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ORIGINAL RESEARCH

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Weaker photosynthetic acclimation to fluctuating than to corresponding steady UVB radiation treatments in grapevines

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Abstract

The effects of transient increases in UVB radiation on plants are not well known; whether cumulative damage dominates or, alternately, an increase in photoprotection and recovery periods ameliorates any negative effects. We investigated photosynthetic capacity and metabolite accumulation of grapevines (*Vitis vinifera* Cabernet Sauvignon) in response to UVB fluctuations under four treatments: fluctuating UVB (FUV) and steady UVB radiation (SUV) at similar total biologically effective UVB dose (2.12 and 2.23 kJ m^{-2} day^{-1}), and their two respective no UVB controls. We found a greater decrease in stomatal conductance under SUV than FUV. There was no decrease in maximum yield of photosystem II (F_v/F_m) or its operational efficiency (ϕ_{PSII}) under the two UVB treatments, and F_v/F_m was higher under SUV than FUV. Photosynthetic capacity was enhanced under FUV in the light-limited region of rapid light-response curves but enhanced by SUV in the light-saturated region. Flavonol content was similarly increased by both UVB treatments. We conclude that, while both FUV and SUV effectively stimulate acclimation to UVB radiation at realistic doses, FUV confers weaker acclimation than SUV. This implies that recovery periods between transient increases in UVB radiation reduce UVB acclimation, compared to an equivalent dose of UVB provided continuously. Thus, caution is needed in interpreting the findings of experiments using steady UVB radiation treatments to infer effects in natural environments, as the stimulatory effect of steady UVB is greater than that of the equivalent fluctuating UVB.

1 | INTRODUCTION

In the natural environment, fluctuating light is more common than steady light because of clouds and because of wind causing canopy movement (Kaiser et al., 2018). Light is one of the most unstable components of plants' environment. Even at the very top of the canopy on a sunny day, steady light conditions are uncommon. Large fluctuations in irradiance (sunlight received) happen over short (less than 1 s; Assmann & Wang, 2001) and long (minutes or longer; Smith & Berry, 2013) timescales. In natural environments, plant canopies are subject to transient changes in cloudiness through the day, producing fast changes in irradiance. These changes prompt the need for mechanisms allowing physiological acclimation to these conditions (Kromdijk et al., 2016; Barnes et al., 2017; Wu et al., 2023).

In the biosphere, UV radiation is a minor portion of sunlight (Björn, 2015), and only UVA (315–400 nm) and UVB (280–315 nm) radiation at wavelengths greater than 290 nm can reach the Earth's surface. Photon irradiance of UVB radiation is equivalent to, at most, 0.33% of the photosynthetically active radiation (PAR, 400–700 nm; Aphalo, 2018). Nevertheless, UVB photons carry the most energy among those spectral regions reaching the Earth's surface. High UVB irradiance can cause detrimental effects on plants, directly or indirectly, inducing damage to DNA (Landry et al., 1997), proteins (Schmelzer et al., 1988; Willekens et al., 1994), and membranes (Britt, 1996). However, testing the effects of UVB radiation in controlled indoor conditions makes any results difficult to extrapolate to the natural environment. Plant defenses are typically well adapted to solar UVB radiation; this means that they acclimate effectively to regional increases in solar UVB radiation caused by stratospheric ozone depletion and changes in atmospheric pollution (Barnes et al., 2023). Hence, the focus of most research has shifted to identifying the role of UVB radiation in regulating plant growth and development (Hideg et al., 2013; Robson et al., 2015).

Grapevine (*Vitis vinifera* L.) is an important berry crop widely grown in many regions of the world (Anderson & Nelgen, 2020). It is a light-demanding species that grows through the whole summer (Coombe, 1995). Cabernet Sauvignon is the most common cultivar worldwide and is planted in more than 80% of countries cultivating grapevine (Anderson & Nelgen, 2020). The amount of solar radiation received by grapevines directly affects canopy photosynthesis, and its spectral quality modifies the accumulation of leaf secondary metabolites (Del-Castillo-Alonso et al., 2016; Petrie et al., 2003). Despite causing stimulation of photoprotective pigments, UVB radiation may also impede carbon fixation by grapevine at high elevations (Berli et al., 2013). This may consequently affect the source-sink balance and allocation to primary *vs* secondary metabolism (Ollat & Gaudillere, 2000). Additionally, the photoassimilate could act as a feedback control that integrates sugar production with environmental factors to regulate photosynthesis (Henry et al., 2020; Lastdrager & Smeekens, 2014).

While the response of plants to short- and long-term exposure to UVB radiation has been compared to assess the induction of photoprotection (Hazard et al., 1997; Martínez-Lüscher et al., 2013), the question of how plants respond to fluctuating UVB radiation remains unresolved. Brief exposure (< 24 h) of non-acclimated plants to UVB radiation can cause photoinhibition and damage through reactive oxygen species (Hazard et al., 1997), whereas plants subject to longer exposure (> 24 h) to UVB radiation typically accumulate UVB-absorbing compounds and display morphological adaptations to high light. These acclimations often ameliorate photoinhibition and alleviate damage caused by acute UVB radiation through the action of antioxidants (Agati et al., 2012; Martínez-Lüscher et al., 2013; Shi et al., 2004; Wargent et al., 2011; Zhao et al., 2020). The phenotype of plants under fluctuating UVB radiation is likely to be a compromise between the rapid responses and long-term acclimation to UVB radiation. It is still unclear whether acute reactions caused by cumulative transient periods of high light dominate or if the periods in-between UV exposure are utilized to relax from stress. Discovering the answer to this question is particularly relevant to field crops, which grow under naturally fluctuating UVB irradiance. A step towards more confidently inferring the role of UVB radiation in acclimation *vs*. damage to the photosynthetic apparatus under natural conditions is thus to study how plants respond to well-defined artificial fluctuations in UVB irradiance.

Combinations of UVB radiation and PAR were used here to investigate the acclimation response of grapevine leaves to fluctuating and steady UVB radiation. The fluctuating UVB radiation treatment was alternately on and off for 15-minute periods, which is within the range of sun/shade patterns from sunflecks or under broken cloud (Smith & Berry, 2013). Both steady and fluctuating UVB treatments provided the same average biologically effective UVB irradiance on a daily basis. A background treatment of PAR was provided to allow normal plant processes of growth and defence, while not being so strong that potential effects of UVB radiation might be expected to be masked by responses to PAR (Roeber et al., 2021). This experiment addresses three questions: (1) how does photosynthetic capacity differ in response to fluctuating and steady UVB radiation; (2) are pigments, sugars and starch accumulation affected differently by fluctuating and steady UVB radiation; and (3) are there differences in the relationship between photosynthesis and metabolite contents under fluctuating and steady UVB radiation?

2 | MATERIALS AND METHODS

2.1 | Plant materials and light treatments

One-year-old grapevine plants (*Vitis vinifera* L. cv Cabernet Sauvignon) grafted to 110R rootstocks (Viveros Provedo) were planted in 10 L pots containing a 7:2:2 mix of peat: sand: vermiculite. Specifically, these are F6 peat (Kekkilä); blowing sand 0.5–1.2 mm (Weber); Agra-vermiculite 0–2 mm (Pull Rhenen).

The grapevines were grown in a greenhouse (17/02/2023–23/04/2023) divided into compartments partitioned by curtains made from black-white plastic film blocking solar radiation. The greenhouse air temperature was maintained at 25°C/16°C day/night and relative humidity at 45%/75% day/night. Plants were grown under LED lamps (AP67 and AP3, Valoya Oy), giving steady photosynthetically active radiation (PAR; 170 μmol m^{-2} s^{-1}) from 05:00 to 21:00 (local time).

The UVB treatments commenced when the eighth leaf (counting from the base to the apex) of each grapevine became mature, and were administered ±3 h from 12:00 (09:00 to 15:00). The UVB treatments were as follows: (1: FUV) fluctuating UVB radiation treatment (1.115 ± 0.112 μmol m^{-2} s^{-1}), the fluctuating UVB radiation treatment was on for 15 min and then turned off for 15 min, and that the cycle was repeated during the photoperiod for six hours, and (2: cFUV) its no UVB radiation control; (3: SUV) steady UVB radiation treatment (0.508 ± 0.065 μmol m^{-2} s^{-1}) and (4: cSUV) its no UVB radiation control. Plants under both the FUV and SUV treatments received the equivalent daily integrated dose of UVB radiation: total daily photon irradiance of unweighted UVB radiation was 12.05 ± 1.21 and 10.98 ± 1.41 mmol m^{-2} day^{-1} calculated as 2.23 ± 0.25 and 2.12 ± 0.35 kJ m^{-2} day^{-1} biologically effective UVB energy irradiance (Table S1); weighted according to Green's formulation of

Caldwell's Generalized Plant Action Spectrum normalized to the same irradiance value as the unweighted measurement at 300 nm (Aphalo et al., 2012). Broadband UVB-313-nm fluorescent tubes (Q-Lab Europe, Ltd.) were used to produce four UVB radiation treatments. A polyester filter (0.125 mm thick, Autostat CT5; Thermoplast), which blocks UVB radiation up to 315 nm wavelengths, was used to create controls without UVB radiation. A cellulose-diacetate filter (0.095 mm thick, Kotelo-Rauma Oy), which blocks UV-C radiation but transmits UV radiation over 280 nm, was used to create the UVB treatments. The cellulose diacetate film was changed weekly to avoid the transmittance reduction caused by its photodegradation. UVB treatments and controls were created by wrapping filter material around half the length of each UVB tube. The two halves of each fluorescent tube were divided using a polyester filter curtain. Each treatment was replicated twice and compartmentalized using black-white plastic film; the white side always facing the plants. Three plants were grown under four light treatments in two replicate compartments (i.e., 3 plants × 4 treatments × 2 replicate blocks = in total 24 plants). The UVB irradiance was recorded (Figure S1 and Table S1) with a calibrated array spectroradiometer (Maya 2000 Pro, Ocean Optics Inc.) using a protocol devised for this purpose detailed in Robson & Aphalo (2019).

2.2 | Measurements of photosynthetic parameters

Photosynthetic parameters were measured on one leaf from each plant (on the fourth to sixth leaf from the base to the apex). Stomatal conductance (g_s) and operating efficiency of photosystem II (ϕ_{PSII}) were measured under the light treatments using a leaf porometer/fluorometer (LI-600, LI-COR). To obtain a time series through the day, measurements of g_s and ϕ_{PSII} were taken every 15 min; 5 min after each change of fluctuating UVB radiation. Two measurements were made before the UVB radiation began (at 07:30 and 08:30) and two after the end of daily UVB radiation (15:30 and 16:30), every three days from the first day after UVB treatment commenced for five measurement days in total.

Chlorophyll fluorescence fast-transient analysis (OJIP) was measured using a FluorPen FR 100 (Photon Systems Instruments (PSI)) in order to examine the dynamics of photochemistry under fluctuating light. Leaves were dark-adapted for 30 min using darkening clips, then exposed to a saturating pulse of photon irradiance 2700 µmol m^{-2} s^{-1} (peak at 470 nm wavelength). The fluorescence intensities at 50 µs (F_o), 2 ms (J-step, F_j), 60 ms (I-step, F_i) and maximum variable fluorescence at 300 ms (F_m) were used for this calculation. Relative variable fluorescence at the J-step and I-step were calculated as $V_j = (F_j - F_o)/(F_m - F_o)$ and $V_i = (F_i - F_o)/(F_m - F_o)$. The maximum quantum yield of photosystem II (F_v/F_m) was calculated as $(F_m - F_o)/F_m$. The OJIP curves were recorded three times at 08:30 (morning), 12:00 (midday) and 15:30 (afternoon) on the same days as ϕ_{PSII}.

Electron transport rate (*ETR*) and ϕ_{PSII} were recorded during rapid light-response curves (RLCs), where the actinic illumination increased stepwise from 10 to 1500 µmol photons m^{-2} s^{-1} every 30 s (mini-PAM, Walz GmbH). The *ETR* PAR-response curve fits the function of Eilers & Peeters (1988):

$$ETR = \frac{PAR}{a \times PAR^2 + b \times PAR + c} \quad (1)$$

where *ETR* and PAR are assessed by mini-PAM from the measurements; *a*, *b* and *c* are nondimensional fundamental parameters. From these, the initial slope in the light-limiting region (α), maximum electron transport rate (ETR_{max}), maximum saturating irradiance (I_k) and operating saturating irradiance (I_m) were calculated as:

$$\alpha = 1/c \quad (2)$$

$$ETR_{max} = \frac{1}{b + 2\sqrt{ac}} \quad (3)$$

$$I_k = \frac{c}{b + 2\sqrt{ac}} \quad (4)$$

$$I_m = \sqrt{\frac{c}{a}} \quad (5)$$

Measurements were carried out from 09:30 to 11:00 for each grapevine on the last day of measurements.

2.3 | Measurements of leaf pigments

Optical indices of chlorophyll content and epidermal phenolics (flavonols and anthocyanins) were non-destructively measured by Dualex Scientific + (FORCE-A) on a per area basis (Cerovic et al., 2012). The same leaf was used for leaf pigment measurement and photosynthetic parameters on each plant, and all pigments were recorded at 12:00, every three days from the first day after UVB treatment commenced and five measurement days in total.

2.4 | Analysis of Photoassimilates

Glucose, fructose, sucrose, and starch were analyzed from grapevine leaves harvested at the end of the daily UVB radiation treatment (15:00), 7 and 13 days after the treatments commenced. A different leaf from that chosen for photosynthesis parameters and pigments was measured (but of the same age), frozen in liquid nitrogen immediately after harvesting, then freeze-dried. Sugars and starch were extracted and measured as described in Stitt et al. (1989). The supernatants of ethanolic extracts were used to analyze glucose, fructose and sucrose using a Megazyme Sucrose, D-Fructose and D-Glucose kit (Megazyme). The insoluble pellets were used to analyze starch (Stitt et al., 1989).

2.5 | Data Analysis

Statistical analyses were performed using R version 4.1.3 (R Core Team, 2022). A linear model was used to test for the effects of

treatments against the parameters of the RLC (a more-complex model was not used so as to avoid overfitting). All other analyses were done using linear mixed-effects (LME) models with the package *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017). The two replicate blocks were considered random-effects factors, and repeated measurements were made on the same leaf. The specific effects on photosynthetic parameters and metabolite contents of treatments, the time course (as days after the treatments commenced), time of day, and their interactions, were investigated using a Type II ANOVA with Satterthwaite's method (Kuznetsova et al., 2017). To accommodate minor differences in the incident PAR received by leaves caused by small differences in their display angle or height, PAR (recorded by LI-600) was treated as a covariate in the LME model for g_s and ϕ_{PSII}. *Post-hoc* pairwise comparisons were performed to test differences among factors with packages *car* (Fox & Weisberg, 2019), *emmeans* (Searle et al., 1980), and *multcomp* (Hothorn et al., 2008). Only the difference between steady and fluctuating UVB treatments, and between UVB treatment and control, were of interest; thus, the adjusted *P*-values (adj. *P*) were calculated only for the following contrasts: FUV-SUV, cFUV-cSUV, FUV-cFUV and SUV-cSUV.

Relationships between photosynthetic parameters and metabolite concentrations were examined using linear models. Pigments, measured once on each measurement day, were compared against a daily average for each sample of g_s, ϕ_{PSII} and F_v/F_m to test for correlation. Sugars and starch, analysed once at the end of daily UVB radiation on 7 and 13 days after the treatments commenced, were correlated against the last of g_s, ϕ_{PSII} and F_v/F_m recordings taken during the daily UVB radiation period on these days. Differences were considered statistically significant when $P < 0.05$.

3 | RESULTS

3.1 | Response of stomatal conductance to fluctuating and steady UVB radiation

On average through the day, leaf stomatal conductance (g_s) was 21.9% lower (adj. $P < 0.001$) in the steady UVB (SUV) treatment compared to its control (cSUV; Figure 1a; Table S2a,b), and likewise lower than fluctuating UVB (FUV) by 11.2% (adj. $P < 0.001$); while g_s was only 8.7% lower (adj. $P < 0.001$) in FUV compared to its control (cFUV). The diurnal pattern of g_s involved a decline in all treatments after 11:45 compared to the first daily measurement at 7:30 (adj. $P = 0.028$). The g_s under SUV was lower than that under cSUV from the first day of UVB treatment (Figure 1b), and it remained consistently lowest in this treatment during the whole experiment. The difference between the effects of SUV and cSUV mostly remained between 10–20%, except on Day 7 when it increased to 28.6%. The overall decrease in g_s due to UVB radiation was greater for SUV than for FUV (related to their respective controls) over the whole experiment. The results indicated that steady UVB, and to a lesser extent fluctuating UVB treatments, suppressed leaf gas exchange and that the effect was sustained over several days of treatments.

3.2 | Response of photosynthetic apparatus to fluctuating and steady UVB radiation

Both FUV and SUV radiation treatments were accompanied by increases in operating efficiency of photosystem II (ϕ_{PSII}) in grapevine leaves compared with their no UVB controls (Figure 2). Under FUV,

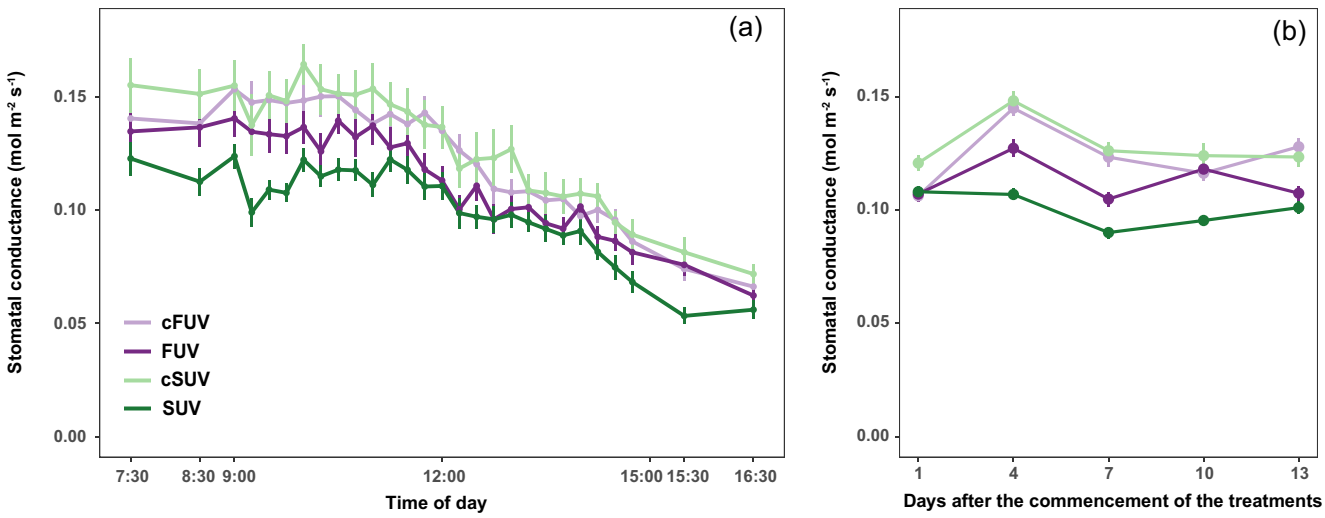

FIGURE 1 Stomatal conductance (g_s) of grapevine leaves grown under fluctuating and steady UVB treatments and controls. (a) Changes in g_s during the day. (b) Changes in g_s with days after the UVB treatment commenced. Measurements on grapevine leaves grown under fluctuating UVB radiation (FUV, dark purple) and its attenuated UVB controls (cFUV, light purple), steady UVB radiation (SUV, dark green) and its attenuated UVB controls (cSUV, light green). One day before the UVB radiation commenced, the daily average of g_s was 0.110 ± 0.004, with no significant difference across the treatment combinations. Data presented are the mean ± SE across replicate blocks (n = 6 replicate compartments in which plants were measured in (a) and n = 168 total daily measurements in (b)).

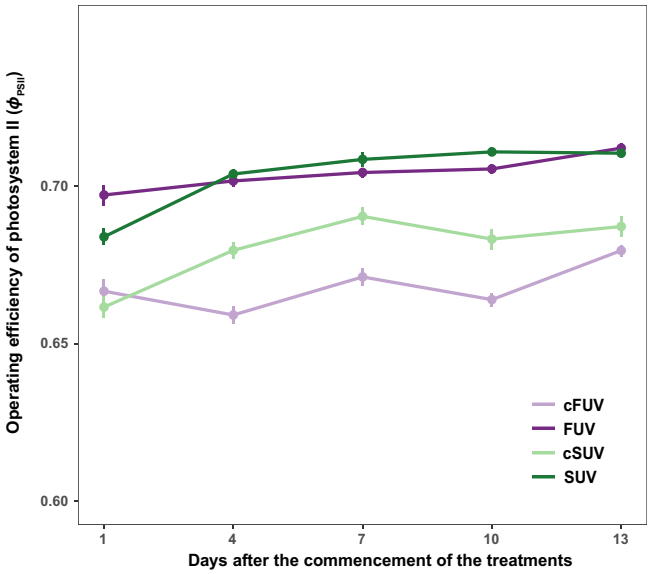

FIGURE 2 Operating efficiency of photosystem II (ϕ_{PSII}) of grapevine leaves grown under fluctuating and steady UVB treatments and controls. Treatments are fluctuating UVB radiation (FUV, dark purple) and its attenuated UVB controls (cFUV, light purple), steady UVB radiation (SUV, dark green) and its attenuated UVB controls (cSUV, light green). Data are the mean ± SE across replicate blocks (n = 168 total daily measurements).

mean ϕ_{PSII} was 0.70 ±< 0.01 and under cFUV 0.67 ±< 0.01 (adj. $P < 0.001$; Table S3a,b). Under SUV mean ϕ_{PSII} was 0.70 ±< 0.01 and under cSUV 0.68 ±< 0.01 (adj. $P < 0.001$). The difference in ϕ_{PSII} between FUV and SUV is statistically significant (marginally higher in SUV adj. $P < 0.001$), but unlikely to be biologically important. Over the course of the experiment, ϕ_{PSII} increased in all four treatments ($F = 38.0$; $P < 0.001$). Notably, the gradual increase in ϕ_{PSII} caused by FUV (adj. $P < 0.001$) and SUV (adj. $P < 0.001$) compared to each of the control treatment was apparent from the first day of UVB treatment (Figure 2).

The chlorophyll *a* fluorescence transient (OJIP) was further used to probe the function of photosystem (PS) II and photosynthetic electron transport (Figure 3a). When kinetics profiles were normalized to F_o, the increase in intensity of chlorophyll fluorescence was larger in SUV than in FUV, and both were larger than their corresponding controls. Analysis of relative shapes of OJIP profiles revealed significantly lower relative variable fluorescence at the J-step (V_j) and the I-step (V_i) in SUV compared to cSUV (adj. $P = 0.028$, 0.010), and lower V_j in SUV compared to FUV (adj. $P = 0.001$) (Figure 3b,c; Table S4a,b). A similar but smaller difference was found in V_i of FUV compared to cFUV (adj. $P = 0.002$). Taken together, these results indicated different redox states of the photosynthetic electron transport chain under different treatments. There was an increase in maximum quantum yield of PSII (F_v/F_m) in SUV (adj. $P < 0.001$) and FUV (adj. $P < 0.001$) compared with their respective no UVB controls (Figure 3d; Table S4a,b). Additionally, the increase in F_v/F_m under SUV (0.75 ±< 0.01) was marginally higher than FUV (0.74 ± < 0.01, adj. $P = 0.060$). The OJIP curves and pulse-saturation census both found that UVB radiation increased the maximum quantum yield of PSII, particularly under SUV.

The parameters of light-limited and light-saturated regions of rapid light curves (RLCs) were calculated to investigate the effect of UVB radiation on photosynthetic capacity (Tables 1 & S5). Plants grown under FUV responded to RLCs with a steeper initial slope (α) than plants from cFUV (adj. $P = 0.021$), but this was not the case when comparing SUV and cSUV (adj. $P = 0.228$). However, the maximum electron transport rate (ETR_{max}), maximum saturating irradiance (I_k) and the operating saturating irradiance (I_m) increased in SUV compared to cSUV (adj. $P = 0.008$, 0.009 and 0.026 respectively), but not in FUV compared to cFUV (adj. $P = 0.641$, 0.486 and 0.542 respectively; Table S5B). Thus, FUV mainly enhanced the light-limited period, and SUV enhanced the light-saturated region of RLCs.

3.3 | Response of chlorophyll and epidermal pigments to fluctuating and steady UVB radiation

Epidermal flavonols accumulated during the treatment period; more so under the FUV (adj. $P = 0.008$) and SUV (adj. $P = 0.009$) radiation treatments than their controls from 7 days after treatment commenced onwards (Table S6a,c), while there was no significant difference between the FUV and SUV overall (adj. $P = 0.294$; Figure 4a; Table S6b). Epidermal flavonol and leaf chlorophyll accumulation covaried over the UVB treatment period ($R = 0.76$, $P < 0.001$; Figure S2a). The chlorophyll content in both FUV (32.4 ± 0.5) and SUV (34.2 ± 0.7) was significantly higher than that in cFUV (28.3 ± 0.8, adj. $P < 0.001$) and cSUV respectively (29.9 ± 1.0, adj. $P < 0.001$; Figure 4b; Table S6a,b). However, the accumulation of epidermal anthocyanins in response to UVB radiation responded generally in the opposite way to epidermal flavonols ($R = -0.66$, $P = 0.002$; Figure S2b). The concentration of anthocyanins in the upper epidermis of FUV (0.231 ± 0.002) and SUV (0.226 ± 0.003) leaves was lower than cFUV (0.251 ± 0.004, adj. $P < 0.001$) and cSUV (0.247 ± 0.005, adj. $P < 0.001$) respectively. Additionally, pigment concentrations were correlated with chlorophyll fluorescence parameters in grapevine leaves (Figure 5). Epidermal flavonol and leaf chlorophyll concentrations were positively correlated, and epidermal anthocyanins negatively correlated with both F_v/F_m ($R = 0.49$, $P = 0.027$; $R = 0.59$, $P = 0.006$; $R = -0.65$, $P = 0.002$, respectively) and ϕ_{PSII} ($R = 0.76$, $P < 0.001$; $R = 0.87$, $P < 0.001$; $R = -0.82$, $P < 0.001$, respectively). Over the whole experiment, FUV and SUV had similar effects on pigment accumulation of mature grapevine leaves: increases in chlorophylls and epidermal flavonols, and a decrease in epidermal anthocyanins.

3.4 | Response of leaf photoassimilate content to fluctuating and steady UVB radiation

Leaf glucose accumulated more under SUV than cSUV both 7 days (by 76.1%, adj. $P = 0.039$) and 13 days (by 54.2%, adj. $P < 0.001$) after

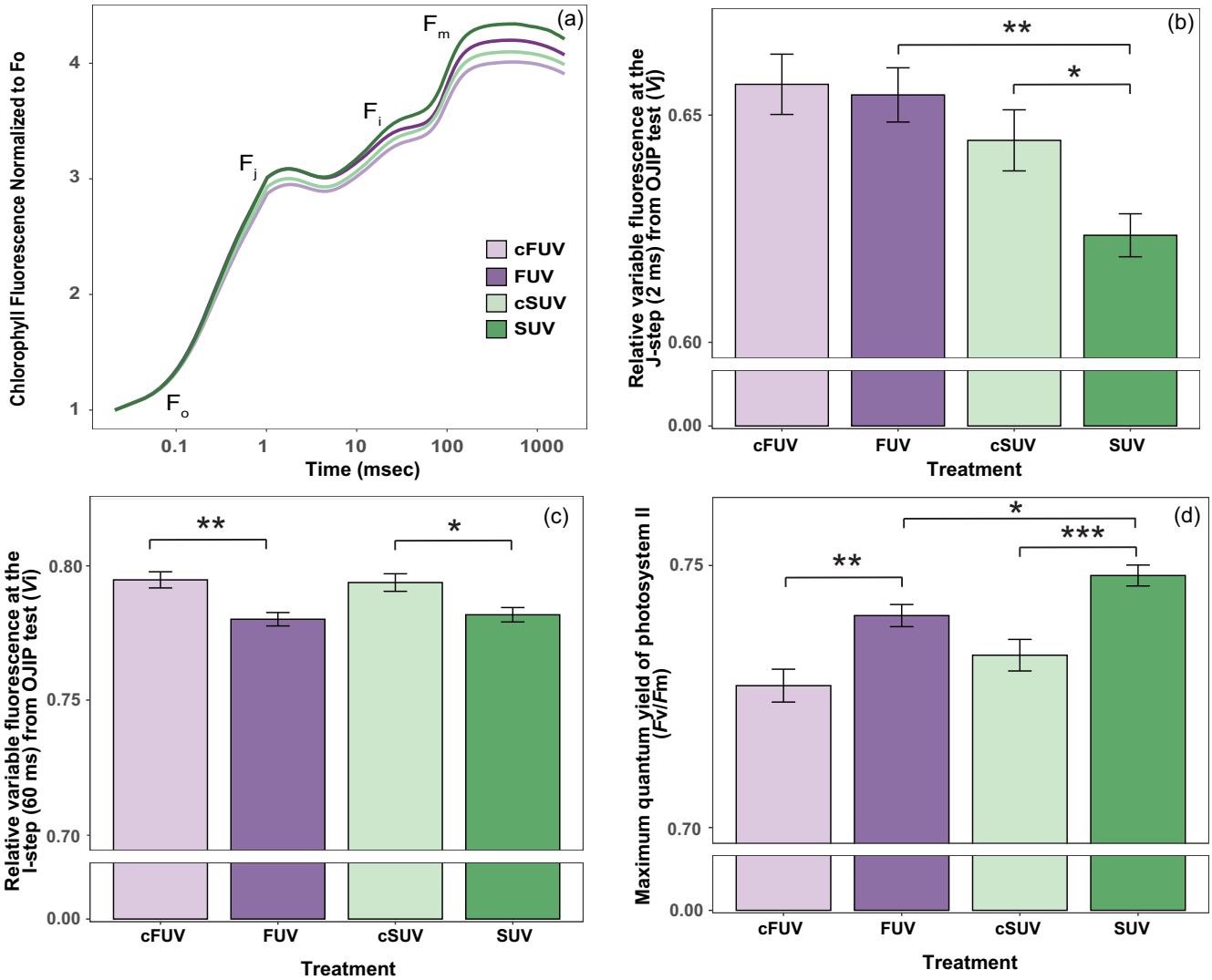

FIGURE 3 Chlorophyll fluorescence fast-transient analysis (OJIP) of grapevine leaves grown under fluctuating and steady UVB treatments and controls. (a) Kinetics of OJIP, normalized to F_o, with time plotted on a logarithmic axis. (b) Relative variable fluorescence at the J-step (V_j). (c) Relative variable fluorescence at the I-step (V_i). (d) Maximum quantum yield of photosystem II (F_v/F_m). Measurements were taken in the 30 min dark-adapted leaves grown under fluctuating UVB radiation (FUV, dark purple) and its attenuated UVB controls (cFUV, light purple), steady UVB radiation (SUV, dark green) and its attenuated UVB controls (cSUV, light green). Data in b, c, d are presented as the mean ± SE across replicate blocks (n = 90 OJIP curves). Significant difference for adjusted *P*-values (adj. *P*) *< 0.05, **< 0.01 and ***< 0.001.

TABLE 1 Initial slope in the light limiting region (α), maximum electron transport rate (ETR_{max}), maximum saturating irradiance (I_k) and operating saturating irradiance (I_m) derived from rapid light-response curves. Data represent mean values ± SE with n = 6 for each treatment.

Treatments	α electron/photons	ETR_{max} µmol electrons·m^{-2} s^{-1}	I_k µmol electrons·m^{-2} s^{-1}	I_m µmol electrons·m^{-2} s^{-1}
cFUV	0.306 ± 0.007	116.1 ± 8.2	380.3 ± 28.8	1076.0 ± 89.0
FUV	0.337 ± 0.007	120.4 ± 6.4	357.8 ± 20.8	997.6 ± 49.3
cSUV	0.314 ± 0.007	110.4 ± 6.8	323.6 ± 22.4	1044.9 ± 50.8
SUV	0.331 ± 0.007	142.9 ± 3.2	434.8 ± 15.6	1297.1 ± 64.7

UVB radiation commenced (Figure 6a; Table S7a,b). Similarly, leaf fructose content under SUV was significantly higher than under cSUV on Day 13 (adj. $P < 0.001$; Figure 6b; Table S7a,b). Additionally, the pattern of leaf sucrose accumulation in response to SUV differed from those of glucose and fructose. Although sucrose concentration appeared to have decreased slightly after 7 days under SUV compared to cSUV, any change was not statistically significant (adj. $P = 0.292$; Figure 6c; Table S7a,b). However, after 13 days under SUV there was

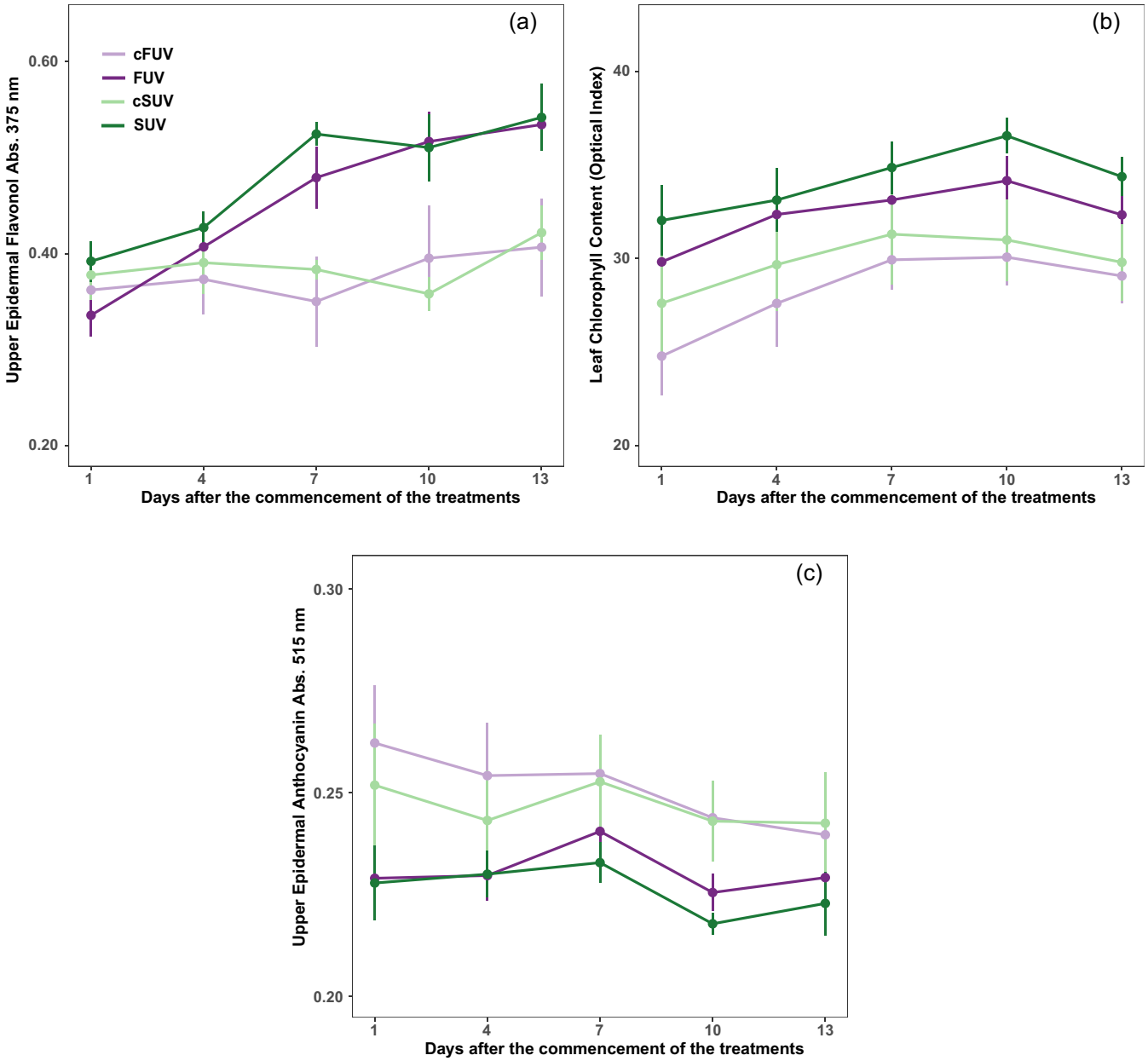

FIGURE 4 Pigments content in grapevine leaves grown under fluctuating and steady UVB treatments and controls. The *y*-axis scale is the optical index from Dualex Scientific + (arbitrary units) on a per-leaf-area basis. (a) Epidermal flavonols, (b) leaf chlorophyll, (c) epidermal anthocyanin. Measurements were taken under fluctuating UVB radiation (FUV, dark purple) and its attenuated UVB controls (cFUV, light purple), steady UVB radiation (SUV, dark green) and its attenuated UVB controls (cSUV, light green). Data are the mean ± SE across replicate blocks (n = 6). One day prior to the UVB radiation commenced, the daily averages were: epidermal flavonols 0.32 ± 0.01, leaf chlorophyll 28.07 ± 0.77 and epidermal anthocyanin 0.213 ± 0.006, with no significant difference across the treatment combination.

a clear effect of SUV (45.3% relative reduction, adj. $P = 0.009$). Likewise, leaves in SUV contained, on average, 45.2% less starch than those in cSUV (adj. $P = 0.008$; Figure 6d; Table S7a,b). Overall, differences between the FUV and cFUV, while presenting similar trends to those of SUV and cSUV, were much less pronounced and generally not significant (Figure 6). With respect to the contents of photoassimilates (sugars and starch), no significant correlation with photosynthetic capacity was detected (Figure S3).

4 | DISCUSSION

4.1 | Steady UVB stimulates stomatal closure more than equivalent fluctuating UVB

Stomatal movements are one of the main processes regulating photosynthesis in higher plants. In this study, a decrease in g_s of grapevine leaves was recorded under steady UVB relative to its control, whereas a generally smaller decline in g_s was caused by fluctuating UVB than

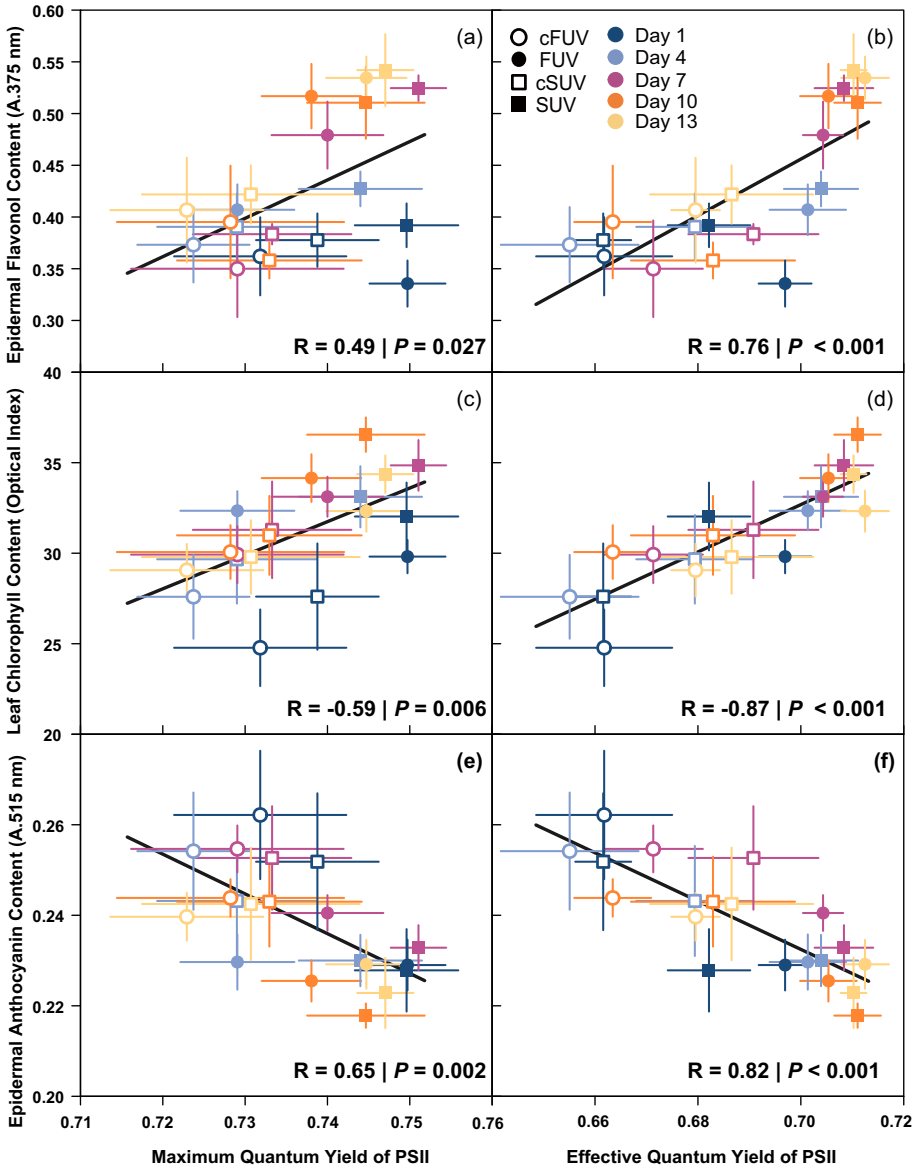

FIGURE 5 The relationship between photosynthetic capacities and pigments content in the grapevine leaves grown under fluctuating and steady UVB treatments and controls. Panels present photosynthetic capacities (maximum quantum yield of photosystem II, F_v/F_m, and operating efficiency of photosystem II, ϕ_{PSII}) against epidermal flavonol index (a, b), chlorophyll content index (c, d) and epidermal anthocyanin index (e, f). Leaves were grown under fluctuating UVB radiation (FUV, filled circles) and its attenuated UVB controls (cFUV, open circles), steady UVB radiation (SUV, filled squares) and its attenuated UVB controls (cSUV, open squares). Measurement days are represented by the colour scale. Data are the mean ± SE across replicate blocks, n = 6 for pigments, n = 90 recorded (3 times per day, 5 days in total) for F_v/F_m and n = 168 for ϕ_{PSII}. The fitted lines and statistics square root of the coefficient of determination and significance (*P*-values) are given from linear regression (R) based on the mean across replicates for each treatment combination and date.

its control (Figure 1). This decrease in g_s caused by UVB radiation is in line with that reported for leaves of *Vitis vinifera* L. cv. Malbec (Berli et al., 2013). Because g_s depends in part on the stomatal aperture, this result is also consistent with the decrease in stomatal aperture from around 2.8 to 2.2 μm reported for *Arabidopsis thaliana* after a UVB treatment of 3 h at 1.5 μmol m^{-2} s^{-1}, or 1 h at 5.5 μmol m^{-2} s^{-1} (Tossi et al., 2014). In our study, FUV and SUV treatments, at equivalent total integrated UVB doses, produced different effects on g_s of grapevines. This implies that not only the total UVB dose but also the highest transient UVB irradiance and the exposure period all affect g_s response. A recent meta-analysis suggests that g_s is mediated by a network of UVB-regulated signalling pathways (Jansen et al., 2022; He et al., 2013). The reduced effect of FUV on g_s, compared with SUV, may occur because 15 min UVB radiation in FUV is not long enough to induce a signal that would suppress stomatal conductance. In SUV, g_s was lower than in its control on the first day after the start of UVB irradiation, and it continued to decline from Day 1 to Day 7. The comparative dynamics of these responses indicate that the establishment of stomatal signalling pathways may result in persistent suppression of g_s, but the mechanism of these pathways is yet to be determined. Recovery following transient UV radiation has been reported to increase stomatal density and aperture in *Mentha spicata* L., allowing greater stomatal control in UV treatment plants than in plants never exposed to UV radiation (Crestani et al., 2023). Accordingly, a 15-min relaxation period following UVB in FUV treatments may ameliorate UVB-induced effects on the functioning and/or development of stomata.

4.2 | Steady and fluctuating UVB differ in the extent to which they affect photosynthesis

The effects of fluctuating and steady UVB radiation on photosynthetic capacity were compared through measurements of quantum

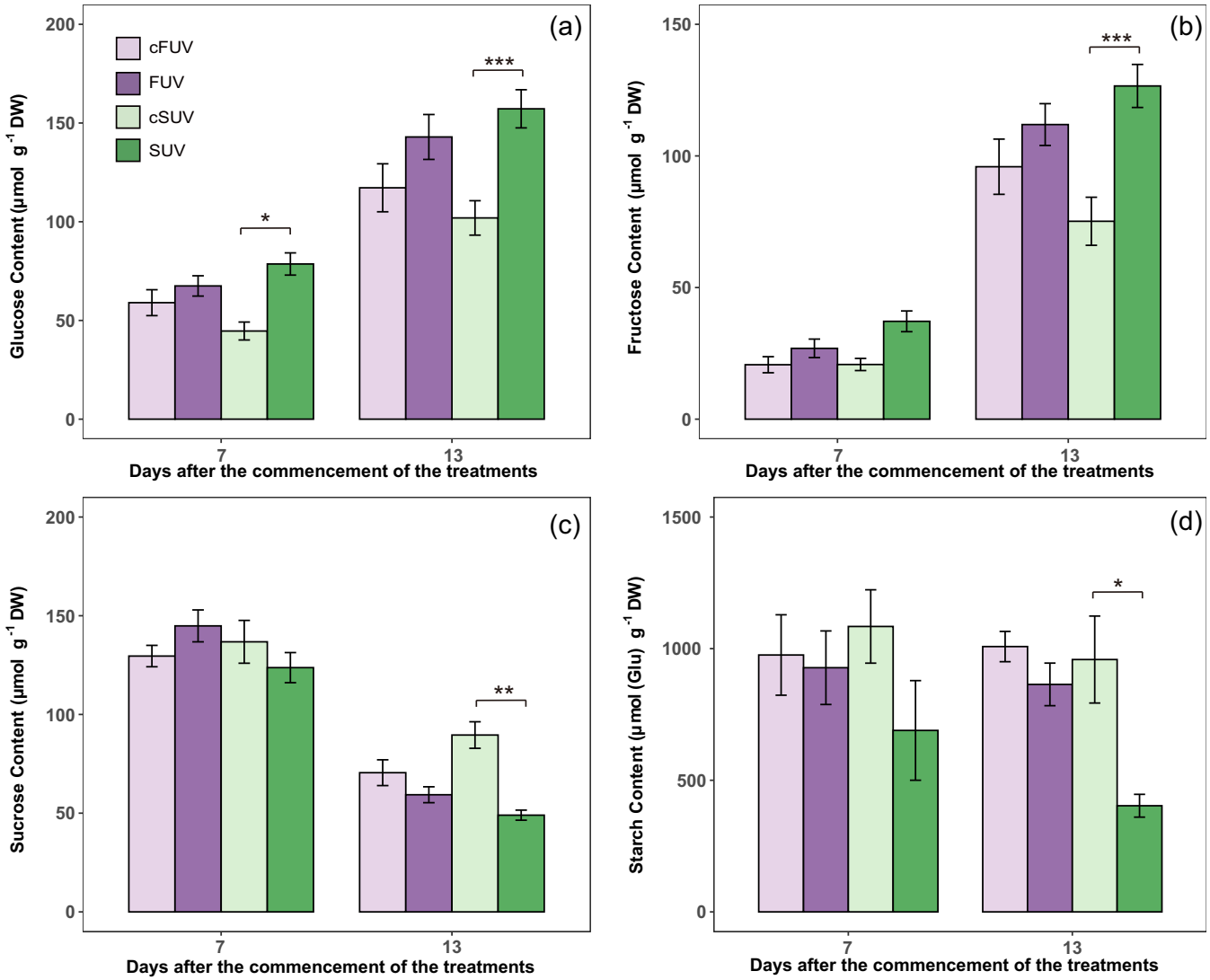

FIGURE 6 Sugar and starch contents in grapevine leaves grown under fluctuating and steady UVB treatments and controls. Treatments are fluctuating UVB radiation (FUV, dark purple) and its attenuated UVB controls (cFUV, light purple), steady UVB radiation (SUV, dark green) and its attenuated UVB controls (cSUV, light green). Leaves were harvested at the end of daily period of UVB radiation (15:00), after 7 and 13 days of UV treatment. Data are the mean ± SE across replicate blocks (n = 6). Significant difference for adjusted *P*-values (adj. *P*) *< 0.05, **< 0.01 and ***< 0.001.

yield, OJIP kinetics and RLCs. Small increases in ϕ_{PSII} and F_v/F_m were found under both UVB radiation treatments compared with controls (Figures 2 and 3d), which runs contrary to the negative effects of UVB radiation often reported (Albert et al., 2011; Allen et al., 1998; Surabhi et al., 2009). While it might be expected that high-UVB irradiance could produce photoinhibition (Kilian et al., 2007; Brosché & Strid, 2003), this is not necessarily the case. There is evidence that net photosynthetic rate (A_{net}) can actually increase under UVB radiation; as found in *Lactuca sativa*, where A_{net} increased by >2 µmol m^{-2} s^{-1} (21% higher) and recovery of F_v/F_m following high PAR was faster in plants receiving solar UV-B radiation than those under UVB attenuated treatments (Wargent et al., 2011; Wargent et al., 2015). Besides, plants growing under artificial PAR and UVB treatments do not receive all of the spectral information perceived by photoreceptors in the full solar spectrum, potentially imposing limitations on photosynthetic performance and acclimation (Landi et al., 2019). Supplemental UVB radiation stimulating UV RESISTANCE LOCUS 8 (UVR8)-mediated responses (O'Hara et al., 2019) may account for the higher values of F_v/F_m and ϕ_{PSII} attained once plants are acclimated to the UVB radiation treatments.

OJIP transients were used to assess the effects of the treatments on the functions of PSII and the photosynthetic electron transport chain (Figure 3). UVB treatments affected the shape of chlorophyll fluorescence dynamics at all phases, including the earliest photochemical phase (F_o-F_j) that reflects processes inside the PSII reaction centre (Figure 3a-c). This, together with higher F_v/F_m (Figure 3d) and ϕ_{PSII} (Figure 2), indicated that SUV, and to a lesser extent FUV, were accompanied by higher efficiency of PSII light harvesting. The

mechanisms whereby UVB radiation enhanced PSII function remain unknown but may be linked to altered abundance and/or connectivity of light-harvesting complex II (LHCII) antennae. LHCII function is supported by higher chlorophyll content, as was found under both SUV and FUV treatment (Figure 4c) because LHCII complexes contain the largest fraction of cellular chlorophyll. Involvement of LHCII is consistent with reported increases in oligomeric forms of LHCII caused by UVB radiation (Sfichi & Kotzabasis, 2004). In addition to LHCII abundance, UVB treatments may alter the distribution of LHCII between PSII and PSI. Dynamic reversible relocations of the mobile LHCII pool between PSII (state 1; St1) and PSI (state 2; St2), the so-called state transitions, allow acclimation of photosynthesis under changing spectral composition by modifying the light absorption properties of the two photosystems (Longoni & Goldschmidt-Clermont, 2021). Our results suggest that UVB treatments promoted the transition to St1 (Figure 3a), decreasing spillover of excitation energy to PSII and increasing PSII light capture and, thus, the PSII photochemical and fluorescence yield (He et al., 2015). Interestingly, FUV had a smaller effect than SUV on promoting St1 than their respective no UVB controls. The fluctuation period of 15 min is within the range over which state transitions occur (Goldschmidt-Clermont & Bass, 2015), meaning that the "recovery period" between UVB doses may offer the opportunity for the grapevine to partially shift back from St1 to St2. Additionally, once the plants have adapted to their treatment conditions, an equilibrium between the excitation of St1 and St2 is likely to be reached (Su et al., 2019). Once this equilibrium is established in SUV, the shift to St1 caused by UVB may be greater than that in FUV.

The effect of sunlight on plants not only depends on the average irradiance but also on the range of low/high light intensity and the duration of fluctuations (Flannery et al., 2021; Wu et al., 2023; Zhu et al., 2010). *Arabidopsis thaliana* plants subjected to steady light from LED lamps in a controlled chamber are reported to have a greater photosynthetic capacity than plants grown at the same total irradiances under LED lamps simulating diurnal fluctuations in irradiance (Vialet-Chabrand et al., 2017). Likewise, in our experiment, the ϕ_{PSII} increase in grapevine leaves was lower under FUV than under SUV (Figure 2), even though overall UVB irradiance was equivalent across the two treatments. The 15 min light fluctuations used here may have increased energy dissipation, meaning that less energy could be directed toward carbon assimilation (Alter et al., 2012; Vialet-Chabrand et al., 2017). A greenhouse experiment with grapevines reported significant reductions in ϕ_{PSII}, g_s and net photosynthesis during the first 20 days of exposure to 9.66 KJ m^{-2} d^{-1} of UVB; effects which were ameliorated after 75 days of treatments (Martínez-Lüscher et al., 2013). While negative effects on photosynthesis were not apparent following initiation of the 2.2 kJ m^{-2} day^{-1} biologically effective UVB radiation treatments used here, this and other studies reporting improved performance over the time course of experiments (Bassman et al., 2002; Shi et al., 2004) provide evidence for long-term acclimation to chronic UVB radiation at moderate UVB doses against low background PAR.

Grapevine leaves under FUV and SUV differed in their response to light-limited and light-saturated conditions during RLCs (Table 1). Under light-limiting irradiance, the RLC in FUV had a steeper initial slope; this indicated a higher efficiency for light capture (Schreiber, 2004). Whereas, under light-saturated irradiance, leaves pre-conditioned to SUV had a higher maximum electron transport rate, maximum saturating irradiance and operating saturating irradiance. Together, this suggests that a higher photosynthetic rate can be maintained under strong irradiance in the leaves of plants grown under SUV than FUV (Ralph & Gademann, 2005), and FUV leaves became light-saturated more easily; findings which have parallels with acclimation to irradiance conditions during growth (e.g., "sun and shade leaves; Earles et al., 2017).

4.3 | Metabolite content correlated with photosynthesis under fluctuating and steady UVB radiation

High leaf flavonol concentration is often associated with acclimation to UVB radiation (Agati et al., 2012) and has previously been found in grapevine leaves in response to UVB treatments (Berli et al., 2013; Majer and Hideg, 2012; Del-Castillo-Alonso et al., 2015; Del-Castillo-Alonso et al., 2016). Increases in flavonol accumulation under UVB radiation measured as increases in epidermal flavonol index can happen quickly (within one day), as reported in okra (*Abelmoschus esculentus*) (Neugart et al., 2021). Still, there are large differences in the ability of different species to quickly adjust their epidermal UV-screening, and grapevine is not among those species considered to produce diurnal changes in flavonol content (Barnes et al., 2015; Barnes et al., 2016). While a small decrease in flavonols in grapevine leaves from morning to evening has been reported, this decrease was not correlated with diurnal solar UVB radiation (Csepregi et al., 2019). Thus, flavonol accumulation in grapevine may typically take days of UVB radiation to be fully manifested. In our experiment, UVB treatments had positive effects on leaf epidermal flavonol accumulation, but there were no significant differences between the effects of FUV and SUV (Figure 4a). This suggests that the accumulation of epidermal UV-screening flavonols may acclimate to the average UVB radiation dose rather than the maximum dose, as found when tracking seasonal change in forest understorey plants (Hartikainen et al., 2020). Flavonols absorb UV radiation and scavenge reactive oxygen species non-enzymatically to protect chloroplasts from photooxidative damage (Agati et al., 2012; Ioku et al., 1995). There was a positive relationship between the accumulation of epidermal flavonols and chlorophyll (Figure S2a), and likewise with F_v/F_m and ϕ_{PSII} (Figure 5a-d). High photosynthetic capacity and pigment content imply that the energy from absorbed light is used efficiently, which is beneficial for plants under low light. A few studies have found anthocyanin content to increase under UVB treatments in some species (Liu-Gitz et al., 1995; Newsham et al., 2005), but no effect is typically found in grapevine (Berli et al., 2010; Majer & Hideg, 2012). This confirms that

anthocyanins are not among the major protective pigments responding to UVB radiation in mature grapevine leaves.

The increases reported in glucose and fructose under our UVB treatments are consistent with the typical effects of UVB radiation on these soluble sugars (Barsig et al., 1998; Hilal et al., 2004; Phoenix et al., 2000; Figure 6a,b). Soluble sugars play pivotal roles in primary metabolism, cellular- and plant-level carbon allocation and signalling pathways controlling, among other functions, photosynthesis (Henry et al., 2020; Lastdrager & Smeekens, 2014; Sheen, 1990; Yan et al., 2019). However, no significant correlations were found between soluble sugar (glucose, fructose and sucrose) concentrations and F_v/F_m or ϕ_{PSII} (Figure S3). This implies that photosynthesis was not affected by the increase in glucose and fructose, or the decrease in sucrose, reported here. In *Betula pendula*, increased bark glucose content under UVB treatments can be associated with a reduced sink demand for photosynthate (Tegelberg et al., 2002). A similar reduced sink strength may provide an alternative explanation for the increase of glucose in grapevine leaves also found under our UVB radiation treatments. Starch accumulates as a carbon reserve during the day in chloroplasts and is depleted during the night to sustain growth and metabolism of the plants (Kölling et al., 2015; Robinson, 1996). Here, there was a reduction in starch accumulation under UVB radiation (Figure 6d), which is consistent with other studies into the effects of UVB radiation showing, e.g., a higher ratio of soluble-sugars-to-starch reported in *Calamagrostis purpurea* (Gwynn-Jones, 2001), reduced starch in *Vaccinium ulginiosum* (Phoenix et al., 2000), and a decrease in starch volume density in *Brassica napus* and *Helianthus annuus* (Fagerberg & Bornman, 1997; Fagerberg, 2007).

5 | CONCLUSIONS

Most studies into the effects of UVB radiation on plants are conducted under steady UVB radiation in controlled conditions rather than fluctuating light outdoors. Even field experiments with UV filters or modulated lamp systems cannot specifically focus on the effect of fluctuating compared with equivalent steady UVB radiation. Here, a major cultivar of grapevine (*Vitis vinifera* L. cv Cabernet Sauvignon) was used to investigate how equivalent doses of fluctuating (FUV) *vs* steady (SUV) UVB radiation affect photosynthesis and metabolite accumulation. It was found that: (1) SUV decreased g_s more than FUV; (2) there was no evidence of damage to PSII caused by the UVB treatments but even a small increase in the F_v/F_m and ϕ_{PSII}; (3) epidermal flavonol content was increased by both SUV and FUV compared with their controls.

Overall, acclimation to FUV was weaker than to SUV across the parameters measured. This implies that experiments examining UVB responses of plants in controlled conditions under steady irradiance may overestimate the effect size when compared to the same daily average of biologically effective UVB radiation in natural environments where UVB radiation is subject to fluctuations. To obtain more reliable estimates, future experiments should consider the total UVB dose, the amplitude of fluctuations in irradiance, and the duration and frequency of these fluctuations when assessing the factors governing plant responses to UVB radiation. This approach would allow a systematic understanding of plants' acclimation mechanisms under the kind of patterns of UVB radiation found in nature, to which plants have adapted over many generations.

AUTHOR CONTRIBUTIONS

Chenxing Su-Zhou designed and carried out the experiment, analyzed the data and wrote the manuscript. Maxime Durand helped with data collection, writing and ideas behind the manuscript. Pedro J. Aphalo advised data analysis. Javier Martinez-Abaigar provided grapevines and expertise on their growth. Alexey Shapiguzov helped design and measure and interpret the OJIP survey. hirofumi ishihara helped with sugar and starch analysis. T. Matthew Robson proposed and designed the original experiment and supervised all the work. All authors edited and approved the manuscript.

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DATA AVAILABILITY STATEMENT

The raw data from this experiment are available in the Appendix.

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REFERENCES

Agati, G., Azzarello, E., Pollastri, S. & Tattini, M. (2012) Flavonoids as antioxidants in plants: location and functional significance. *Plant Science: An International Journal of Experimental Plant Biology*, 196, 67–76.

Albert, K.R., Mikkelsen, T.N., Ro-Poulsen, H., Arndal, M.F. & Michelsen, A. (2011) Ambient UV-B radiation reduces PSII performance and net photosynthesis in high Arctic *Salix arctica*. *Environmental and Experimental Botany*, 72, 439–447.

Allen, D.J., Nogués, S. & Baker, N.R. (1998) Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *Journal of Experimental Botany*, 49, 1775–1788.

Alter, P., Dreissen, A., Luo, F.L. & Matsubara, S. (2012) Acclimatory responses of *Arabidopsis* to fluctuating light environment: comparison of different sunfleck regimes and accessions. *Photosynthesis Research*, 113(1–3), 221–237.

Anderson, K. & Nelgen, S. (2020) *Which Winegrape Varieties Are Grown Where: A Global Empirical Picture*. (Revised ed). University of Adelaide Press: Adelaide, Australia.

Aphalo, P.J. (2018) Exploring temporal and latitudinal variation in the solar spectrum at ground level with the TUV model. *UV4Plants Bulletin*, 2018(2), 45–56.

Aphalo, P.J., Albert, A., Björn, L.-O., McLeod, A., Robson, T.M., & Rosenqvist, E., (2012) Beyond the visible: A handbook of best practice in plant UV photobiology. *COST Action* FA0906. ISBN 1479133442.

Assmann, S.M. & Wang, X.Q. (2001) From milliseconds to millions of years: guard cells and environmental responses. *Current Opinion in Plant Biology*, 4(5), 421–428.

Barnes, P.W., Flint, S.D., Tobler, M.A. & Ryel, R.J. (2016) Diurnal adjustment in ultraviolet sunscreen protection is widespread among higher plants. *Oecologia*, 181, 55–63.

Barnes, P.W., Robson, T.M., Tobler, M.A., Bottger, I.N. & Flint, S.D. (2017) Plant responses to fluctuating UV environments. Chapter 6 in B. Jordan. *Plant UV Biology*, CABI publishers, 72–89.

Barnes, P.W., Robson, T.M., Zepp, R.G., Bornman, J.F., Jansen, M.A.K., Ossola, R., Wang, Q.W., Robinson, S.A., Foereid, B., Klekociuk, A.R., Martinez-Abaigar, J., Hou, W.C., Mackenzie, R. & Paul, N.D. (2023) Interactive effects of changes in UV radiation and climate on terrestrial ecosystems, biogeochemical cycles, and feedbacks to the climate system. *Photochemical & Photobiological Sciences*, 22(5), 1049–1091.

Barsig, M., Schneider, K. & Gehrke, C. (1998) Effects of UV-B radiation on fine structure, carbohydrates, and pigments in *Polytrichum commune*. *Bryologist*, 101, 358–365.

Bassman, J., Edwards, G. & Robberecht, R. (2002) Long-term exposure to enhanced UV-B radiation is not detrimental to growth and photosynthesis in *Douglas-fir*. *New Phytologist*, 154, 107–120.

Bates, D., Mächler, M., Bolker, B. & Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1–48.

Berli, F. J., Moreno, D., Piccoli, P., Hespanhol-Viana, L., Silva, M. F., Bressan-Smith, R., Cavagnaro, J. B. & Bottini, R. (2010) Abscisic acid is involved in the response of grape (*Vitis vinifera* L.) cv. Malbec leaf tissues to ultraviolet-B radiation by enhancing ultraviolet-absorbing compounds, antioxidant enzymes and membrane sterols. *Plant, Cell & Environment*, 33(1), 1–10.

Berli, F.J., Alonso, R., Bressan-Smith, R. & Bottini, R. (2013) UV-B impairs growth and gas exchange in grapevines grown in high altitude. *Physiologia Plantarum*, 149(1), 127–140.

Björn, L.O. (2015) Ultraviolet-A, B, and C. *UV4Plants Bulletin*, 2015(1) 17–18.

Britt, A.B. (1996) DNA damage and repair in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 47, 75–100.

Brosché, M. & Strid, Å. (2003) Molecular events following perception of ultraviolet-B radiation by plants. *Physiologia Plantarum*, 117, 1–10.

Cerovic, Z.G., Masdoumier, G., Ghozlen, N.B. & Latouche, G. (2012) A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf chlorophyll and epidermal flavonoids. *Physiologia Plantarum*, 146, 251–260.

Coombe, B.G. (1995) Growth stages of the grapevine: Adoption of a system for identifying grapevine growth stages. *Australian Journal of Grape and Wine Research*, 1, 104–110.

Crestani, G., Cunningham, N., Csepregi, K., Badmus, U.O. & Jansen, M.A.K. (2023) From stressor to protector, UV-induced abiotic stress resistance. *Photochemical & Photobiological Sciences*, 22(9), 2189–2204.

Csepregi, K., Teszlák, P., Kőrösi, L. & Hideg, É. (2019) Changes in grapevine leaf phenolic profiles during the day are temperature rather than irradiance driven. *Plant Physiology and Biochemistry*, 137, 169–178.

Del-Castillo-Alonso, M.Á., Diago, M.P., Monforte, L., Tardaguila, J., Martínez-Abaigar, J. & Núñez-Olivera, E. (2015) Effects of UV exclusion on the physiology and phenolic composition of leaves and berries of *Vitis vinifera* cv. *Graciano*. *Journal of the Science of Food and Agriculture*, 95(2), 409–416.

Del-Castillo-Alonso, M.Á., Diago, M.P., Tomás-Las-Heras, R., Monforte, L., Soriano, G., Martínez-Abaigar, J. & Núñez-Olivera, E. (2016) Effects of ambient solar UV radiation on grapevine leaf physiology and berry phenolic composition along one entire season under Mediterranean field conditions. *Plant Physiology and Biochemistry*, 109, 374–386.

Earles, J.M., Théroux-Rancourt, G., Gilbert, M.E., McElrone, A.J. & Brodersen, C.R. (2017) Excess diffuse light absorption in upper mesophyll limits CO_2 drawdown and depresses photosynthesis. *Plant Physiology*, 174(2), 1082–1096.

Eilers, P.H.C. & Peeters, J.C.H. (1988) A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modelling*, 42, 199–215.

Fagerberg, W.R. & Bornman, J.F. (1997) Ultraviolet-B radiation causes shade-type ultrastructural changes in *Brassica napus*. *Physiologia Plantarum*, 101, 833–844.

Fagerberg, W.R. (2007) Below-ambient levels of UV induce chloroplast structural change and alter starch metabolism. *Protoplasma*, 230(1–2), 51–59.

Flannery, S.E., Hepworth, C., Wood, W.H.J., Pastorelli, F., Hunter, C.N., Dickman, M.J., Jackson, P.J. & Johnson, M.P. (2021) Developmental acclimation of the thylakoid proteome to light intensity in *Arabidopsis*. *Plant Journal*, 105, 223–244.

Fox, J. & Weisberg, S. (2019) *An R Companion to Applied Regression*, Third edition. Sage, Thousand Oaks CA.

Goldschmidt-Clermont, M. & Bassi, R. (2015) Sharing light between two photosystems: mechanism of state transitions. *Current Opinion in Plant Biology*, 25, 71–78.

Gwynn-Jones, D. (2001) Short-term impacts of enhanced UV-B radiation on photo-assimilate allocation and metabolism: a possible interpretation for time-dependent inhibition of growth. *Plant Ecology*, 154, 67–73.

Hartikainen, S.M., Pieristè, M., Lassila, J. & Robson, T.M. (2020) Seasonal Patterns in Spectral Irradiance and Leaf UV-A Absorbance Under Forest Canopies. *Frontiers in Plant Science*, 10, 1762.

Hazard, C., Lesser, M.P. & Kinzie, R.A. (1997) Effects of Ultraviolet Radiation on Photosynthesis in the Subtropical Marine Diatom, *Chaetoceros gracilis* (Bacillariophyceae). *Journal of Phycology*, 33, 960–968.

He, J.M., Ma, X.G., Zhang, Y., Sun, T.F., Xu, F.F., Chen, Y.P., Liu, X. & Yue, M. (2013) Role and interrelationship of Ga protein, hydrogen peroxide, and nitric oxide in ultraviolet B-induced stomatal closure in *Arabidopsis* leaves. *Plant Physiology*, 161, 1570–1583.

He, J., Yang, W., Qin, L., Fan, D.-J. & Chow, W.S. (2015) Photoinactivation of Photosystem II in wild-type and chlorophyll *b*-less barley leaves: which mechanism dominates depends on experimental circumstances. *Photosynthesis Research*, 126, 399–407.

Henry, C., Watson-Lazowski, A., Oszvald, M., Griffiths, C., Paul, M.J., Furbank, R.T. & Ghannoum, O. (2020) Sugar sensing responses to low and high light in leaves of the C_4 model grass *Setaria viridis*. *Journal of Experimental Botany*, 71(3), 1039–1052.

Hideg, E., Jansen, M.A. & Strid, A. (2013) UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? *Trends in Plant Science*, 18(2), 107–115.

Hilal, M., Parrado, M.F., Rosa, M., Gallardo, M., Orce, L., Massa, E.M., González, J.A. & Prado, F.E. (2004) Epidermal lignin deposition in quinoa cotyledons in response to UV-B radiation. *Photochemistry and Photobiology*, 79(2), 205–210.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal*, 50(3), 346–363.

Ioku, K., Tsushida, T., Takei, Y., Nakatani, N. & Terao, J. (1995) Antioxidative activity of quercetin and quercetin monoglucosides in solution and phospholipid bilayers. *Biochimica et Biophysica Acta*, 1234(1), 99–104.

Jansen, M.A.K., Ač, A., Klem, K. & Urban, O. (2022) A meta-analysis of the interactive effects of UV and drought on plants. *Plant, Cell & Environment*, 45(1), 41–54.

Kaiser, E., Morales, A. & Harbinson, J. (2018) Fluctuating Light Takes Crop Photosynthesis on a Rollercoaster Ride. *Plant Physiology*, 176(2), 977–989.

Kilian, J., Whitehead, D., Horak, J., Wanke, D., Weinl, S., Batistic, O., D'Angelo, C., Bornberg-Bauer, E., Kudla, J. & Harter, K. (2007) The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. *The Plant Journal*, 50(2), 347–363.

Kölling, K., Thalmann, M., Müller, A., Jenny, C. & Zeeman, S.C. (2015) Carbon partitioning in *Arabidopsis thaliana* is a dynamic process controlled by the plant's metabolic status and its circadian clock. *Plant, Cell & Environment*, 38(10), 1965–1979.

Kromdijk, J., Głowacka, K., Leonelli, L., Gabilly, S.T., Iwai, M., Niyogi, K.K. & Long, S.P. (2016) Improving photosynthesis and crop productivity by accelerating recovery from photoprotection. *Science*, 354(6314), 857–861.

Kuznetsova, A., Brockhoff, P.B. & Christensen, R.H.B. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, 82(13), 1–26.

Landi, M., Živčák, M., Sytar, O., Brestič, M. & Allakhverdiev, S.I. (2019) Plasticity of photosynthetic processes and the accumulation of secondary metabolites in plants in response to monochromatic light environments: A review. *Biochimica et Biophysica Acta. Bioenergetics*, 1861, 148131.

Landry, L., Stapleton, A., Lim, J., Hoffman, P., Hays, J. B., Walbot, V. & Last, R. (1997) An *Arabidopsis* photolyase mutant is hypersensitive to UV-B radiation. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 328–332.

Lastdrager, J., Hanson, J. & Smeekens, S. (2014) Sugar signals and the control of plant growth and development. *Journal of Experimental Botany*, 65, 799–807.

Liu-Gitz, L., Gitz, D. & McClure, J.W. (1995) Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Physiologia Plantarum*, 93, 725–733.

Longoni, F.P. & Goldschmidt-Clermont, M. (2021) Thylakoid Protein Phosphorylation in Chloroplasts. *Plant & Cell Physiology*, 62(7), 1094–1107.

Majer, P. & Hideg, E. (2012) Developmental stage is an important factor that determines the antioxidant responses of young and old grapevine leaves under UV irradiation in a green-house. *Plant Physiology and Biochemistry*, 50(1), 15–23.

Martínez-Lüscher, J., Morales, F., Delrot, S., Sánchez-Díaz, M., Gomés, E., Aguirreolea, J. & Pascual, I. (2013) Short- and long-term physiological responses of grapevine leaves to UV-B radiation. *Plant Science*, 213, 114–122.

Neugart, S., Tobler, M.A. & Barnes, P.W. (2021) Rapid adjustment in epidermal UV sunscreen: Comparison of optical measurement techniques and response to changing solar UV radiation conditions. *Physiologia Plantarum*, 173(3), 725–735.

Newsham, K., Geissler, P.A., Nicolson, M.J., Peat, H. & Lewis-Smith, R. (2005) Sequential reduction of UV-B radiation in the field alters the pigmentation of an Antarctic leafy liverwort. *Environmental and Experimental Botany*, 54, 22–32.

O'Hara, A., Headland, L., Diaz, A., Morales, L., Strid, Å. & Jenkins, G. (2019) Regulation of *Arabidopsis* gene expression by low fluence rate UV-B independently of UVR8 and stress signaling. *Photochemical and Photobiological Sciences*, 18, 1675–1684.

Ollat, N. & Gaudillere, J.P. (2000) Carbon balance in developing grapevine berries. *Acta Horticulturae*, 526, 345–350.

Petrie, P.R., Trought, M.C., Howell, G.S. & Buchan, G.D. (2003) The effect of leaf removal and canopy height on whole-vine gas exchange and fruit development of *Vitis vinifera* L. Sauvignon Blanc. *Functional Plant Biology*, 30, 711–717.

Phoenix, G.K., Gwynn-Jones, D., Lee, J.A. & Callaghan, T.V. (2000) The impacts of UV-B radiation on the regeneration of a sub-arctic heath community. *Plant Ecology*, 146, 67–75.

R Core Team (2022) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ralph, P.J. & Gademann, R. (2005) Rapid light curves: A powerful tool to assess photosynthetic activity. *Aquatic Botany*, 82, 222–237.

Robinson, J.M. (1996) Leaflet photosynthesis rate and carbon metabolite accumulation patterns in nitrogen-limited, vegetative soybean plants. *Photosynthesis research*, 50(2), 133–148.

Robson, T.M. & Aphalo, P.J. (2019) Transmission of ultraviolet, visible and near-infrared solar radiation to plants within a seasonal snow pack. *Photochemical & Photobiological Sciences*, 18(8), 1963–1971.

Robson, T.M., Klem, K., Urban, O. & Jansen, M.A. (2015) Re-interpreting plant morphological responses to UV-B radiation. *Plant, Cell & Environment*, 38(5), 856–866.

Roeber, V.M., Bajaj, I., Rohde, M., Schmülling, T. & Cortleven, A. (2021) Light acts as a stressor and influences abiotic and biotic stress responses in plants. *Plant, Cell & Environment*, 44(3), 645–664.

Schmelzer, E., Jahnen, W. & Hahlbrock, K. (1988) In situ localization of light-induced chalcone synthase mRNA, chalcone synthase, and flavonoid end products in epidermal cells of parsley leaves. *Proceedings of the National Academy of Sciences of the United States of America*, 85(9), 2989–2993.

Schreiber, U. (2004) Pulse-amplitude (PAM) fluorometry and saturation pulse method. In: Papageorgiou, G., Govindjee, (Eds.), *Chlorophyll fluorescence: A Signature of Photosynthesis*. Advances in Photosynthesis and Respiration Series. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Searle, S.R., Speed, F.M. & Milliken, G.A. (1980) Population marginal means in the linear model: an alternative to least squares means. *The American Statistician*, 34: 216–221.

Sfichi, L., Ioannidis, N. & Kotzabasis, K. (2004) Thylakoid-associated polyamines adjust the UV-B sensitivity of the photosynthetic apparatus by means of light-harvesting complex II changes. *Photochemistry and Photobiology*, 80(3), 499–506.

Sheen, J. (1990) Metabolic repression of transcription in higher plants. *The Plant cell*, 2(10), 1027–1038.

Shi, S., Zhu, W., Li, H., Zhou, D., Han, F., Zhao, X. & Tang, Y. (2004) Photosynthesis of *Saussurea superba* and *Gentiana straminea* is not reduced after long-term enhancement of UV-B radiation. *Environmental and Experimental Botany*, 51, 75–83.

Smith, W.K. & Berry, Z.C. (2013) Sunflecks? *Tree Physiology*, 33, 233–237.

Stitt, M., Lilley, R. M., Gerhardt, R. & Heldt, H. W. (1989) Metabolite levels in specific cells and subcellular compartments of plant-leaves. *Methods in Enzymology*, 174, 518–552.

Su, X., Ma, J., Pan, X., Zhao, X., Chang, W., Liu, Z., Zhang, X. & Li, M. (2019) Antenna arrangement and energy transfer pathways of a green algal photosystem-I-LHCI supercomplex. *Nature Plants*, 5(3), 273–281.

Surabhi, G., Reddy, K.R. & Singh, S.K. (2009) Photosynthesis, fluorescence, shoot biomass and seed weight responses of three cowpea (*Vigna unguiculata* (L.) Walp.) cultivars with contrasting sensitivity to UV-B radiation. *Environmental and Experimental Botany*, 66, 160–171.

Tegelberg, R., Aphalo, P.J. & Julkunen-Tiitto, R. (2002) Effects of long-term, elevated ultraviolet-B radiation on phytochemicals in the bark of silver birch (*Betula pendula*). *Tree Physiology*, 22, 1257–1263.

Tossi, V., Lamattina, L., Jenkins, G. I. & Cassia, R. O. (2014) Ultraviolet-B-induced stomatal closure in *Arabidopsis* is regulated by the UV RESISTANCE LOCUS8 photoreceptor in a nitric oxide-dependent mechanism. *Plant physiology*, 164(4), 2220–2230.

Vialet-Chabrand, S., Matthews, J.S., Simkin, A.J., Raines, C. A. & Lawson, T. (2017) Importance of fluctuations in light on plant photosynthetic acclimation. *Plant Physiology*, 173(4), 2163–2179.

Wargent, J.J., Elfadly, E.M., Moore, J.P. & Paul, N.D. (2011) Increased exposure to UV-B radiation during early development leads to enhanced photoprotection and improved long-term performance in *Lactuca sativa*. *Plant, Cell & Environment*, 34(8), 1401–1413.

Wargent, J.J., Nelson, B.C.W., Mcghie, T.K. & Barnes, P.W. (2015) Acclimation to UV-B radiation and visible light in *Lactuca sativa* involves up-regulation of photosynthetic performance and orchestration of metabolome-wide responses. *Plant, Cell & Environment*, 38, 929–940.

Willekens, H., Van Camp, W., Van Montagu, M., Inze, D., Langebartels, C. & Sandermann, H. (1994) Ozone, sulfur dioxide, and ultraviolet B have similar effects on mRNA accumulation of antioxidant genes in *Nicotiana plumbaginifolia* L. *Plant Physiology*, 106(3), 1007–1014.

Wu, H.Y., Qiao, M.Y., Zhang, Y.J., Kang, W.J., Ma, Q.H., Gao, H.Y., Zhang, W.F. & Jiang, C.D. (2023) Photosynthetic mechanism of maize yield under fluctuating light environments in the field. *Plant Physiology*, 191(2), 957–973.

Yan, Y., Stoddard, F.L., Neugart, S., Sadras, V.O., Lindfors, A., Morales, L. O. & Aphalo, P. J. (2019) Responses of flavonoid profile and associated gene expression to solar blue and UV radiation in two accessions of *Vicia faba* L. from contrasting UV environments. *Photochemical & Photobiological Sciences*, 18(2), 434–447.

Zhao, B., Wang, L., Pang, S., Jia, Z., Wang, L., Li, W. & Jin, B. (2020) UV-B promotes flavonoid synthesis in *Ginkgo biloba* leaves. *Industrial Crops and Products*, 151, 112483.

Zhu, X.G., Long, S.P. & Ort, D.R. (2010) Improving photosynthetic efficiency for greater yield. *Annual review of plant biology*, 61, 235–261.

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