PHOTOSYNTHESIS OF GRAPEVINE LEAVES INFECTED BY DOWNY MILDEW

PHOTOSYNTHÈSE DES FEUILLES DE VIGNE INFECTÉES PAR LE MILDIOU

S. ORLANDINI and A. GIUNTOLI

CNR-IATA (National Research Council - Institute of Agrometeorology and Environmental Analysis for Agriculture), Piazzale delle Cascine 18, 50144 Firenze (Italy)

Summary : A correct approach to crop protection must consider the effect of disease on plant activity and its impact in terms of economical losses. These evaluations can drive the farmers to a quantification of plant responses to pathogen attack and to the application of economical thresholds for fungicide sprays. For grapevine (*Vitis vinifera* L.), the knowledge of plant responses to disease is still very limited independently from the level of investigation (physiology, growth, development, etc.). The aim of this research was to evaluate the impact of downy mildew (*Plasmopara viticola* Berl. et De Toni) on grapevine photosynthesis under controlled and field conditions. Downy mildew was shown to reduce to a negative value net photosynthesis in the oilspot, as well as to affect the physiology of gas exchanges of the tissues surrounding the oilspot. The daily trend under field condition pointed out a normal trend of assimilation, without differences between green portions of healthy and diseased leaves. The consequences on source-sink relationships were discussed, as well as the relation between visible symptoms and physiological alterations.

Résumé : Une méthode correcte pour le contrôle des infections doit considérer les effets du pathogène sur l'activité de la plante et son poids en termes économiques. D'un point de vue pratique, l'évaluation de ces effets est utile pour une meilleure quantification des dommages et pour déterminer le seuil des applications des anticryptogamiques. Pour obtenir ces résultats, il faudrait analyser la réponse de la culture au pathogène à différents niveaux, par rapport à nombreux processus : photosynthèse, translocation, transpiration, croissance, développement. L'analyse de ces réponses physiologiques est très importante, car elle offre la possibilité de confronter des résultats similaires, obtenus par plusieurs types de stress. La connaissance des altérations physiologiques produites par les phytopathogènes sur la vigne est encore limitée ; en particulier par rapport aux maladies cryptogamiques. La finalité de cette recherche a été l'analyse des conséquences du mildiou sur la photosynthèse de la vigne. Afin de rendre visibles ces effets on a mesuré les échanges gazeux en plein champ et en conditions contrôlées. Les mesures en plein champ ont été effectuées dans la but de vérifier les effets de la maladie même en présence d'autres stress, tandis que ces facteurs étaient réduits en conditions contrôlées. En particulier, les mesures effectuées en conditions contrôlées montrent que le mildiou endommage gravement la photosynthèse en réduisant la transpiration de la surface objet des dommages macroscopiques (tache d'huile). De plus, la maladie endommage les échanges gazeux même immédiatement à l'extérieur de la tache d'huile (« effet bord »). Ces données montrent aussi qu'il n'existe pas de grandes différences entre les performances photosynthétiques du tissu sain restant de la feuille malade et de la feuille entièrement saine. La diminution de l'assimilation et de la transpiration est étroitement liée au pourcentage de tissu endommagé, inclus dans la surface de la cuvette. Cette régression montre une valeur élevée de l'intercept qui confirme l'hypothèse de la présence d'un « effet bord ». La corrélation est confirmée même en cas de régression entre le périmètre de la tache et la photosynthèse. Les mesures des échanges gazeux effectuées en plein champ confirment qu'il n'y a aucune différence entre le tissu indemne des feuilles malades et les feuilles saines. Celles-ci ont montré tout de même un niveau d'assimilation légèrement plus élevé. Malheureusement ces mesures ne permettent pas la mise en évidence de l'origine de l'« effet bord », mais plusieurs hypothèses peuvent être formulées. Ces résultats sont discutés pour vérifier la possibilité de les utiliser pour la détermination d'un seuil d'intervention qui considère l'existence de « l'effet bord » et de la possibilité que les feuilles, une fois soumises à une attaque cryptogamique, ont, même avec un pourcentage environ de 60 p. cent, de convertir leur rôle de source à celui de puits. En particulier la définition de ce niveau représente un résultat significatif pour l'amélioration de la méthodologie d'intervention anticryptogamique et de la capacité de simuler par des modèles de croissance l'accumulation de matières sèches et les coefficients de répartition du carbone sur les plantes attaquées par le mildiou.

Key words : *Plasmopara viticola*, *Vitis vinifera*, gas exchange, disease intensity, source-sink relationships.

Mots clés : *Plasmopara viticola*, *Vitis vinifera*, échange gazeux, intensité de la maladie, relation source-puits.

1 : correspondance : orlandi@sunserver.iata.fi.cnr.it

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INTRODUCTION

The recent improvements of crop protection require the knowledge of several elements, among those the epidemiology of diseases, the trend of biological cycle, the variation of pathogen population during the season play a fundamental role. However they could be integrated with other parameters describing the inter-relationships between host and pathogen in order to take into account the real effect of disease on plant activity (ORLANDINI, 1996). So fungicide application should be timed according to the real consequences of disease infection on final yield, both in terms of quality and quantity aspects of production. On these bases, economical thresholds of treatment can be implemented, further improving the efficacy of crop protection techniques (CAMPBELL et MADDEN, 1990).

To reach this goal, the responses of crop to pathogen should be investigated at different levels, according to the considered processes: photosynthesis, translocation, biomass accumulation, growth, development, etc. (RABBINGE *et al.*, 1989). The analysis of physiological responses assumes a high importance because of the possibility of describing and understanding the first phases of host-pathogen relationships. This represents a basic step in the assessment of plant responses to the pathogen and it also allows characterising similar dynamics to other stresses. In such a way progress in research can be facilitated and the results of isolated studies can be used in different biotic and a-biotic stress analyses (AYRES, 1992). Moreover, the observed crop physiological responses to disease attack can be quantified and implemented to formulate simulation models for the effect of disease on growth and development of plants. In particular, existing growth models could be modified to simulate growth of plants subjected to a disease attack including specific parameters which value could be tuned according to the state and the intensity of infection (ROSSING *et al.*, 1992).

Among the physiological processes, photosynthesis is one of most sensible to different conditions of stress, and its measurement is frequently used to analyse and describe stress responses of crops (CORREIA *et al.*, 1990 ; QUICK *et al.*, 1992). Diseases, such as downy mildew (*Plasmopara viticola* Berl. et De Toni), are known to induce many alterations at the physiological level, but the exact location and mechanisms of damage have been identified only for few patho-systems (SCHOLES, 1992). Alteration of photosynthetic performances are typically localised in the area of the disease attack, but there have also been evidences of reduction of assimilation rate outside the spot or the area attacked by disease. Several studies describe this effect, called border effect, for different patho-systems as the result of the presence of a virtual lesions, i.e. a physiological alteration of the leaf tissues without a corresponding visible symptom (BASTIAANS, 1991). Alterations of the area surrounding the lesions have been also detected through modifications of reflectance signature, but the physiological meaning has not been yet investigated (BACCI *et al.*, 1993).

As concerning grapevine (*Vitis vinifera* L.), the knowledge of the real impact of diseases on the plant is still very limited and only a few studies have been devoted to the analysis of physiological plant responses (LAKSO *et al.*, 1982 ; BREM *et al.*, 1986 ; GOODWIN *et al.*, 1988), as well as to the assessment of quantitative and qualitative losses related to disease severity (DUSO et BELVINI, 1992 ; KOBLET *et al.*, 1993 ; ORLANDINI, 1997). In the last few years, several studies have been carried out to describe and explain some aspects of grapevine responses to viruses (BALO *et al.*, 1997) and to the attack of several pests (CANDOLFI *et al.*, 1993).

This paper presents the results obtained during 1996, when the responses of photosynthesis, transpiration and other physiological aspects were studied on grapevine leaves during a downy mildew attack which naturally developed under field conditions. With respect to this pathogen of grapevine, its biology and epidemiology have been studied since many years, and this knowledge has been analysed in several simulation models developed for the main viticultural regions (MORIONDO *et al.*, 1997). On the contrary, the lack of understanding of direct grapevine responses to downy mildew requires a deeper investigation to find more precise relationships and to propose economical thresholds of damage based on visible symptoms (JERMINI *et al.*, 1994). With this aim, in controlled conditions photosynthesis was measured on healthy leaves and on the green and the oilspot portions of diseased leaves, to outline the responses of plant and the presence of virtual lesions and their relationships with the area of visible symptoms. Additionally, under field conditions, measurements were performed on the green portion of healthy and diseased leaves to investigate the diurnal daily trend of gas exchanges.

MATERIALS AND METHODS

This work was carried out in the experimental farm Mondeggi (Bagno a Ripoli - central Italy). 20 years old grapevine plants (cultivar Sangiovese) in well nutrition condition were cordon trained and spur pruned (9-11 trellised shoots per plant). In three vineyard plots randomly chosen (1000 m^2 each), downy mildew

attacks were allowed by the absence of fungicide application, while treatments against powdery mildew (*Uncinula necator*) were applied at fixed intervals to avoid any possible infection and damage interactions. The following products were chosen, to avoid any interference with downy mildew: wettable sulphur and dinocap. Any other product was applied since the absence of other diseases and pests. Control plants were located in three plots with the same characteristics and area, and they received treatments against all pests and diseases at identical intervals.

During all measurements, either in controlled environment or in the field, the rates of photosynthesis, stomatal conductance and transpiration were measured on healthy and diseased leaves of similar age, still attached to field plants, with a portable gas-analyser (Ciras-1, PP-system, Hitchin). It was equipped with a Parkinson cuvette, that had a shape of a circle with an area of approximately 3.1 cm^2. Diseased leaves were chosen among those having a similar intensity of infection, which was never higher than 15 p. cent, because of the low intensity of downy mildew infection during the season.

At first, to avoid cutting the leaves for the measurements and, at the same time, to standardise measurement conditions, they were conducted by introducing through the window shoots still attached to plant, inside a car equipped with an air conditioning system providing a controlled environment. During these measurements, light was provided by a halogen lamp connected to the cuvette which gave a PPF (Photosynthetic Photon Flux) of about 1500 µmol m^{-2} s^{-1}, water vapour pressure deficit (VPD) in the cuvette was kept under 1 kPa and temperature at 27 ± 1 °C. During three consecutive days, measurements were conducted on healthy leaves as well as on the green portion and on the oilspot of diseased leaves. For each plot a total of seven leaves was chosen for every position (table I).

After measurements, leaves were cut and the percentage of oilspot area included into the leaf chamber was estimated using three different methods :

- by visual estimation of skilled technicians ;

- by means of a leaf area meter (LI-COR), photocopying the leaves and then cutting the oilspot area included into the cuvette ;

- by means of a software for image analysis, using the scanned photocopies of the leaves and determining the grey levels due to the green and the oilspot tissues. This method also permitted the calculation of the oilspot perimeter.

Then the assimilation rate was also measured under field condition to evaluate the trend of gas exchanges, as the results of environmental conditions on grapevine physiology. During several days from June to September, photosynthesis was monitored every two hours on ten leaves per plot. Leaves were always randomly chosen among those well orientated towards the sun, to obtain maximum PPF, and having a similar age, degree of attack and development of oilspots.

RESULTS AND DISCUSSION

Under controlled condition, comparison of photosynthetic performances of the green portion of healthy and diseased leaves, pointed out that the photosynthetic rate was not significantly affected by the fungus (table I), though assimilation level was lower in the green portion of diseased leaves than in the healthy leaves. The impact of disease was evident when measurements were carried out directly on the spot. Because of the low intensity of downy mildew infection, it was difficult on every measurement to find oilspots as large as the area of the cuvette. Thus, many times, green and oilspot tissues were both included into the cuvette, determining the positive value of assimilation in the diseased samples. On the contrary, when photosynthesis was measured only on the oilspot, the observed level of photosynthesis was well below zero (table I).

The values of photosynthesis measured on the oilspot and green tissue were used to analyse the relationship with the infected area. Relative values (p. cent) of photosynthesis were preferred to normalise the variability among different leaves. The ratio between the assimilation rate measured on oilspot and green tissue and the assimilation measured on the green portion of the same leaf was linearly correlated with the disco-

TABLE I

Assimilation rate of healthy and diseased leaves.

AVG = average value (µmol m^{-2} s^{-1}), SE = standard error. Different letters indicate statistically significant differences (analysis of variance and Bonferroni test with a significance level of .05).

Tableau I – Taux d'assimilation des feuilles saines et malades.

AVG = valeur moyenne (µmol m^{-2} s^{-1}), SE = erreur standard. Les différentes lettres indiquent les différences significatives d'un point de vue statistique (analyse de variance et test Bonferroni avec un degré significatif de 0,05).

Position	AVG	SE
Oilspot	-2.20 A	0.93
Oilspot and green portion	2.17 B	0.70
Green portion of diseased leaves	11.82 C	0.84
Healthy leaves	14.02 C	1.13

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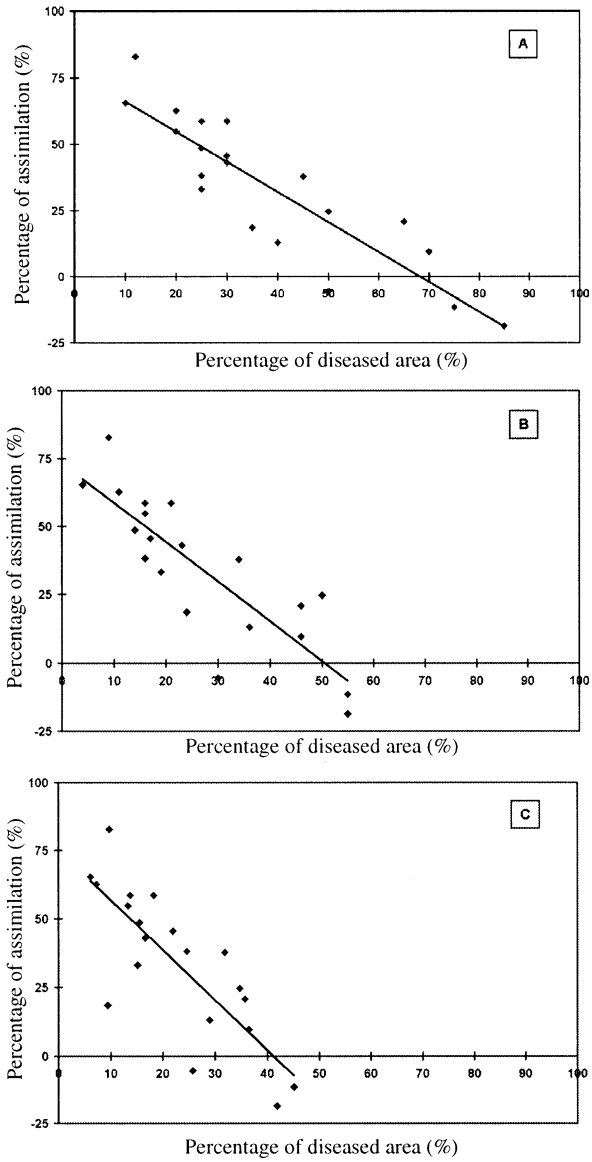

Fig. 1 - Linear regression of reduction of assimilation rate vs. area of the cuvette occupied by oilspot tissue estimated by visual method (A), leaf area meter (B) and software for image analysis (C).

Fig. 1 – Régression linéaire de la réduction du taux d'assimilation en rapport avec la surface de la cuvette occupée par le tissu de la tache d'huile estimée par la méthode visuelle (A), la mesure de la surface de la feuille (B) et le logiciel pour l'analyse de l'image (C).

loured area included into the cuvette, independently from the method for the estimation of diseased area (figure 1 and table II). According to this method, the intercept of the linear functions fitted to the data, ranged from 73 to 77 p. cent. Accordingly when the area of the infection approached zero, the reduction in photosynthesis was already about the 23-27 p. cent, suggesting that the disease was in some way affecting the physiology without showing any visible symptoms. Since all the leaves had the disease at a similar developmental stage, it is possible to assume from the hypothesis that there was a border effect that could account for the missing assimilation, as BAASTIANS (1991) showed for other patho-systems. Moreover, when the percentage of the diseased portion increased to 40-70 p. cent of the cuvette surface area (depending on which method of estimation was used), the total assimilation was close to zero. This could be the result, considering that the oilspot had a large impact on the total photosynthetic rate, probably due to the negative values of assimilation in the lesions as shown earlier. The large variability showed by the slope of the regression functions was probably due to the level of subjectivity of the disease estimation methods. This level could be the highest in the visual method, lower using the leaf area meter (due to the manual cutting of oilspot area), and it was the lowest in the image analysis (related to the choose of grey level thresholds). However, considering that the final output of the functions led to similar qualitative conclusions, the visual method carried on by skilled technicians confirmed its usefulness mainly due to the simplicity and rapidity of application.

The perimeter of the oilspot was similarly well related to the reduction in assimilation (figure 2), but the intercept (table II) was much closer to the assimilation rate measured on healthy leaves (value of about 100 p. cent). This could point out that the perimeter allowed a better estimation of the combined effect of visual (oilspot) and virtual (border) lesions.

Stomatal conductance was also linearly related to the area of oilspot included into the cuvette (figure 3).

TABLE II

Linear regression functions, R squared and F ratio ($p < .001$) of assimilation rate versus the area or the perimeter of oilspot included into the cuvette.

Tableau II – Fonctions de régression linéaires, R carré et F rapport ($p < .001$) du taux d'assimilation en rapport avec la surface ou le périmètre de la tache d'huile comprise dans la cuvette.

Type of disease estimation	Linear function	R squared	F
Visual	y = -1.13x + 77.6	0.78	63.77
Leaf area meter	y = -1.45x + 73.3	0.71	44.75
Image analysis of area	y = -1.83x + 75.3	0.63	31.16
Image analysis of perimeter	y = -1.73x + 104.5	0.62	29.85

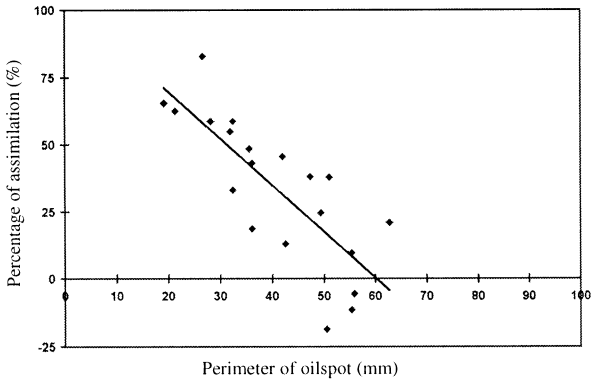

Fig. 2 - Linear regression of reduction of assimilation rate vs. perimeter of oilspot estimated by software for image analysis.

Fig. 2 - Régression linéaire de la réduction du taux d'assimilation en rapport avec le périmètre de la tache d'huile estimée avec le logiciel pour l'analyse de l'image.

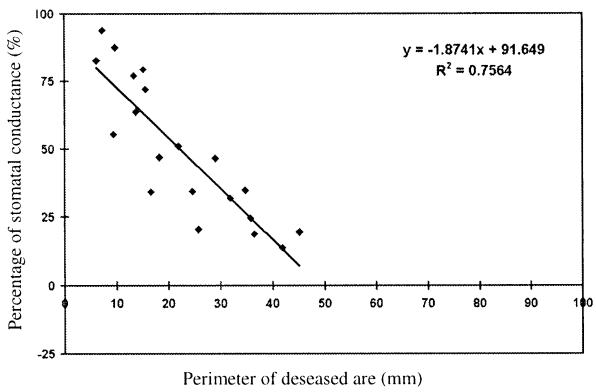

Fig. 3 - Linear regression of reduction of stomatal conductance vs. area of the cuvette occupied by oilspot tissue estimated by software for image analysis.

Fig. 3 – Régression linéaire de la réduction de la conductivité des stomates en rapport avec la surface de la cuvette occupée par le tissu de la tache d'huile estimée avec le logiciel pour l'analyse de l'image.

The function fitted to the data showed that already a 50 p. cent of infected area (estimated by using the image analysis method) reduced stomatal conductance to a very low level. Nevertheless data collected directly on the oilspot had a low, but still measurable, level of stomatal conductance (about 18 mmol m^{-2} s^{-1}) with a high rate of respiration (data not shown).

When gas exchange measurements were conducted in open air on the green portion of healthy and diseased leaves, the typical diurnal trend of was observed (DURING, 1991). The 14th of August was used as example to show the trends observed during all the measurements days (figure 4). The data collected showed that all the considered physiological parameter (assimilation, stomatal conductance, transpiration) were quite constantly higher on the healthy leaves than on the diseased ones, particularly during the afternoon. It can be hypothesised that diseased leaves were more susceptible to environmental stress (high temperature and water deficit) that induced a higher stomatal closure in the afternoon. However they were never significantly different ($p \leq 0.05$) confirming the results obtained under controlled condition (table I).

CONCLUSIONS

Downy mildew determined a negative net rate of photosynthesis in the oilspot, as well as had a strong impact on the border area of lesions. These results are in agreement with others obtained for the different patho-systems ; however, the measurements neither clarify the mechanisms nor the exact location of this effect. Several hypotheses can be proposed, such as the production of toxic compounds, alteration of water and assimilate transport. To increase the knowledge of these effects, this preliminary investigation should be integrated with other analyses to draw a complete description of plant disease inter-relationships during infection periods. In such a perspective the measurements of other physiological parameters (fluorescence, leaf water potential, etc.), the assessment of growth and development of diseased plant, the histological analyses of infected and healthy tissues seem to represent useful tools.

The direct consequences of these results can be a different evaluation of infection intensity, because of the impact of disease involves a leaf area higher than that showing the typical lesion. On these basis the assessment of economical threshold of fungicide application can be carried out with higher accuracy, as well as the estimation of the infection impact on the development, growth and final yield. In such a perspective, simulation model can be calibrated taking into account the modified physiological parameters, the reduction of leaf area due to presence of lesion including oilspot and border effect, and the presence of a specific thresholds of infection intensity to which the leaf may change its role, from a source to a sink organ. The estimation of this level (about 60 p. cent of disease attack from the previously reported data) represents an important goal for the improvement of crop protection, as well as for modelling activity, to simulate both biomass accumulation and coefficients of partitioning in diseased plants.

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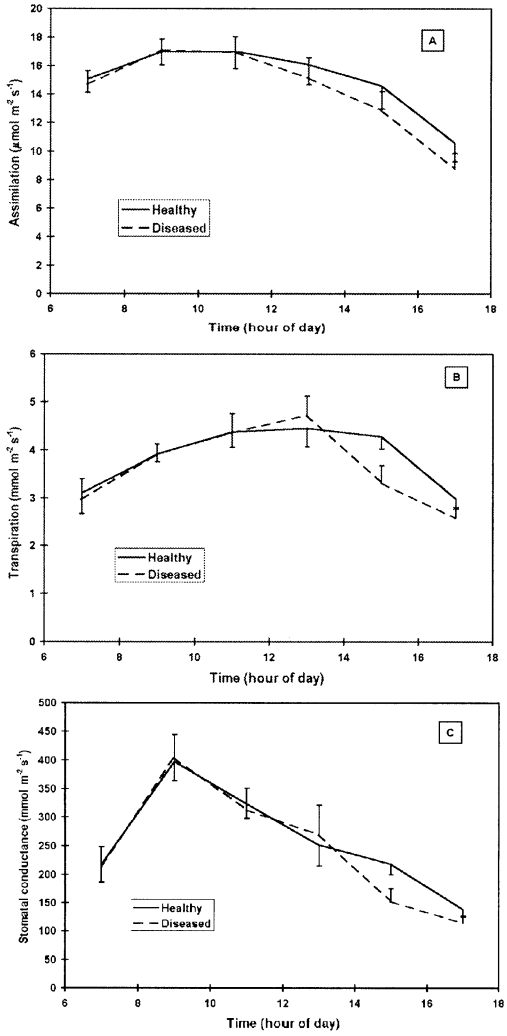

Fig. 4 - Rate of assimilation (A), transpiration (B) and stomatal conductance (C) measured throughout the 14th of August on the green portion of healthy and diseased leaves. Standard errors are indicated.

Fig. 4 – Taux d'assimilation (A), transpiration (B) et conductivité des stomates (C) mesuré toute la journée du 14 août sur la portion verte des feuilles saines et malades. Les erreurs standard sont indiquées.

REFERENCES

AYRES P.G., 1992. Plants versus pests and pathogens : an old story but the same story ? *In: P. G. AYRES* (Eds.) Pests and pathogens - plant responses to foliar attack. BIOS, Oxford, 1-10.

BACCI L., GIUNTOLI A., GOZZINI B., MASELLI F., ORLANDINI S., RAPI B. et ZIPOLI G., 1993. Analisi della riflettanza nella valutazione di infezioni fungine su foglie di vite. *In: Proceedings of the Workshop « Protezione delle colture: osservazione, previsione, decisione »*. Pescara, Italy, October 7-8, 129-143.

BALO B., VERADI G.Y., PAPP E., MUERDY L.A. et POLIK D., 1997. Structural and functional alteration in photosynthetic apparatus of virus infected « Chardonnay » vines. *In: Program and abstract of Ve Int. Symp. on grapevine physiology*. Jerusalem, Israel, May 25-30, 66.

BASTIAANS L., 1991. Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology*, **81**, 611-615.

BREM S., RAST D.M. et RUFFNER H.P., 1986. Partitioning of photosynthate in leaves of *Vitis vinifera* infected with *Uncinula necator* or *Plasmospora viticola*. *Physiological and Molecular Plant Pathology*, 29, 285-291

CAMPBELL C.L. et MADDEN L.V., 1990. *Introduction to plant disease epidemiology*. John Wiley & Sons ed., New York.

CANDOLFI M.P., WERMELINGER B. et BOLLER E.F., 1993. Influence of the European red mite (*Panonychus ulmi* KOCH) on yield, fruit quality and plant vigour of three *Vitis vinifera* varieties. *Vitic. Enol. Sci.*, **48**, 161-164.

CORREIA M.J., CHAVES M.M. et PEREIRA J.S., 1990. Afternoon depression in photosynthesis in grapevine leaves : evidence for a high light stress effect. *J. Experimental Botany*, **41**, 417-426.

DURING H., 1991. Determination of the photosynthetic capacity of grapevine leaves. *Vitis*, **30**, 49-56.

DUSO C. et BELVINI P., 1992. Simulazione dei danni da parassiti sulla vite. *Vignevini*, **7-8**, 33-37.

GOODWIN P.H., DE VAY J.E. et MEREDITH C.P., 1988. Physiological responses of *Vitis vinifera* cv. « Chardonnay » to infection by the Pierce's disease bacterium. *Physiol. Molecular Plant Pathology*, **32**, 17-32.

KOBLET W., CANDOLFI-VASCONCELOS M.C., AESCHIMANN E. et HOWELL G.S., 1993. Influence of defoliation, rootstock, and training system on Pinot noir grapevines. I. Mobilisation and reaccumulation of assimilates in woody tissue. *Vitic. Enol. Sci.*, **48**, 104-108.

JERMINI M., GESSLER C. et BLAISE P., 1994. Preliminary investigation on the impact of Plasmopara viticola on the yield quantity and quality of *Vitis vinifera*. *In : Proc. of II Workshop on grapevine downy and powdery mildew modelling* (Hill, G. K. and Kassemeyer, H. H.). Freiburg im Breisgau (Germany), 29 August-1 September.

LAKSO A.N., PRATT C., PEARSON R.C., POOL R.M., SEEM R.C. et WELSER M.J., 1982. Photosynthesis, transpiration, and water use efficiency of mature grape leaves infected with *Uncinula necator* (Powdery mildew). *Phytopathology*, **72**, 232-236.

MORIONDO M., ORLANDINI S. et ZIPOLI G., 1997. Analysis of models for grapevine phenology, diseases

and pests. *Intermediate Report of Working group on phenology, pest and disease of crops*. COST Action 711, European Union (in press).

ORLANDINI S., 1996. Agrometeorological models for crop protection. *In : Int. Symp. in Applied Agrometeorology Agroclimatology*. Volos (Greece), 24-26 April, (in press).

ORLANDINI S., 1997. Preliminary evaluation of foliar diseases effect on grapevine growth. *In : Program and abstract of Ve Int. Symp. on grapevine physiology*. Jerusalem, Israel, May 25-30, 69.

QUICK W. P., CHAVES M.M., PEREIRA J.S., WENDLER R., DAVID M., RODRIGUES M.L., PASSAHARINHO J. A., ADCOCK M.D., LEEGOOD R.C. et STITT M., 1992. The effect of water stress on photosynthetic carbon metabolism in four species growth under field conditions. *Plant Cell Environment*, **15**, 25-35.

RABBINGE R., WARD S.A. et VAN LAAR H.H., 1989. *Simulation and system management in crop protection*. PUDOC, Wageningen, pp 420.

ROSSING W. A.H., VAN OIJEN M., VAN DER WERF W., BASTIAANS E.L. et RABBINGE R., 1992. Modelling the effect of foliar pests and pathogens on light interception, photosynthesis, growth rate and yield of field crops. *In : P. G. AYRES (Eds.) Pests and pathogens - plant responses to foliar attack*. BIOS, Oxford, 161-180.

SCHOLES J.D., 1992. Photosynthesis: cellular and tissue aspects in diseased leaves. *In : P. G. AYRES (Eds.) Pests and pathogens - plant responses to foliar attack.* BIOS, Oxford, 85-101.

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