EFFECT OF SOLAR RADIATION LEVELS ON GRAPEVINE LEAF POLYPHENOLIC CONTENT AND INTERACTION WITH PLASMOPARA VITICOLA

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Key words

Downy mildew, flavonoid, chlorophyll fluorescence (ChlF), shading, Sangiovese, dualex.

Abstract

In the experimental parcel two light environments were applied to induce differences in total polyphenolic content in grapevine leaves (*Vitis vinifera*, cv. Sangiovese) and to assess if the resistance to downy mildew was affected. Shaded environment was obtained using 70% shading nets placed 3 m above ground. The canopy microclimate, evaluated as air and leaf temperature, relative humidity leaf wetness and different wavelength of solar radiation was measured. Leaf epidermal polyphenols, mainly flavonoids, under the two light regimes were non-destructively measured during the growing season by the dualex optical device. Epidermal polyphenols were found to be considerably higher (80%) in sunny leaves with respect to shaded ones independently from leaf age. Both full developed shade and sun leaves were then inoculated with a sporangial suspension of downy mildew (*Plasmopara viticola*) and infection severity was assessed as percentage of leaf infected area. Downy mildew development showed to be inversely correlated with epidermal polyphenolic content with 15% and 5% of severity value on shaded and sunny leaves respectively.

Introduction

In Italy, as well as in all the main viticultural areas of the world, downy mildew (*Plasmopara viticola*) represents one of the most important disease of grapevine (*Vitis vinifera*), causing severe

damages on the crop every year, and affecting both quantity and quality of wine production (Agrios, 1997).

It is known that incoming radiation affects humidity and leaf wetness within the canopy, which are key variables for downy mildew development. At the same time, quantity and quality of light determine a complex set of both direct and indirect effects on plant-pathogen interactions. Different studies were carried out to understand how ultraviolet radiation (UV) B and photosynthetic active radiation (PAR) behave differently in canopies. PAR penetration into canopies is often higher then UV but in less dense canopies the situation can be reversed. In grapevine this fact is particularly important due to the extreme different managements and conditions under which it is cultivated. Microclimatic conditions within plant canopies are mainly determined by topography, crop structure, trellising and pruning systems (Dalla Marta, in press). More precisely, canopy structure affects light environment and radiative balance and leads temperature, humidity and wind regimes within the crop canopy.

Solar irradiation determines the production in the leaves of several secondary metabolites that may affect the host disease resistance. For example, it is widely accepted that phenolic compounds, including flavonoids, accumulate in response to pathogen attack (Dai et al. 1995; Dixon and Paiva, 1995). On the other hand, preformed constitutive flavonoids are also involved in several host-pathogen interactions (Treutter, 2006) and their antifungal activity is well recognized (Harborne and Williams, 2000). The biosynthesis of flavonoids is largely affected by the intensity and the spectral composition of leaf irradiance (Rozema et al., 1997; Kolb et al., 2001). This aspect can contribute to the overall mechanisms by which light influences plant–pathogen interactions (Raviv and Antignus, 2004; Roberts and Paul, 2006). Interestingly, there is evidence of an inverse correlation between infection severity and intensity of preinoculation light treatments (Shafia et al., 2001). Recently, a low susceptibility to powdery mildew (Uncinula necator) in grapevine was related to high constitutive leaf phenolic compounds, mainly flavonol glycosides (Keller et al., 2003).

Superficial leaf phenolic (Phen) can now be assessed non-destructively using new portable devices, based on the comparison of chlorophyll fluorescence (ChlF) signals at two different excitation wavelengths (Goulas et al., 2004; Kolb et al., 2005). Furthermore, it has been showed that the optical measurements of (EPhen) are representative of total leaf phenolic (Kolb and Pfündel, 2005). Therefore,monitoring of leaf phenolic evolution in the field over a large number of the same samples is possible and can be used to easily select plants with different phenolic contents. On the basis of these consideration, in this study two light environments were applied to induce differences in total polyphenolic content in grapevine leaves (cv Sangiovese) and to assess if the resistance to downy mildew after leaf inoculation was affected. Results were also analysed in order to determine microclimatic variations due to radiation effects and their possible impact on infection.

Materials and methods

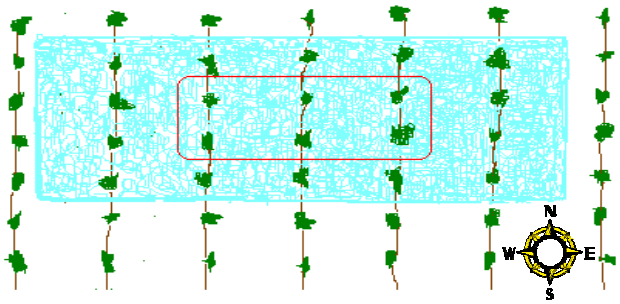


Figure 1: shading net (grey), perpendicular to the rows. The central rectangle shows plants used for measurements.

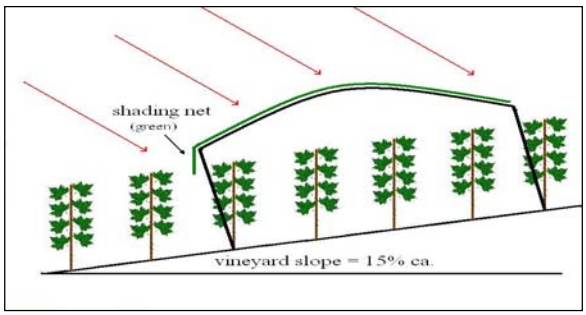


Figure 2: arrangement of the shading tunnel over the vineyard slope.

The field experiment was conducted during the whole 2006 growing season, in the experimental farm of Mondeggi Lappeggi, located in the northern part of the Chianti region in Tuscany (Central Italy, Lat. 43°47′ N, Long. 11°35′ E). The vineyard, called Paretaio, faced south; the elevation was 180 m a.s.l. and the slope 15%.

Grapevines of Sangiovese variety were cordon trained and spur pruned (4-5 spurs with two buds per vine), rows were oriented north-south and the spacing was 1 m in the rows and 3 m between the rows. The canopy was trained in a single curtain between 90 and 210 cm from ground level. The two different light environments were provided using exposed to natural light environment and shaded parcels. The experimental vineyard was arranged using shading net perpendicular to the rows. Each parcel included about 20 vines and measurements were performed on the 6 central ones.

The transmittance of the shading nets (full webbing), measured in laboratory, was about 30% and constant. A complete agrometeorological station was installed over turf, near the lower part of the Paretaio vineyard. Air temperature, relative humidity, solar radiation, leaf temperature and leaf wetness were measured outside and under tunnels to evaluate potential effects of shading on canopy microclimate.

Air temperature and relative humidity were measured by Hobo thermometric microstations (Onset Computer Corporation; thermistor resolution of 0.02 °C and precision of 0.1 °C). UVA (400-315 nm), UVB (320–280 nm), PAR (photosynthetic active radiation) and global radiation (composed by the direct component of sunlight and the diffuse component of skylight) were measured using a double monochromator spectroradiometer (model SR9910-PC, Macam Photometric Ltd, Livingstone, Scotland) with a 100 mm focal length, a spectral range of 200-800 nm, a wavelength accuracy of 0.5 nm and equipped with a diffuser connected by a 1.8 m long optical fiber to the input slit of the monochromator. The diffuser error associated with the cosine response was less than ~3% for a solar zenith angle up to 70°. The spectroradiometer, which has an operating ambient

temperature of +10 °C/+40 °C was calibrated in March 2004 by the manufacturer and then periodically verified using a SR 903 standard unit (Macam Photometrics Ltd, Livingstone, Scotland). Leaf temperature was measured using an handheld infrared thermometer (Everest interscience inc., model 100.3ZL) on both sides of the rows.

Leaf wetness was estimated by visual observations repeated every half hour during the night of 10th of July from sunset till sunshine. Epidermal polyphenols (EPhen) were optically estimated in situ using a portable leaf-clip device, the dualex (Force-A, Orsay, France; Goulas et al., 2004; Cartelat et al., 2005). The instrument determines the epidermal absorbance in the UV-A, mainly due to flavonoids, by comparing the chlorophyll fluorescence (ChlF) signals at two different excitation wavelengths (375 and 650 nm). In order to limit variability in EPhen due to leaf age, for each branch only two fully expanded leaves positioned just above the relative bunches were considered.

The chosen leaves for such measure were the 5th and the 6th leaf of the vine-shoot. The measure was carried out on 40 leaves per parcel (2 leaves on 4 vine-shoots on 5 plants per parcel). Two adaxial and two abaxial measurements were recorded in sequence from the middle part of the leaf avoiding the main vascular bundles. EPhen content of single leaves was defined as the sum of the adaxial and abaxial values, and these were the average of the two measurements taken from each side. EPhen was expressed in absorbance units as directly given by the dualex.

Leaves located at the low-middle part of the shoots (between the 10th and 15th from the base) were inoculated on the 20th of July by spraying their adaxial and abaxial surfaces with a sporangial suspension of *P. viticola* and then enclosed in polyethylene bags for 12 h during the night. The sporangial suspension contained 1.6 10^3 sporangia ml^{-1}. Severity of downy mildew infection was measured by visual observations as percentage of infected leaf area 18 days after inoculation.

Results and discussion

Temperature and relative humidity

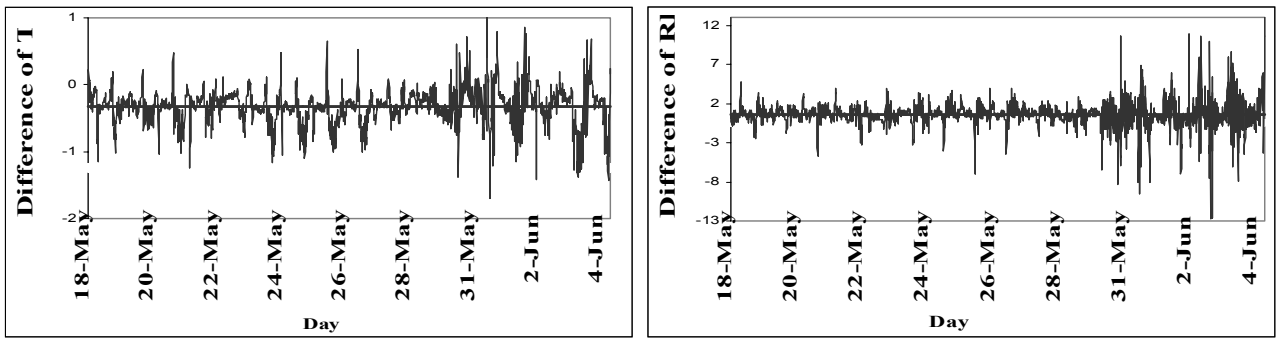


Figure 3: differences in air temperature (T) and relative humidity (RH) between full light and tunnel environments.

Hobo thermometric microstations recorded data every 10 minutes for the complete duration of the experiment. Figure 3 shows the results of the monitoring in terms of differences in air temperature and relative humidity between sun and shade sites. There are minimal differences between the two treatments and the maximum differences were recorded at dawn and sunset.

Radiation

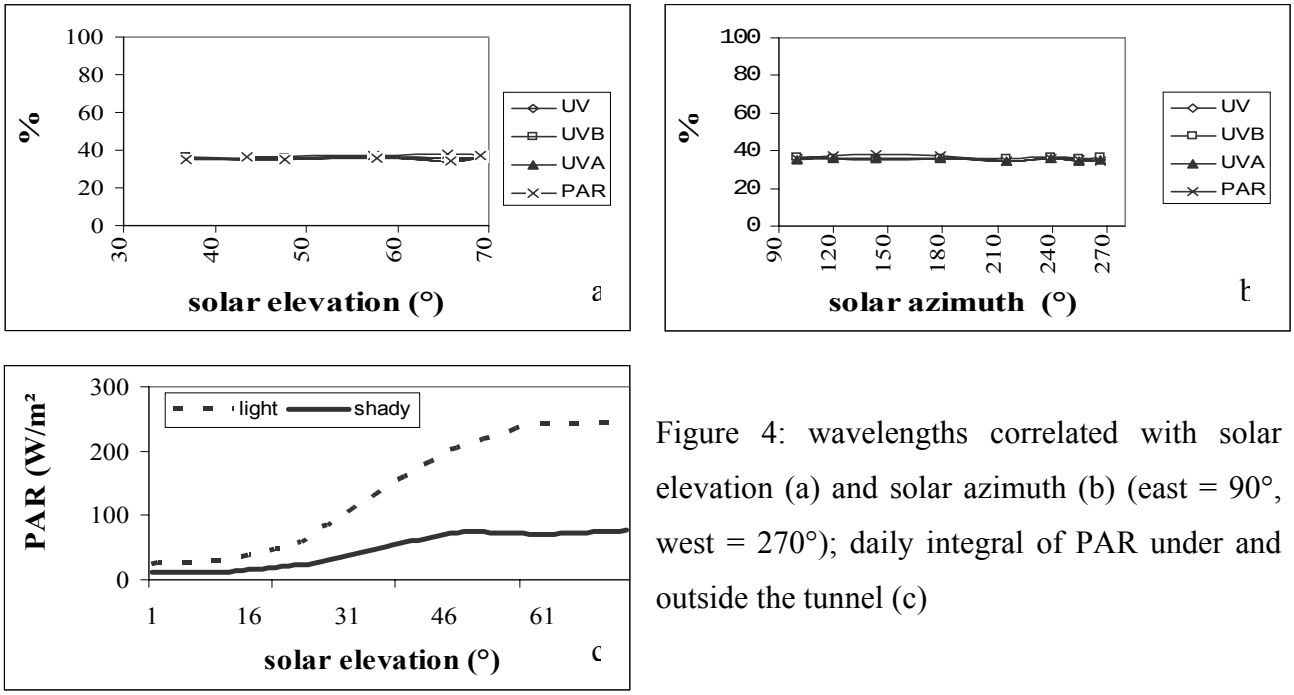
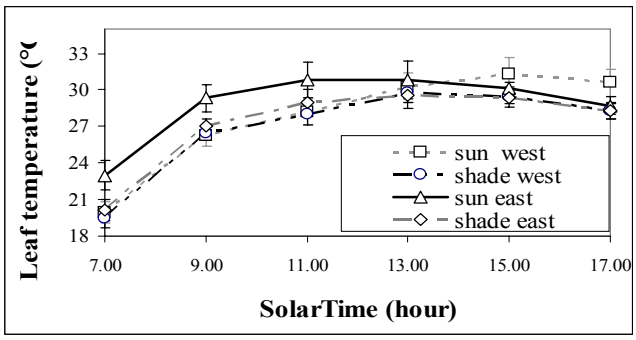


Figure 4: wavelengths correlated with solar elevation (a) and solar azimuth (b) (east = 90°, west = 270°); daily integral of PAR under and outside the tunnel (c)

Using the spectroradiometer measurements with the sensor set on a horizontal plane we have verified that during the day (and therefore at various solar elevations and azimuth), the percentage of solar radiation under the tunnels was approximately 36% of direct light (figure 4). Moreover it is possible to notice that such percentage is almost the same for all the value solar radiation visible (PAR) and not visible (UV). The constancy in the shading relative level with solar elevation, permits to apply such a value to the entire period of the measurement campaign. The trend of the wavelengths with the sensor facing east and west showed differences in percentage in the shady condition only on the PAR. Calculating the values of daily integral, the levels of daily PAR under the tunnel were approximately constant (70% of the full light value, figure 4c). This percentage seems not to change into the various solar elevations and therefore to be independent from the period of the year.

Leaf temperature

As expected, the temperature is greater from the 11:00 to 14:30 when the solar radiation is greater and in the case that the leaves were exposed to the direct solar radiation (figure 5).



However the range of observed differences of temperature cannot be considered responsible of modification in infection intensity of downy mildew.

Figure 5: diurnal change in temperature (°C) of t leaves west and east oriented.

Leaf wetness

During observation of night, leaf wetness was not noticed under the tunnels, whereas outside the tunnels wetting was observed from 2:30 a.m. to 7:30 a.m. The survey was repeated every half of hour. This evidence can be explained as due to a mechanical barrier of the net.

Polyphenols

The values of the polyphenols contained in the leaves are clearly greater in the sunny parcel. In fact, as it is well-known the UVB solar radiation stimulate the production EPhen (figure 6). For both sun and shade conditions there is a significant variation with time of EPhen ($p < 0.0005$, one-way ANOVA), however, in sun leaves there is a tendency to increase the EPhen content from the beginning to the end of the monitoring period. This is also evidenced by the trend in the sun/shade ratio. It is worth noting that the maximum in EPhen was found at DOY 171 corresponding to the maximal annual solar irradiance. The average value of EPhen for sun leaves at the end of the monitored period was about 70% higher than that for shade leaves. This result is in accordance with the radiation-induced synthesis of polyphenols (Kolb et al. 2001).

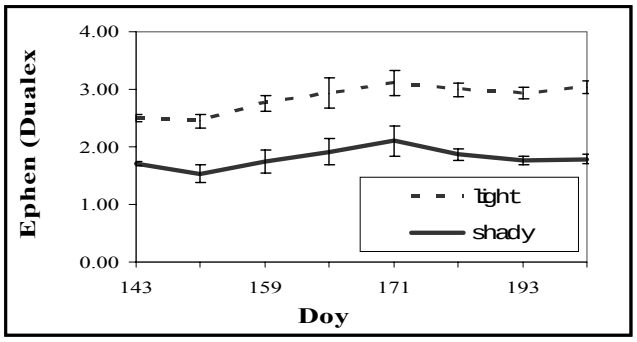


Figure 6: time evolution of Ephen (expressed as Dualex value) in sun and shade leaves.

Downy mildew infection

Seventy-two leaves (36 sun and 36 shade leaves) located at the low-middle part of the shoots (between the 10th and 15th from the base) were chosen to test plant resistance to downy mildew in relation to the light regime. The average value of EPhen in sun and shade leaves before inoculation was 2.44 (STD = 0.29) and 1.37 (STD = 0.21), respectively. Severity of downy mildew infection was larger ($p<0.0005$) in shade leaves with respect to sun leaves, being the attacked leaf area 15.7 ± 8.4 (average ± STD) and 5.0 ± 3.6, respectively (figure 7). Our results indicate that susceptibility towards downy mildew infection in grapevines under full solar radiation is lower than in shaded

(35% of solar radiation) plants. Accordingly, viticultural practices aimed to improve plant irradiances should be adopted. Canopy microclimate under the two experimental light regimes was similar and, therefore, it can not be considered responsible for the different development of the downy mildew disease. It is likely that the higher resistance observed in sun exposed versus shade leaves come from the higher content in polyphenols, which are known to possess antifungal properties (Harborne and Williams, 2000). However, since differences in the leaf dry mass per area ratio between sun and shade leaves were observed, a contribution of the morphology to the light-induced to fungal infection can not be ruled out.

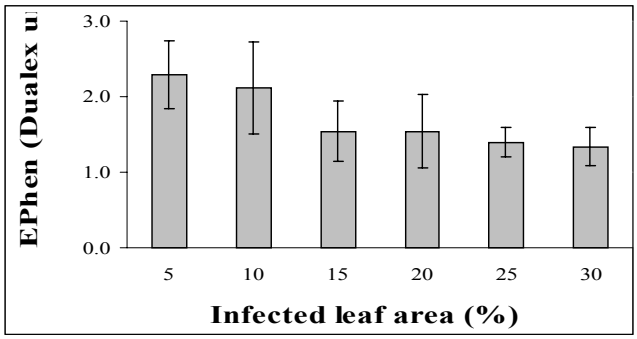


Figure 7: relationship between Ephen and severity of downy mildew infection.

Conclusions

The results show that solar radiation affects downy mildew susceptibility of grapevine likely by increasing the content of leaf polyphenols. For this reason knowledge about radiative conditions in the vineyard could provide a spin off for agricultural activities. This information could allow to improve management practices (pruning techniques, row orientation, etc.) to obtain a natural enhancement of constitutive defences (such as polyphenols) against diseases. The non destructive monitoring of leaf polyphenols may be a useful tool for disease prediction.

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