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Nitrogen and ultraviolet radiation modify grapevines' susceptibility to powdery mildew

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Summary

Potted Cabernet Sauvignon and Chardonnay grapevines were grown in southeastern Australia under either ambient or reduced ultraviolet (UV) radiation to test the effect of UV on powdery mildew (*Uncinula necator*) susceptibility. Diacetate films were used to screen out UV-B (280-315 nm) and UV-A (315-400 nm). Two nitrogen treatments (0 or 3 g N per plant) were applied at bloom to test interactions of nitrogen status with UV radiation. Cabernet Sauvignon was much less susceptible to *U. necator* and also responded less to UV and nitrogen. *U. necator* incidence and severity on leaves were increased dramatically when high nitrogen supply was combined with low UV, particularly in Chardonnay. The differences in infection were not due to variation in canopy microclimate (temperature and humidity) caused by experimental treatments. However, high disease susceptibility in response to high nitrogen status and low UV radiation was related to low concentrations of constitutive phenolic compounds (flavonol glycosides and, to a lesser extent, hydroxy-cinnamic acid derivatives), high leaf-nitrogen status and photosynthetic rates, high succulence, and reduced cuticular wax deposition. No stilbene phytoalexins could be detected following infection, suggesting that stilbenes are not involved in *U. necator* resistance in grapevines. These results show that UV radiation affects disease susceptibility of grapevine cultivars and that this susceptibility is modulated by nitrogen supply.

K e y w o r d s : biotroph; disease resistance; flavonoids; stilbenes; genotype; *Vitis vinifera*.

Introduction

Ultraviolet (UV)-B is a component of the 'ambient' light environment and also is strongly attenuated within plant canopies (Caldwell *et al*. 1983; Paul 2001). UV-B radiation on earth varies strongly with latitude, altitude, season, and time of day. In addition, UV-B is increasing at earth's surface due to the depletion of stratospheric ozone (O_3), although these processes are thought to be close to their maximum (McKenzie *et al*. 1999). The increase in UV-B is particularly important for Australia, because UV radiation in the Southern Hemisphere historically has been 10-15 % higher than in the Northern Hemisphere. Moreover, ozone depletion in the south occurs year-round, whereas in the north the depletion is less severe in summer, when solar UV radiation is most intense. Nevertheless, the natural variation in UV radiation greatly exceeds the increase of UV-B due to ozone depletion (Rozema *et al*. 1997). Within a given geographic region the greatest variability in surface UV radiation is due to cloud cover (Lubin *et al.* 1998). Thus it is important to assess the effects of ambient variation in UV-B.

We have a very poor understanding of the influence of UV radiation on plant-pathogen interactions (Paul 2000). High UV could change rates of pathogen development, modify host resistance, and alter the physiology of host-pathogen interactions (Manning and von Tiedemann 1995; Paul *et al*. 1997). UV-B has been reported to affect fungi both negatively (inhibition of spore germination and mycelial growth) and positively (stimulation of growth and sporulation) (Paul *et al*. 1997). In a recent review Krupa *et al*. (1998) discussed a possible decrease in the incidence of fungal diseases due to increased UV. However, the available evidence suggests that the impact of UV on disease development depends on crop species, cultivar, age, affected plant organ, pathogen type, inoculum level, and the timing and duration of UV exposure (Krupa *et al*. 1998; Paul 2000). Reduced photosynthesis and premature senescence could result in a decrease in diseases caused by biotrophs such as *Uncinula necator* [Schwein.] Burr., and an increase in those caused by necrotrophs, such as *Botrytis cinerea* Pers.:Fr. Manning and von Tiedemann (1995) argued that the main effects of elevated UV-B on plant diseases would be via alterations in host plants; for example increased secondary metabolites such as flavonoids could lead to improved disease resistance. Flavonoids may increase as UV-B increases (Rozema *et al*. 1997; Jansen *et al*. 1998), but this is not invariably the case, and there is only limited evidence that such changes have significant effects on disease incidence. There is no specific evidence that UV-B enhances plant resistance to fungal pathogens (Paul *et al*. 1997).

U. necator is one of the most ubiquitous pathogens of grapevines. It can develop on all green plant parts. The powdery mildew fungus develops on both the upper and lower surface of leaves, but thrives in shade and often develops in the interior of dense canopies (Nicholas *et al*. 1998). Willocquet *et al*. (1997) recently found that UV-B inhibited both spore germination and mycelial growth of *U. necator*

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on grapevine leaves. However, their study did not allow them to determine if the effect was directly on the fungus, on the leaves, or both. Moreover, little is known about the activation of defense responses during infection. The involvement of phenolic phytoalexins (stilbenes) in the resistance of grapevine leaves to two other major pathogens, *B. cinerea* and *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni, is well documented (DERCKS *et al*. 1995), but there is no evidence that stilbenes are a factor in the resistance to *U. necator*. We do not even know if stilbenes accumulate in the leaves following infection. Only an indirect correlation between stilbenes and *U. necator* resistance has been reported (BAVARESCO and EIBACH 1987). If stilbenes are indeed involved in *U. necator* resistance, then UV exposure could potentially reduce resistance due to the need for accumulation of UV-protective flavonoids, which might lead to competition for substrates between the two key enzymes, chalcone synthase and stilbene synthase (PAUL 2000). To test this hypothesis, we conducted an experiment with two cultivars of intact grapevines under natural conditions. The plants were exposed to two levels of UV radiation, combined with two levels of nitrogen (N) nutrition to test the effects of genotype by environment interactions on powdery mildew development and vine response.

Material and Methods

Plant material and experimental conditions: Two-year-old, own-rooted grapevines (*Vitis vinifera* L.) cvs Cabernet Sauvignon (clone 125) and Chardonnay (clone V3) were grown in 22-l pots outdoors under either ambient (+UV) or reduced (–UV) UV in Wagga Wagga, New South Wales, Australia, during the late 1998 growing season. The potting mix consisted of equal proportions of loam, sand, and composted humus, plus 10 % (v/v) cocopeat. Vines were arranged in north-south oriented rows spaced 1 m apart, and grown with three shoots bearing two clusters each. Shoots were trained vertically for uniform light exposure, and no fungicides were applied. Budbreak occurred on 3 March, the first flowers opened on 25 March, full bloom (100 % capfall) was between 2 and 8 April, and the end of bloom (most stamen necrotic or dropped) occurred on 12 April. UV was reduced with diacetate films (Triphan UV, Lonza-Folien, Weil am Rhein, Germany) suspended parallel with the ground, approximately 50 cm above the top of the vines and extending 1 m beyond the northern end of the rows, so that all sides of the vines were exposed to free air movement. Light transmission of the UV absorbing film, as determined by SCHULTZ (2000), was approximately 2 % for both UV-B (280 to 315 nm) and UV-A (315 to 400 nm), and 93 % for photosynthetically active radiation (PAR, 400 to 700 nm) and far-red to near-infrared (tested to 1100 nm). The two rainfall events (2 mm on 5 March and 0.2 mm on 10 April) that occurred on exposed vines during the experimental period were simulated simultaneously under the UV-blocking plastic by pressure-sprayer application of deionized water that had been left standing in an open glass container to reduce the pH to that of rainwater (measured pH 5.7) by absorption of atmospheric CO_2. Two N treatments were imposed to test potential interactions of plant N status with UV radiation. Nitrogen was applied as NH_4NO_3 three times during bloom, amounting to 0 (–N) or 3 (+N) g N per vine in addition to N available from the potting mix. These treatments previously had been found to result in large differences in N uptake in potted grapevines, leading to a range of leaf-N contents similar to field-grown vines (KELLER *et al*. 1995). All treatment combinations (cultivar *x* UV *x* N) were replicated with six individual plants randomly selected within rows.

Environmental conditions and leaf gas exchange: Solar radiation (daily global irradiance) data were obtained from the Commonwealth Bureau of Meteorology's Wagga Wagga weather station (Station Number 72150, 35.16 °S, 147.46 ºE, elevation 212 m). As direct measurements of plant-effective UV exposure (COOHILL 1989) were not available, UV radiation (daily total erythemal exposure) data were obtained from the NASA Goddard Space Flight Center's Earth Probe TOMS (Total Ozone Mapping Spectrometer). UV radiation also was measured using a portable radiometer (VLX-3W; Vilber Lourmat, Marne-la-Vallée, France) with silicon photo-electric cell sensors with a half bandwidth of 12 ± 2 nm for UV-B (312 nm) and UV-A (365 nm). Values reached approximately 3.5 W m^{-2} (UV-B) and 20 W m^{-2} (UV-A) at midday.

To assess the potential effects of the diacetate film screens and N supply on canopy microclimate, micrometeorological measurements were recorded. Air temperatures above 31 ºC (GADOURY and PEARSON 1990) and relative humidities below 40 % (PEARSON and GOHEEN 1998) have been found to inhibit *U. necator* infection. Transpiration can significantly increase humidity adjacent to leaves, creating a more favorable microclimate for *U. necator*. Air temperature and relative humidity above the canopy, leaf temperature and relative humidity at the lower leaf surface, and photosynthetic photon flux (PPF) were measured along with leaf gas-exchange (photosynthesis and transpiration), using an LCA-4 system (Analytical Development Company, Hoddesdon, Herts, England). A thermocouple was connected to the leaf chamber for the determination of leaf temperature. Measurements were conducted weekly between 10.00 and 13.00 hrs under clear-sky conditions (PPF in excess of 1000 µmol m^{-2} s^{-1}).

Disease and leaf properties: Nearby vineyards provided abundant inoculum for a natural *U. necator* infection to occur towards the end of bloom. Incidence (% leaves with at least one lesion) and severity (mean number of lesions per leaf or per surface area) were assessed on 11 April by counting the number of lesions on the underside of each leaf on one shoot per plant. *U. necator* can develop on both leaf surfaces, and visual assessment confirmed that disease levels were similar on both surfaces. However, leaves transmit only a negligible fraction of UV (CALDWELL *et al.* 1983). Because we were interested primarily in plant responses to UV and their effect on disease expression, we monitored only the abaxial surface, which was protected from direct exposure to UV. This approach minimized UV effects on the pathogen *per se*. Following the assessment of *U. necator* incidence and severity, fresh disks (4.02 cm^2) from mature leaves were used to measure relative

water content (RWC) as described by PATAKAS *et al*. (1997). Additional disks taken from the same leaves were frozen in liquid N and stored at -80 °C for chlorophyll determination. Chlorophyll was extracted at 5 °C overnight with 100 ml g^{-1} aqueous acetone (80 % v/v) with 0.5 % (v/v) concentrated NH_3, and measured with a UV-265 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) as described by SCHOPFER (1989). Leaves 5 (old) and 11 (young) from the shoot base were removed, frozen in liquid N, and stored at -80 °C for analysis of stilbenes, flavonoids, and cuticular wax. Phenolic compounds were extracted from 20 to 30 mg leaf tissue with 1 ml ethyl acetate and analyzed by HPLC as described previously (KELLER *et al*. 2000). Cuticular wax was quantified gravimetrically on chloroform extracts (1 h) of leaf disks and observed using scanning electron microscopy (SEM). Leaf disks were prepared for SEM by immersing them in liquid N, freeze drying, attaching to aluminium stubs, and sputter-coating with gold at 0.05 mb and 25 mA for 3 min.

Statistical analysis: The Statistica software package (StatSoft, Tulsa, OK, USA) was used for statistical data analysis. Results were tested using factorial analysis of variance (ANOVA) and *F* test. Duncan's multiple range test was used for *post-hoc* comparisons of means where appropriate. Selected parameters also were subjected to product-moment (linear) correlation analysis. Curves were fitted using the distance-weighted least squares method.

Results

Disease expression: The natural *U. necator* infection was relatively uniform within treatments with no apparent spatial gradient, indicating that there was no effect of wind direction or vine position other than treatment effects. All treatment factors, however, had a highly significant effect on both incidence and severity of powdery mildew on grapevine foliage (Fig. 1). Chardonnay was much more susceptible than Cabernet Sauvignon, with incidence 31 % higher and severity three times higher. Moreover, both UV and N influenced the cultivar response, indicating significant genotype by environment interactions. Nitrogen markedly increased both incidence (+64 %) and severity (+205 %), especially in Chardonnay. UV screening also strongly increased incidence (+63 %) and severity (+240 %) compared with ambient conditions (Fig. 1). Therefore, low UV in combination with high N supply additively favored disease development by increasing both the number of infected leaves and the number of colonies on each of the affected leaves.

UV exposure and canopy microclimate: Both daily global irradiance and daily total UV exposure gradually decreased during the course of the experiment due to decreasing daylength and increasing solar zenith angle (Fig. 2). Ambient global irradiance, which was measured at 2 m, correlated very closely ($r = 0.93$, $P < 0.001$) with incident UV as estimated from satellite data. The UV-protective film absorbed virtually all UV while only marginally reducing total solar radiation (Fig. 2). Air and leaf temperatures were closely correlated, regardless of cultivar and treatment ($r = 0.98$, $P < 0.001$). Leaf temperature was generally 1-2 ºC above air temperature in the lower temperature range, but the two converged as temperature increased. Mean leaf temperature at midday did not exceed 32 ºC, although individual values varied from 21.6 to 35.3 ºC. Of the temperature measurements taken during the course of the experiment (mean ± standard error = 29.5 ± 0.1 ºC), 53 % were in the range 20 to 30 ºC and 44 % in the range 30 to 35 ºC. All of the temperatures above 35 ºC occurred at least two weeks before the disease outbreak. Neither N nor UV had a significant effect on leaf temperature (Tab. 1).

Compared to the temperature data, the correlation between the relative humidity above the vine canopy and that

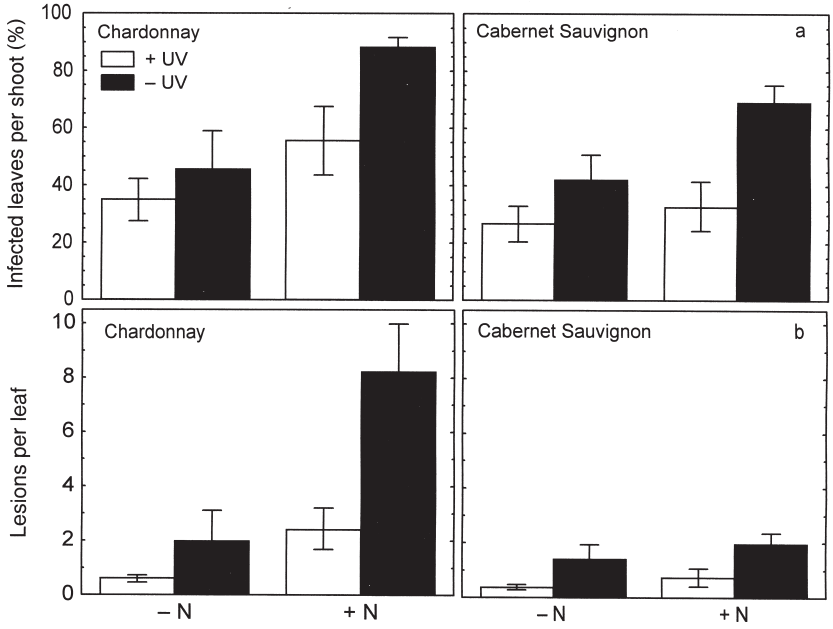

Fig. 1: Incidence (**a**) and severity (**b**) of *U. necator* infection on grapevine leaves as affected by cultivar, UV radiation, and N availability (means ± standard errors). ANOVA effects, a: cultivar $P = 0.035$; UV $P < 0.001$; N $P < 0.001$; b: cultivar $P < 0.001$; UV $P < 0.001$; N $P < 0.001$; interactions: cultivar *x* UV $P = 0.043$; cultivar *x* N $P = 0.004$.

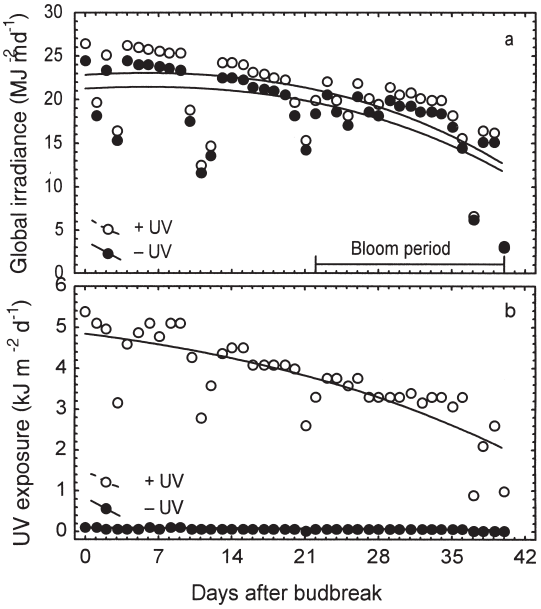

Fig. 2: Daily global irradiance (**a**) and daily total erythemal UV exposure (**b**) in Wagga Wagga, NSW, Australia. Grapevines were grown with (+) and without (–) UV radiation, using diacetate films suspended above the vine canopy. The bloom period lasted from 22 to 40 d after budbreak and *U. necator* infection was assessed 39 d after budbreak.

adjacent to the leaves was not as high ($r = 0.78$, $P < 0.001$) due to the modulating influence of transpiration. The majority (71 %) of values above the vine canopy was below 40 % relative humidity, while all of the values around the leaves were above 40 %. The humidity adjacent to the leaves was unaffected by cultivar, UV, and N (Tab. 1), indicating that the presence of the UV screen and N supply did not change the canopy microclimate.

Leaf properties: Cabernet Sauvignon leaves contained 62 % more total chlorophyll than Chardonnay leaves. Nitrogen addition strongly increased chlorophyll content in both cultivars, but UV had no effect on leaf chlorophyll (Tab. 2). Despite their lower chlorophyll content, Chardonnay leaves photosynthesized at a significantly higher rate compared with Cabernet Sauvignon (Tab. 2), suggesting that the latter has a lower photosynthetic efficiency. Photosynthesis also was enhanced by N supply, but there was no significant response to UV. Neither the cultivar nor applied N or UV affected the leaves' transpiration rate (Tab. 2), confirming that humidity was not altered due to experimental treatments.

No stilbenes could be detected in any leaf material, whether healthy or infected (Tab. 3). Using larger quantities of sample material, we subsequently did find *trans*-piceid at concentrations ranging from 20 to 60 µg g^{-1} fresh weight in very severely infected Chardonnay leaves, but no other stilbene derivatives were detected in these leaves. However, -UV strongly reduced the concentration of flavonol-glycosides (particularly glycosylated quercetin derivatives) in leaves, while not affecting hydroxy-cinnamic acid derivatives (mainly caffeyl and *p*-coumaryl tartaric acids) (Tab. 3). High N markedly decreased the concentration of all phenolics, exacerbating the -UV effect. Moreover, the significant UV *x* N interaction reflects the fact that the UV effect was more pronounced under –N than under +N. Young Cabernet Sauvignon (but not Chardonnay) leaves had significantly higher concentrations of soluble phenolic compounds than old leaves, although overall the two cultivars had comparable levels of leaf phenolics (Tab. 3).

Leaf surfaces in the +UV treatment became distinctly glossy during the experiment, while leaves under -UV remained mat. This was related to variation in the amount and deposition pattern of cuticular wax. There was a strong and significant ($P < 0.001$) cultivar effect on the amount of wax regardless of treatment (Fig. 3). Cabernet Sauvignon leaves contained 24 % more wax (409 µg cm^{-2}) than Chardonnay leaves (330 µg cm^{-2}). Vines exposed to ambient UV also had significantly ($P = 0.004$) more (+22 %) cuticular wax than the –UV vines, but there was no influence ($P = 0.439$) of N availability on cuticular wax. Moreover, SEM revealed an increase in epicuticular wax platelets on both the upper and lower

Table 1

Microclimatic variables for two grape cultivars as affected by UV radiation and N availability. Values are means ± standard error (n = 6) averaged from measurements over the three weeks preceding *U. necator* monitoring. Interactions were not significant, therefore only main effects are shown. ns = not significant

Treatment[a]	Air temperature above canopy (ºC)	Leaf temperature (ºC)	Rel. humidity above canopy (%)	Rel. humidity below leaf (%)
Cultivar	ns	ns	ns	ns
Chardonnay	28.8 ± 0.2	29.7 ± 0.2	34.0 ± 0.5	62.4 ± 0.5
Cabernet Sauvignon	28.3 ± 0.4	29.3 ± 0.3	35.1 ± 0.9	61.6 ± 0.7
UV radiation	ns	ns	ns	ns
Ambient (+UV)	28.4 ± 0.3	29.6 ± 0.3	34.8 ± 0.7	63.2 ± 0.6
Reduced (–UV)	28.7 ± 0.3	29.5 ± 0.3	34.3 ± 0.7	60.7 ± 0.6
Nitrogen	ns	ns	ns	ns
High (+N)	28.3 ± 0.3	29.4 ± 0.3	34.0 ± 0.8	62.1 ± 0.7
Low (–N)	28.9 ± 0.3	29.6 ± 0.3	35.1 ± 0.6	61.9 ± 0.5

[a] Ambient (+UV) or 2 % (-UV) of ambient UV radiation and 3 g (+N) or 0 g (-N) N per plant.

Table 2

Chlorophyll concentration and gas exchange of mature leaves of two grape cultivars as affected by UV radiation and N availability. Values are means ± standard error (n = 6), gas-exchange data were averaged from measurements over the three weeks preceding *U. necator* monitoring. Interactions were not significant, therefore only main effects are shown. ns = not significant; *** = $P < 0.001$

Treatment[a]	Chlorophyll$_{a+b}$ (mg g^{-1} fw)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})
Cultivar	***	***	ns
Chardonnay	1.09 ± 0.22	14.3 ± 0.2	4.6 ± 0.1
Cabernet Sauvignon	1.77 ± 0.21	12.9 ± 0.2	4.3 ± 0.1
UV radiation	ns	ns	ns
Ambient (+UV)	1.49 ± 0.23	13.7 ± 0.2	4.6 ± 0.1
Reduced (–UV)	1.38 ± 0.24	13.5 ± 0.2	4.3 ± 0.1
Nitrogen	***	***	ns
High (+N)	2.07 ± 0.17	14.1 ± 0.2	4.3 ± 0.1
Low (–N)	0.80 ± 0.11	13.1 ± 0.2	4.6 ± 0.1

[a] see Tab. 1.

Table 3

Phenolic compounds in mature leaves of two grape cultivars as affected by UV radiation and N availability. Values are means ± standard error (n = 6). Main effects and significant interactions are shown. ns = not significant; * = $P < 0.05$; *** = $P < 0.001$; values followed by the same letter do not differ significantly

Treatment[a]	Flavonol glycosides (mg g^{-1} fw)	Cinnamic acid derivatives (mg g^{-1} fw)	Stilbene derivatives (µg g^{-1} fw)
Cultivar	ns	ns	
Chardonnay	1.16 ± 0.10	0.35 ± 0.03	not detected
Cabernet Sauvignon	1.07 ± 0.07	0.36 ± 0.02	not detected
UV radiation	***	ns	
Ambient (+UV)	1.31 ± 0.09	0.35 ± 0.03	not detected
Reduced (–UV)	0.92 ± 0.06	0.36 ± 0.03	not detected
Nitrogen	***	***	
High (+N)	0.86 ± 0.06	0.29 ± 0.02	not detected
Low (–N)	1.38 ± 0.07	0.43 ± 0.02	not detected
Leaf age	*	ns	
Old	1.04 ± 0.09	0.36 ± 0.02	not detected
Young	1.20 ± 0.08	0.36 ± 0.03	not detected
UV *x* N	*	ns	
+UV/+N	0.98 ± 0.06 b		
+UV/–N	1.65 ± 0.08 a		
–UV/+N	0.74 ± 0.09 c		
–UV/–N	1.11 ± 0.05 b		
Cultivar *x* leaf-age	*	*	
Chardonnay/old	1.16 ± 0.13 a	0.39 ± 0.03 a	
Chardonnay/young	1.17 ± 0.16 a	0.32 ± 0.05 b	
Cabernet/old	0.91 ± 0.11 b	0.33 ± 0.03 b	
Cabernet/young	1.22 ± 0.07 a	0.40 ± 0.04 a	

[a] see Tab. 1.

surfaces of mature Chardonnay (but not Cabernet Sauvignon) leaves grown in +UV (Fig. 4). However, no wax platelets were detected on young leaves near the shoot tip. Leaf RWC was 7 % higher for Chardonnay than for Cabernet Sauvignon ($P < 0.001$) and also was slightly higher in -UV compared with +UV ($P = 0.004$), but N ($P = 0.646$) and leaf age ($P = 0.113$) did not affect RWC. There were significant interactions between cultivar and UV ($P = 0.049$), and cultivar

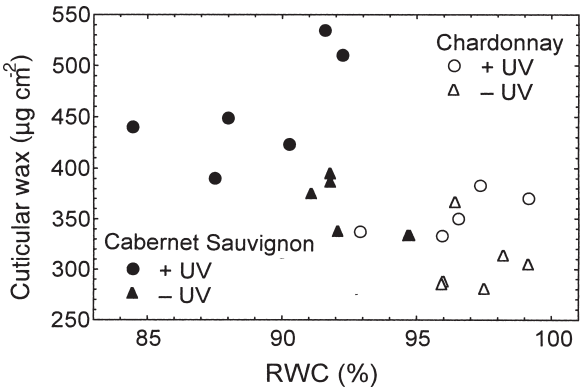

Fig. 3: Relationship between leaf RWC (relative water content) and cuticular wax on leaves of two grape cultivars as affected by UV radiation (r = -0.65, $P < 0.001$).

and N ($P = 0.015$), indicating that the treatment response of Cabernet Sauvignon was more pronounced than that of Chardonnay. Accordingly, RWC correlated negatively with the amount of cuticular wax (Fig. 3).

Discussion

The course of solar UV exposure during the present study followed the same trend as the mean of the period 1993 to 1997 reported for Melbourne, Australia (UDELHOFEN *et al*. 2000), although it was at a slightly higher level in this study. These UV exposures also correspond well with recent (1999) summertime values for latitudes between 40º and 50º North (MCKENZIE *et al*. 2000). This indicates that, although the experiment was conducted during the late Australian summer, UV exposure was similar to current midsummer values in areas where the majority of the world's vineyards are located.

Powdery mildew susceptibility of two major winegrape cultivars was differentially affected by UV radiation and N nutrition. The high susceptibility of Chardonnay is generally accepted, whereas the behavior of Cabernet Sauvignon has been more variable (DOSTER and SCHNATHORST 1985). Visual assessment of berry infection in the subsequent season confirmed the cultivar difference, with Chardonnay berries being much more severely infected, especially under -UV (unpublished data). However, low UV in combination with high N supply additively favored disease development in both cultivars. Unfortunately it is difficult to separate direct effects of UV on the fungus from indirect effects via its influence on leaf morphology and physiology, because as an obligate biotroph *U. necator* cannot be grown in the absence of living tissue. An effect of UV on the fungus *per se* would be expected on the adaxial leaf surface (WILLOCQUET *et al*. 1997). However, the highly significant UV effect (in addition to cultivar and N effects) on disease severity on

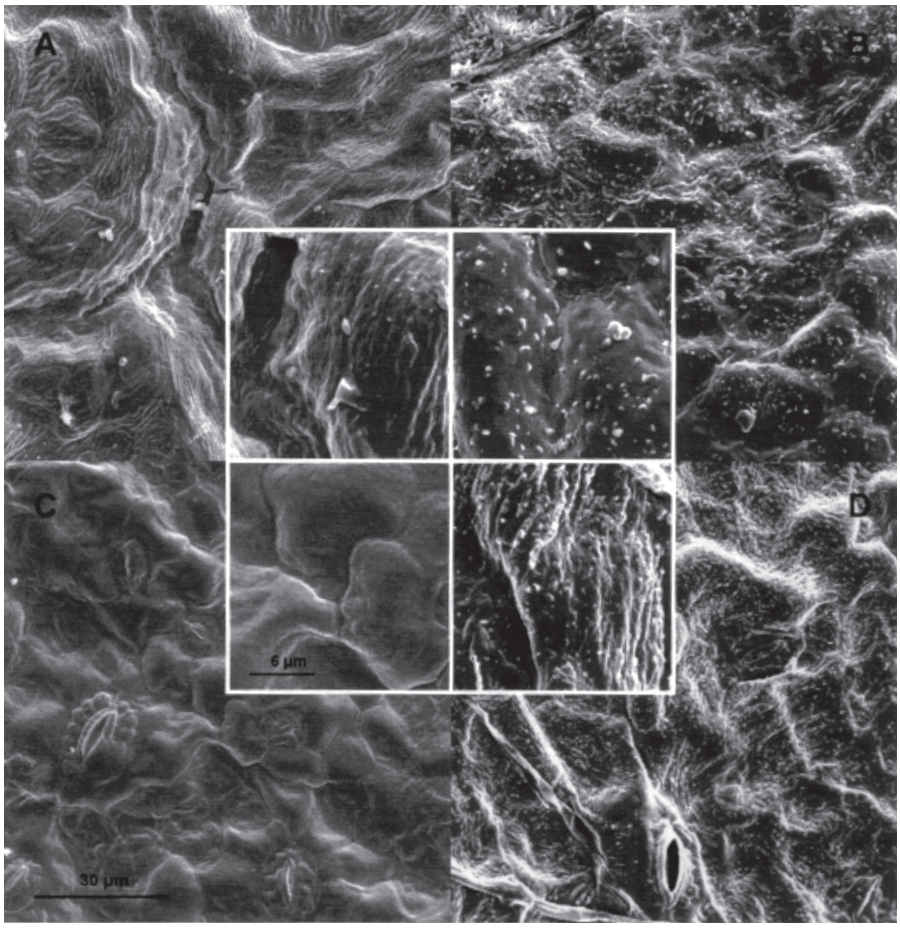

Fig. 4: Differences in cuticular wax deposition on the adaxial (**A**, **B**) and abaxial (**C**, **D**) leaf surfaces of grapevine cv. Chardonnay grown with (**B**, **D**) and without (**A**, **C**) UV radiation. Scanning electron micrographs were taken at 1000 *x* magnification, inserts at 5000 *x* magnification.

the abaxial leaf surface, which was protected from direct UV exposure, suggests that differences in infection were largely due to physiological responses of the plant rather than to direct effects on the fungus. Moreover, the diacetate films did not affect leaf temperature in the present study, and thus the UV effect was not due to temperature differences. Treatment-induced humidity differences also were a concern but, because humidity was, if anything, slightly lower under -UV, one should have expected lower disease severity due to the presence of the films, rather than the reverse. Leaf temperature and relative humidity also were unaffected by applied N. Hence, treatment effects on disease expression were not due to differences in canopy microclimate induced by UV screens or N supply.

Differences in photosynthesis due to differences between cultivars and leaf N status may have contributed to some of the observed variation in powdery mildew infection. Biotrophic pathogens, including *U. necator*, depend on the living host plant for carbohydrate supply and can act as sinks altering carbon distribution in the host to divert assimilates for their own metabolism (Hall and Williams 2000). Powdery mildew-infected leaves generally have increased sugar concentration due to import from uninfected tissue (Brem *et al*. 1986). Thus high photosynthetic rates prior to the arrival of *U. necator* spores could predispose leaves to infection by improving carbon availability for the fungus. It would be interesting to test if the low crop yields currently 'en vogue' for premium wine production also lead to an increase in powdery mildew incidence in the field.

Despite its effect on photosynthesis, the pronounced impact of N on disease expression was unexpected, as it is generally believed that N has no influence on grape powdery mildew (Champagnol 1984). Nevertheless, Bavaresco and Eibach (1987) found a positive correlation between leaf N content and the severity of *U. necator* infection for the grape cultivars Riesling and Kerner. In the present experiment leaf N content was not measured, but N generally correlates closely with chlorophyll (Evans 1989). However, while applied N strongly increased leaf chlorophyll, UV had no effect, and the more susceptible Chardonnay contained far less chlorophyll than Cabernet Sauvignon, making a direct effect of leaf N on *U. necator* unlikely. Bavaresco and Eibach (1987) hypothesized that the N effect reflected a decrease in resistance due to a reduced capacity for stilbene phytoalexin synthesis. Contrary to this study, the above authors did not measure stilbenes in the infected tissue itself, but used mucic acid as an elicitor to trigger stilbene (resveratrol) synthesis. Thus their assumption was based on correlation rather than direct evidence for a causal relationship between stilbene production and *U. necator* resistance. The present results suggest instead that stilbenes are not involved in resistance of *V. vinifera* leaves to the powdery mildew fungus. Indeed, as an obligate biotrophic fungus, *U. necator* would be expected to minimize damage to its host's cells and suppress host defense.

Constitutive soluble phenolic compounds in leaves, on the other hand, corresponded reasonably well with the variation observed in powdery mildew severity, although they did not explain the difference in cultivar susceptibility. The major soluble phenols detected were flavonol-glycosides (particularly glycosylated quercetin derivatives) and hydroxy-cinnamic acid derivatives (particularly caffeyl and *p*-coumaryl tartaric acids). Flavonols are located predominantly in the epidermis and cuticular wax, and accumulate in response to UV exposure, providing a 'sunscreen' for plant tissues (Rozema *et al*. 1997; Jansen *et al*. 1998). Powdery mildew grows on the leaf surfaces and penetrates only epidermal cells (Pearson and Goheen 1998), thus it is conceivable that enhanced synthesis of constitutive phenolic compounds due to high UV and low N contributes to an unfavorable environment for powdery mildew growth. Although this plant stress response could slow down the penetration process, it obviously would be insufficient to prevent infection entirely.

Cuticular wax measurements and SEM confirmed the difference in leaf glossiness between –UV and +UV, indicating that UV radiation enhanced wax production. Moreover, the leaves of the more susceptible Chardonnay also had less wax than Cabernet Sauvignon leaves. The production of cuticular wax, leading to scattering and reflection of radiation, also is thought to be a defense mechanism of higher plants against damage by UV-B radiation (Rozema *et al*. 1997). Though cuticular wax alone cannot have accounted for the profound differences in disease severity, the inverse relationship between cuticular wax and RWC along with the cultivar and UV effects on RWC suggest that more succulent leaves with a thinner cuticular wax cover were more susceptible to powdery mildew. Accordingly, Heintz and Blaich (1989) found a negative linear relationship between cuticle thickness of young grape leaves and *U. necator* sporulation on these leaves.

This study has demonstrated significant genotype by environment interactions. While the two cultivars tested differed considerably in their susceptibility to *U. necator*, both N nutrition and UV radiation modified the level of infection. Reduced susceptibility may be a by-product of grapevine acclimation to low nutrient availability and high UV radiation. However, while each of the factors measured may have added to the variability in disease expression, it is clear that none of these factors alone could account for all of the observed differences. Our data further suggest that UV plays an important role in the natural regulation of powdery mildew under field conditions. In addition to the reduction in visible light and temperature, and the increase in humidity caused by clouds and canopy shade, screening of UV by clouds during overcast conditions and by dense canopies may contribute to favorable conditions for *U. necator* development.

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