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Influence of *Plasmopara viticola* on gas exchange parameters on field-grown *Vitis vinifera* 'Merlot'

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Summary

The impact of downy mildew (*Plasmopara viticola*) epidemic on main and lateral leaf assimilation capacity of *Vitis vinifera* 'Merlot' has been quantified, under field conditions during the ripening phase, by means of leaf gas exchange measurements. The aim was to describe the impact of different disease severity levels on the gas exchange rate of symptomless portions of main and lateral leaves and of the sporulating parts in comparison with healthy leaves. The measurements were carried out on plants normally treated and on plants where only the clusters were treated with a contact fungicide to prevent quantity yield losses. A drastic reduction in the photosynthetic rate was observed on the sporulating area of main and lateral leaf tissues. Stomatal and mesophyll conductance decreased and stomatal resistance increased, indicating the difficulty of CO_2 diffusing through the stomata into the mesophyll to the site of carboxylation. 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 stomatal conductance and, consequently, of the photosynthetic rate, transpiration and water use efficiency was observed already at low severity level with increments of the disease severity on the leaf. At the same time an increase of stomatal resistance on the symptomless area of a lateral leaf was measured. Visual assessment of the diseased leaf area didn'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. The results also emphasized the important role of downy mildew as a stress element for the plant during ripening phase, a source element for carbohydrate production.

K e y w o r d s : Downy mildew, photosynthesis, disease severity, latent lesion, visual assessment.

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, Remund and Boller 1995, Mercader and Isaacs 2003, Linder *et al*. 2009,) and diseases (Lakso *et al*. 1982, Goodwin *et al*. 1988, Shtienberg 1992, Cabaleiro *et al.* 1999, Beltrami *et al.* 2004, Moriondo *et al.* 2005, Nail and Howell 2005). Concerning downy mildew, studies had been carried out by Orlandini *et al*. (1998), Moriondo *et al.* (2005), Allegro *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 lat-

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eral 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 $kg \cdot m^{-2}$, 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 $kg \cdot m^{-2}$, 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 $g \cdot L^{-1}$ difenoconazol) were made starting from bloom to prevent powdery mildew (*Uncinula necator*) and black rot (*Guignardia bidwellii*) infections and one with Switch (25 % fludioxonyl + 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 cm^2 (PLC-B for broad leaf). All measurements were carried out under fully light saturated conditions (> 1200 µmol PAR m-2s-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 d of rainfall with an amount of 1,144 $mm \cdot m^{-2}$ were measured in 1997 and 25 d with 1,108 $mm \cdot m^{-2}$ in 1998. In June, July and August 1997 16, 11 and 9 d with rainfall were measured with a total of 466, 184 and 259 $mm \cdot m^{-2}$, respectively. For the same months of 1998 11, 8 and 6 d with rainfall were measured with a total of 191, 116 and 78 $mm \cdot m^{-2}$, 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 6^{th} and the 9^{th} main

leaf 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). 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 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 leave 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). The partial pressure of the CO_2 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 CO_2 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 (Figure). 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 (Figure). 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 neg-

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 = 1,017) 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 %

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 ± Std dev 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 ± 1.25	4.98 ± 0.51	2.06 ± 0.22	0.199 ± 0.057	5.37 ± 1.45	0.052 ± 0.009	199.94 ± 24.46	37.41 ± 1.19
		Sporulating area	1.81 ± 1,19	2.19 ± 0.87	0.77 ± 0.37	0.042 ± 0.023	33.04 ± 25.36	0.008 ± 0.006	237.03 ± 35.06	41.68 ± 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 ± 1.25	4.72 ± 0.39	1.89 ± 0.21	0.197 ± 0.035	5.29 ± 1.23	0.054 ± 0.010	166.55 ± 10.52	36.95 ± 0.74
		Sporulating area	1.82 ± 1.22	2.69 ± 0.83	0.62 ± 0.32	0.067 ± 0.033	17.65 ± 7.36	0.009 ± 0.007	210.04 ± 22.23	39.86 ± 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 ± 1.20	5.61 ± 0.81	1.81 ± 0.41	0.263 ± 0.086	4.05 ± 0.98	0.925 ± 0.068	219.21 ± 19.89	35.35 ± 0.95
		Sporulating area	1.86 ± 0.97	2.16 ± 0.86	0.85 ± 0.25	0.043 ± 0.029	28.38 ± 11.89	0.108 ± 0.074	240.88 ± 28.09	40.33 ± 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 ± 1.05	3.90 ± 0.38	2.54 ± 0.26	0.172 ± 0.029	5.99 ± 1.10	1.110 ± 0.093	198.05 ± 10.36	33.43 ± 1.26
		Sporulating area	1.27 ± 1.23	1.45 ± 0.65	0.72 ± 0.48	0.032 ± 0.020	39.93 ± 21.21	0.072 ± 0.079	261.33 ± 33.74	38.06 ± 0.73
		P value	< 0.001	< 0.001	< 0.001	< 0.001	0.012	< 0.001	0.003	0.002

A = Photosynthesis (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$); E = Transpiration (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$); WUE_{ins} = Water Use Efficiency instantaneous (µmol CO_2 / mmol $H_2O \cdot m^{-2} \cdot s^{-1}$);
g_s = Stomatal conductance (mol $CO_2 \cdot m^{-2} \cdot s^{-1}$); r_s = Stomatal resistance ($m^2 \cdot s \cdot mol\ CO_2^{-1}$); g_m = Mesophyll conductance (mol $CO_2 \cdot m^{-2} \cdot s^{-1}$);
C_i = Substomatal CO_2 concentration (µmol $CO_2 \cdot mol^{-1}$); Tleaf = Leaf temperature (°C).

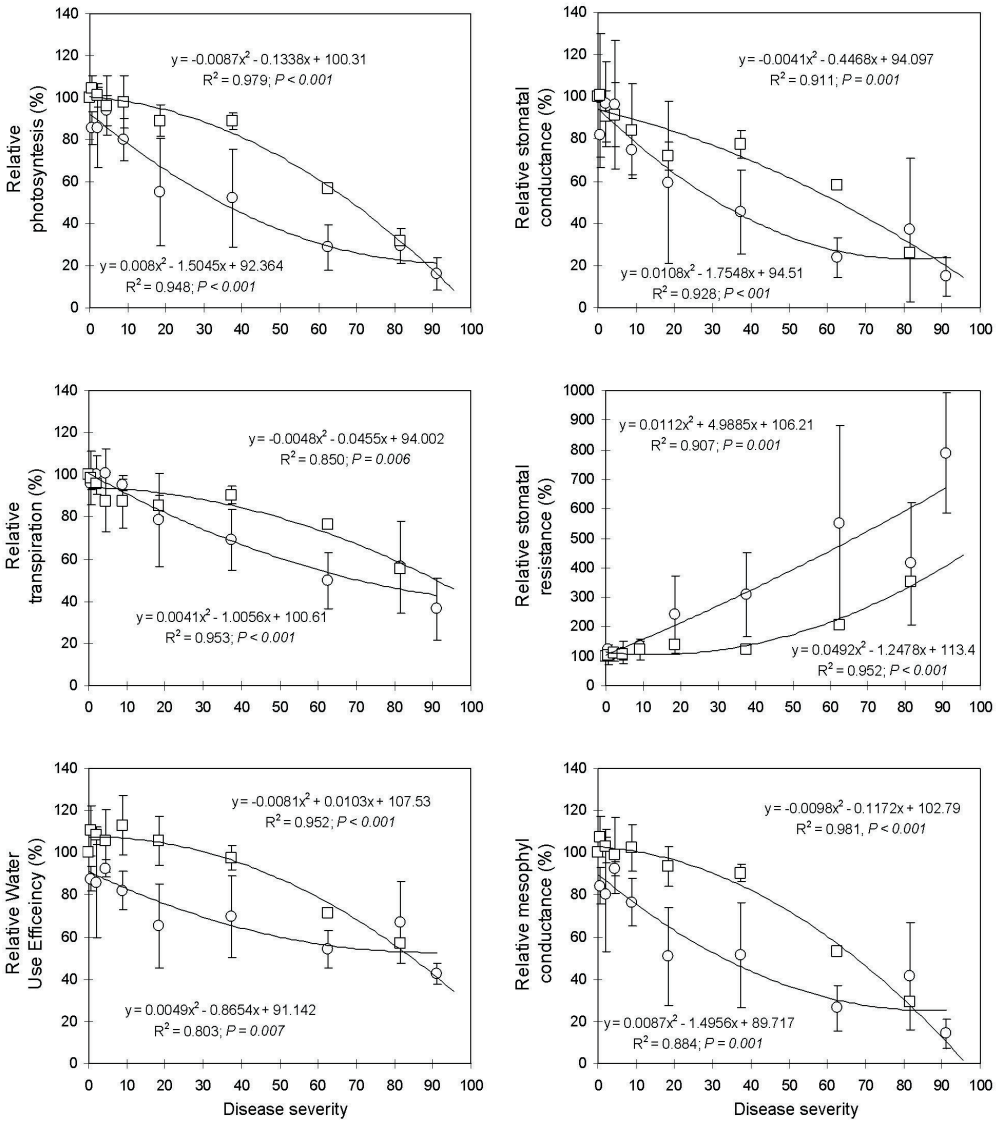

Figure: Relationship between disease severity, defined with the middle value of the modified Horsfall scale, and the photosynthesis, transpiration, water use efficiency, stomatal and mesophyll conductance and stomatal resistance rate expressed as ratio between values measured on symptomless area of infected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

ative 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 (Figure). 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 (Al-

legre *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-Vasconcesos 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-Vasconcesos (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 physiological process 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 CO_2-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.

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