important element.

It is therefore unthinkable to apply the MFS on a large scale without the support of a quantitative model as discussed above because of the risk that an inaccurate timing of the spray application can lead to yield damages as shown in 1999 during the first year of our experimentation. The MFS could be more efficient and pose fewer risks in its application if it is coupled with a sanitation measure consisting of the harvest and elimination of the leaves from the field in the autumn with the aim of reducing the oosporic pool in the soil and therefore the disease inoculum for the next year (Gobbin *et al.*, (2007).

Practical considerations

This work proposes some advice for the winegrower:

- Starting with the downy mildew control after the finding of the first sporulation in the field doesn't necessarily mean a reduction in yield.
- The typical late epidemic progress observed in our region during the second part of the ripening phase doesn't necessarily lead to yield quality losses because the grapevine is able to compensate for this stress situation.
- It is important to avoid multiple stress elements and particularly a high crop load and important defoliations, which can amplify stress situations. Therefore, a good balance between crop load and leaf area is the basis for a correct management of impact of biotic stress elements.

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