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1 Abstract

2

3 Large numbers of studies have reported on the responses of plants that are exposed to a specific 4 dose of ultraviolet-B (UV-B) radiation. However, in the natural environment UV-B is a highly 5 dynamic variable with UV-B intensities depending on, amongst others, geographic, temporal, 6 weather and climatic factors. Furthermore, UV-B effects on plants can potentially be modulated 7 by other environmental variables, and vice versa. This study aimed to characterise UV-B effects 8 on plant morphology and accumulation of UV-screening pigments within the context of an oceanic 9 climate and to assess the potential seasonality of plant UV-B responses. Arabidopsis thaliana was 10 grown outdoors under UV-blocking or transmitting filters. Genotypic differences in the adaptive 11 response to UV-B were assessed at seven time-points over a 12 month period and involved the 12 Arabidopsis accessions Ler, Col-0, and Bur-0. Strong seasonal effects were found on rosette 13 morphology and total UV-screening pigment concentrations across the three accessions. Low 14 temperatures were the main determinant of accumulation of UV-absorbing pigments, with no 15 clear UV-B effect observed at any time throughout the year. There was a significant UV effect on 16 morphology during the summer months, and this was most likely associated with stress. This 17 study shows that UV-effects need to be analysed in the context of weather, and other co-occurring 18 natural factors, and emphasizes the importance of a holistic, multifactorial approach for the 19 investigation of environmentally relevant UV-effects 20 21

22 Keywords

23 Arabidopsis, Morphology, Photosynthesis, Ultraviolet radiation, UV-screening pigments, Weather.

1 <u>Introduction</u> 2

3 UV wavelengths (UV-B 280-315 nm; UV-A 315-400 nm) are only a minor component of the solar 4 spectrum in the biosphere. Yet, these wavelengths can have a disproportionate effect on living 5 organisms due to their energetic nature. There is an extensive body of literature on the potentially 6 harmful effects of high levels of UV-B radiation on a broad range of organisms (Teramura and 7 Sullivan, 1994; Rozema et al., 1997; Jansen et al., 1998). In plants, UV-B can negatively affect 8 several targets, including genetic material and the photosynthetic machinery, triggering 9 production of ROS and impairment of cellular processes. In parallel, UV-B induces expression of 10 protective responses, including enhanced photorepair capacity, accumulation of UV-screening 11 pigments and increases in total antioxidant capacity (Strid et al., 1994; Jansen et al., 1998; Morales 12 et al., 2010). UV-B also induces morphological changes in plants, although it is not fully 13 understood if and how these contribute to plant UV-B protection (Robson et al., 2015). The UV-B 14 photoreceptor UVR8 plays a major role in controlling the plant UV-B response, and UVR8 15 mediated responses can already be observed under very low UV-B intensities (Brown et al., 2005; Brown and Jenkins 2008; Jenkins et al., 2014). As a result of the expression of protective 16 17 responses under low UV-intensities, plants exposed to ambient levels of UV-B rarely display signs 18 of distress (Searles et al., 2001; Ballaré et al., 2011; Jansen et al., 2012).

19

20 Much of what is known about plant UV-B responses comes from indoor studies whereby plants 21 are daily exposed for a set number of hours to a standardised intensity of UV-B. In reality, UV-B 22 is a fluctuating environmental variable, the intensities of which can vary dramatically across 23 multiple timescales. On a daily basis, UV-B intensities vary in a predictable manner with the solar 24 angle, but also in a much less predictable manner depending on factors such as cloud cover, 25 albedo, and air pollution (Madronich et al., 1998; Calbó et al., 2005; Barnes et al., 2017). On a 26 seasonal basis there are similar predictable and less predictable fluctuations in UV-B intensities, 27 depending on geographic, climate and weather factors. This discrepancy between UV-B exposure 28 in laboratory and natural conditions makes it difficult to compare and extrapolate data between 29 the two experimental approaches. Indeed it is frequently found that results from indoor and 30 outdoor experiments differ significantly from each other.

31

32 An additional factor that complicates the comparison of indoor and outdoor studies is that plants 33 under outdoor conditions are simultaneously exposed to a broad range of environmental factors, 34 which may act as stressors and/or signals. Some of these factors induce plant responses 35 independently from UV-B, while others trigger interactive effects with UV-B radiation (Bornman 36 et al., 2015). In principle, the responses driven by different environmental factors can be 37 synergistic, additive or antagonistic (See editorial). For example, UV-B radiation has been shown 38 to impede plant thermomorphogenesis in a UVR8 dependent manner (Hayes et al., 2017), and 39 this can potentially contribute to increased sensitivity to high temperatures. Conversely, UV-B 40 radiation induces production of a range of volatile isoprenes which have been associated with 41 heat tolerance (Liu et al., 2017). Thus, interactions between the responses to UV-B and other 42 environmental variables can be complex. This is also the message that arises from studies on the 43 interaction between UV-B and drought. In some studies it was found that UV-B can diminish the 44 negative effects of drought exposure (Kovacs et al., 2014; Robson et al., 2015;), while in another 45 study it was found that exposure to UV-B and drought synergistically enhanced negative effects 46 on photosynthesis (Doupis et al., 2016). A better understanding of how UV-B can alter plant 47 responses to other environmental variables, and vice versa, is important to fully appreciate the ecological role of UV-B radiation. Furthermore, a better understanding of interactions between
 UV-B and global climate change factors (e.g. extreme temperatures, drought, elevated CO₂) is

- 3 critical for climate change predictions (Bornman et al., 2015).
- 4

5 A third factor that is not commonly considered when studying plant UV-responses is within 6 species genetic variation. The ability of plants to adapt to local geographical and climatic 7 conditions is an important selective force which has led to within species genetic variation 8 (Shindo et al., 2007). Adaptations to local conditions have led to ecologically specialized 9 accessions with optimised performance in a given region. As an example, Arabidopsis thaliana 10 shows considerable genotypic and phenotypic variation, including different degrees of tolerance 11 to stressors such as salinity, drought and extreme temperatures (Koornneef et al., 2004). 12 Arabidopsis has a wide distribution, including Europe, Asia, north America and Africa. Across its 13 natural distribution area there is significant variation in the intensity of UV-B experienced at 14 ground level, depending on latitude and altitude (Liley & Mckenzie, 2006). Substantial differences 15 in protection of photosystem II of photosynthesis amongst UV-exposed Arabidopsis accessions were reported by Jansen et al., (2010). Cooley et al., (2001) detailed differences in morphological 16 17 responses to UV-B by different accessions. Significant understanding of the ecological role of UV-18 B radiation can be gained from studies of natural accessions. However, at present there is 19 insufficient information about the behavior of such accessions under ambient UV-B conditions. 20

In this study we have explored effects of UV-B on plant morphology and UV-screening pigments across an entire calendar year under outdoor conditions. Specifically, we assessed the role of seasonality on plant UV-B responses. It was hypothesised that UV-B effects will be substantially modulated by other environmental factors. Furthermore, UV-responses of *Arabidopsis thaliana* accessions Ler and Col-0 were compared with those by a local accession, Bur-0, to identify differential adaptation to UV-B.

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29 <u>Materials and Methods</u>30

31 <u>Plant Material</u>

33 Seeds of three *Arabidopsis thaliana* accessions were kindly donated by Prof. Koornneef 34 (Wageningen University, The Netherlands and MPIZ, Cologne, Germany), and had been 35 propagated for several generations under controlled conditions prior to use in the described 36 experiments. Burren-0 (Bur-0) originates in the Burren in the west of Ireland. Columbia-0 (Col-37 0) and Landsberg erecta (Ler) are the two accessions of *Arabidopsis thaliana* most commonly used 38 in research.

39

40 Seeds of Arabidopsis thaliana accessions Ler, Col-0 and Bur-0 were cold-treated for a minimum 41 of seven days before being sown into trays containing sieved John Innes No.2 compost (J. Arthur 42 Bowers, William Sinclair Horticulture Ltd., Firth Rd., Lincoln, LN6 7AH). The flats were covered 43 with transparent film and kept at 21°C under a 16 hour light/ 8 hour dark photoperiod, with 60-44 80µmol m⁻² s⁻¹ PAR. The transparent film was taken off once a substantial number of seeds had 45 germinated. The seedlings were pricked out into 200ml pots with John Innes No. 2 compost while 46 still at the cotyledon stage (Fig. 1). When the seedlings had grown in to the 1.02 stage (Boyes et 47 al., 2001) they were moved to a glasshouse and next to a cold frame to facilitate acclimation to 48 natural weather conditions (Fig. 1). Plants were considered to be ready for experimental use at the Boyes 1.04 stage. At the onset of this stage plants were transferred to outdoor conditions for a total of 10 days. This experimental approach was repeated seven times across the year, exposure to outdoor conditions taking place in January, February, May, July, September, October and November.

- 4 5
- 6 <u>UV-exposure Conditions</u>

7 8 A UV-filtration approach, using natural solar light, was used in order to manipulate UV-levels. 9 Three distinct filtration treatments were used; (1) UV-A/B (exposure to visible light + UV-A + UV-10 B) using 95µm thickness UV transparent cellulose acetate filter (Kunststoff-Folien-Vertrieb 11 GmbH, Hamburg, Germany); (2) UV-A (exposure to visible + UVA) using 125µm thickness UV-B 12 blocking mylar filter (Polyester film, Tocana Ltd., Ballymount, Dublin, Ireland); (3) UV-0 13 (exposure to visible) using a UV opaque filter (poly-tunnel plastic, BPI Visqueen, Stevenston, 14 U.K.). The cellulose acetate and mylar were changed after 20 days exposure to solar light to 15 prevent changes of the light spectrum caused by degradation of the plastic. The transmission of 16 the filters was routinely measured using a spectrophotometer (Shimadzu - UV visible 17 spectrophotometer- 160A).

18

Boxes measuring 50cm x 50cm were assembled using opaque corri-board (See Coffey et al., 2017). The lids of these boxes comprised the filters that were positioned above the Arabidopsis 21 plantlets. Each filtration treatment comprised four independent replicates. The boxes were 22 randomly positioned at a sun exposed site in Cork, Ireland (51°53'58"N 8°29'14"W). The boxes 23 were slightly angled to make possible air circulation with the northern edge of the frame raised 24 above the ground. Four individual plants of each accession were placed in each box.

25

26 <u>Plant parameters</u>

After ten days of growth under outdoor conditions, both leaf and rosette morphology were
quantified. Rosettes were first dissected and then immediately photographed for processing with
ImageJ (Abramoff et al., 2004). Morphological parameters such as rosette diameter (mm),
biomass (mg) and leaf area (mm²) were all determined. The smallest leaves (defined as having a
petiole of less than 2mm) were not included in analysis.

33

Total UV-absorbing pigments were extracted from leaf number four and normalized using the area of that specific leaf. Leaves, including the petioles were put in to micro-tubes with 1ml acidified methanol (1%HCL, 20%H₂O, 79% CH₃OH) and incubated in the dark at 4°C for four days. Absorbance was recorded at 330nm on a spectrophotometer (Shimadzu – UV visible spectrophotometer- 160A). Absorbance was normalized per leaf using leaf area.

39

Imaging chlorophyll *a* fluorometry (Imaging PAM Waltz, Germany) was used to determine the maximal quantum yield of photosystem (PS) II (Fv/Fm). Fv/Fm values were determined after plants had been grown for ten days under outdoor conditions under different filtration treatments. Whole rosettes were dark adapted for a minimum of 20 minutes before Fv/Fm was determined using an Imaging PAM (Waltz, Germany). Three measurements were taken at random from each rosette and pooled per rosette.

- 46
- 47 <u>Statistical Analysis</u>
- 48

1 Statistical relationships between plant growth responses and environmental variables such as 2 temperature, hours of sunshine and global solar radiation and UV treatment, were tested using

- 3 multiple regression analysis with IBM SPSS Statistics 21. As a first step, it was established that all
- 4 data sets were suitable for regression analysis and that there was no violation of the assumption
- 5 of linear multicollinearity and homoscedasticity. The meteorological data used for this study were
- 6 obtained from Met Eireann (65/67 Glasnevin Hill, Dublin 9, D09 Y921). It was found that there
- 7 was a high degree of correlation between the independent variables temperature, hours of
- 8 sunshine and global solar radiation. For this reason, these variables were analysed in separate
- 9 regressions.
- 10

To acquire a more comprehensive understanding of the influence of UV treatment under varying weather conditions (seasonality), the months of January and July were selected as case studies and analysed in more detail. Prior to such analysis, all data sets were assessed for normality. The biomass dataset was non-normal, and a square root transformation was applied prior to statistical analysis. All data were analysed statistically using parametric interaction ANOVAs, with multiple comparison test being carried out using Tukey's range test.

17

18

19 <u>Results</u> 20

Arabidopsis plants at the Boyes 1.04 growth stage were transferred to outdoor conditions where the plants were kept for 10 days. In all cases plants grew during the outdoor period, and this was seen as increases in the number of leaves, and rosette diameter.

24

25 The data on plant biomass, rosette diameter and leaf area (Fig. 2A, B, C, respectively) show that 26 the rate of growth is season dependent. Plants in July and September display the greatest rosette 27 diameter, leaf area and aboveground weight. Rosettes are smallest and weigh least in January. A 28 similar seasonality patterns is revealed using chlorophyll *a* fluorometry, with Fv/Fm values as 29 low as 0.65 in January, and greater than 0.80 in October. Concentrations of UV absorbing 30 pigments displayed a distinctly different seasonal pattern (Fig. 3), with high concentrations of 31 pigments measured in the winter, and in many cases some 3-4-fold lower concentration in 32 summer.

33

34 To analyse these plant growth responses in the context of weather conditions, a full set of 35 meteorological data were obtained from the Irish meteorological service (Met Eireann) to cover 36 the period of the growth trials (details see Coffey et al., 2017). Temperatures during the trial period ranged between 3.1 and 15.6°C. Total hours of sunshine and UV-B doses ranged between 37 3 to 7 hours and between 1.18×10^6 to 2.58×10^7 J/m² per day, respectively. Monthly means of 38 global solar radiation during the trial ranged between 6.46 x 10^7 and 5.7 x 10^8 J/m². The 39 meteorological parameters temperature, global solar radiation, hours of sunshine and UV-B 40 41 irradiance were all significantly correlated with each other. The correlation between the 42 meteorological parameters means that they lack independence and have to be analysed in separate multiple regressions. The dependant variables were expressed as follows: Dependent 43 44 variable = constant + $(B1 \times Temp)$ + $(B2 \times uv-a/b)$ + $(B3 \times uv-a)$. B1, 2 and 3 are slope coefficients 45 of the independent variables, used to obtain the R² values, i.e. the percentage variation in the 46 dependent variable which can be attributed to the independent variable. The significance of the 47 relationship and thus the R^2 variable is determined by an F-test. The constant in a multiple

1 regression is the value of the dependent variable when all other variables are zero. As this 2 scenario is outside the scope of the observed data, changes in the constant have not been 3 analysed. Regression analysis of meteorological parameters versus the measured biological responses identified several significant correlations (R² values) (Table 1). Using temperature as 4 5 the independent variable produced the highest R² values indicating that temperature is the 6 strongest determinant of plant size and total UV absorbing pigment content (Table 1). 7 Temperature accounted for between 49 and 74% of the variation in rosette diameter, leaf area, 8 total UV absorbing pigments and Fv/Fm for Ler, Col-0 and Burren-0. Hours of sunshine accounted 9 for between 7 and 41% of the variation in biological responses, while global solar radiation and 10 UV-B irradiance contributed 15 – 49% and 9 - 37%, respectively (Table 1).

11

12 Graphs of rosette diameter as a function of temperature visualize the positive association 13 between growth and temperature, as well as the relative lack of impact of genotype (Fig. 4A, B, 14 C). Further exploration of the correlation between temperature and the biological responses 15 included the UV filters as independent variables (Table 2). The UV-A/B and UV-A treatments were 16 compared to the UV-0 treatment which acted as a control. This approach allowed for 17 identification of the impact that the filters had on the fit of the regression, within the context of 18 the seasonal trend, which was largely dominated by temperature. The slope in table 2 (Beta 19 coefficient) indicates how much the dependant variable varies with each independent variable 20 when all others are held at a constant. The part number squared describes the contribution that 21 each independent variable makes to the total R², and thus to the variation in the dependant 22 variable. From the Part No. Squared it is evident that temperature accounts for a large part of the 23 R^2 value for all biological responses, but there is also evidence that the UV-A/B treatment 24 contributes significantly to the regression (Table 2). Specifically, leaf area and rosette diameter 25 are significantly affected by the UV-A/B treatment, in Ler, Col-0 and Bur-0 (Table 2). Between 3-26 7% of the variation in the R² value is accounted for by the UV-A/B treatment and the sign 27 associated with these values indicates UV has a negative impact (Table 2). Thus, UV-B has a 28 negative impact on the positive slope-values (Fig. 4A, B, C; Table 2) of the relationship between 29 temperature and rosette diameter. The graphs of rosette diameter as a function of total, seasonal 30 UV-dose (Fig. 4 D, E, F) show an overall positive relationship. This reflects the close association 31 between seasonal fluctuation in UV and other growth promoting weather conditions. However, 32 in this instance the, positive association is diminished by the actual UV-filtration treatment (Table 33 2). Thus, in both types of analysis the UV-A/B treatment has negative effect on leaf area and 34 rosette diameter as indicated by the slope values (Table 2).

34 35

36 Across the yearlong study, and across all three accessions studied, there was no significant filter 37 effect on photosynthetic efficiency measured as Fv/Fm (Table 2). Yet, a significant relationship of 38 Fv/Fm with temperature was found, as temperature increases so did the efficiency of PSII (Table 39 2). UV-absorbing pigments were also not affected by filter type in the three accessions (Fig. 3 & 40 Table 2). UV-absorbing pigments increased during the winter months and decreased during the 41 summer months, this trend was the reverse of the trend observed for Fv/Fm and other plant 42 growth parameters (Fig. 2). Graphs of UV-screening pigments as a function of temperature 43 visualize the negative association between pigments and temperature, as well as the near 44 complete lack of impact of genotype (Fig. 5A, B, C). UV-B treatment also has no effect on the slope-45 values (see Table 2) that detail the relationship between temperature and UV screening pigments. 46 Similarly, graphs of pigments as a function of total, seasonal UV-doses (Fig. 5D, E, F) show a weak 47 overall negative relationship, and no impact of filters (Fig. 2 & Table 2).

- 1
- 2 To further explore the dataset, two months were chosen for more detailed analysis. January and
- 3 July were chosen as representative of the months with the highest and the lowest incidents of UV-
- 4 B. In January, there was no significant effect of UV treatment on the morphology of accessions
- 5 (Table 3). There were, however, significant differences between the accessions in rosette
- 6 diameter (F (2, 27) =22.613. p=0.0001) (Table 3). Both Col-0 and Bur-0 had larger rosette
- 7 diameters than Ler, 21% and 16% respectively (Table 3). The only other significant effect was on
- 8 UV-screening pigments; UV-A treated plants had higher total UV-absorbing pigment levels than
- 9 the UV-B treated plants and while this difference was significant the actual difference between
- 10 the treatments was small (F(2, 27) = 0.772, p=0.001) (Table 3).
- 11

12 In July, there was clear evidence of a UV effects on biomass, rosette diameter and leaf area of 13 exposed plants. The biomass of plants grown under the UV transmitting filters was between 42 14 and 52% less than that of plants grown under the UV-0 treatment (F(2, 18)=12.137, p=0.0001) 15 (Table 3). Rosette diameter was between 18 and 37% less (F(2, 18)=670.466, p=0.0001) and leaf 16 area was between 33 and 48% less (F(2,18)=19.929, p=0.0001) compared to plants grown under 17 the UV-0 treatment (Table 3). In July, there was also a significant difference between the rosette 18 diameters of the three accessions, Bur-0 was on average 18% larger than Ler and 17% larger than 19 Col-0 (F(2, 18)=167.933, p=0.05) (Table 3). No effects of accession or filter were found on either

- 20 Fv/Fm or total UV-absorbing compounds.
- 21 22

23 Discussion

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25 <u>Plant growth and development</u>26

This study was set over the course of a year to determine if, and how, plant UV-B responses are moderated by seasonal meteorological factors, and *vice versa*. The Irish climate is described as oceanic and characterised by high levels of rainfall, relatively low hours of direct sunshine, and a lack of temperature extremes. Due to these mild climatic conditions, some plant species display nearly year round growth, enabling the study of interactions between growth and climatic variables such as temperature, hours of sunshine, global radiation and UV irradiance, throughout the four seasons.

34

35 The data show that significant variations in plant growth and concentrations of total UV-36 absorbing pigments occur throughout the year. It was found that variations in growth, 37 morphology, and photosynthesis are predominantly linked to seasonal changes in temperature. 38 Not unexpectedly, biomass, rosette diameter, leaf area and Fv/Fm all increased with higher 39 temperatures and decreased again as temperatures dropped. Overall, the seasonal pattern of 40 growth was not substantially distorted by the three distinct UV-filter treatments. In fact, no effect 41 of the UV-filtration treatment was observed across the winter months. However, there were 42 significant UV mediated differences in biomass and morphology found over the summer months. 43 Arabidopsis thaliana accessions Ler, Col-0 and Bur-0 exhibited a more dwarfed phenotype when 44 grown under UV-A/B or UV-A in the months of May and July. A slightly more compact plant is 45 considered a typical morphological response to UV-B exposure (Robson et al., 2015). The UVR8 46 photoreceptor was first discovered as a mutant unable to undergo such UV-mediated dwarfing 47 (Heijde and Ulm, 2012; Jenkins 2014). However, a more dwarfed phenotype can also be an indication of plant stress. Stress-induced Morphogenic Responses (SIMR) can produce a dwarf 48

1 phenotype in response to exposure to a range of unfavourable conditions (Potters et al., 2007). 2 UV-induced stress is considered rare in plants under ambient conditions, however there are two 3 reasons why stress may have occurred in this study; 1) plants used in this experiment came from 4 a greenhouse and could potentially have experienced an initial UV shock when placed outdoors; 5 2) Arabidopsis thaliana set seed and die back during the summer months, thus would not 6 normally be exposed to high, summer UV-intensities in combination with high levels of PAR and 7 elevated temperatures. Measurements of Fv/Fm generated no evidence of plant stress. Yet, a 8 substantial decrease in accumulated biomass was noted, a feature not typically seen as part of a 9 UVR8 mediated re-direction of plant growth. Furthermore, in a parallel experiment under the 10 same weather conditions it was noted that the Arabidopsis mutant uvr8-1, which lacks functional 11 UVR8, also displayed dwarfing and growth inhibition during the summer months (Coffey et al., 12 2017). Finally, plants grown under UV-A radiation displayed similar dwarf morphology as plants 13 grown under UV-A/B, further emphasizing that the observed change in rosette diameter is not 14 necessarily associated with UV-B, but rather is a general UV-response. Thus, based on the 15 observation of parallel reductions in biomass, leaf area and rosette diameter, plus the observation 16 of a similar response in the uvr8-1 mutant, we conclude that the UV-A/B induced change in 17 morphology is not UVR8 mediated acclimation, but rather a stress response that we speculate to 18 be the result of exposure to high summer temperatures, high PAR, drought in combination with 19 either UV-A or UV-B.

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22 UV-absorbing pigments

24 UV-mediated increases in UV-absorbing pigments are amongst the most commonly reported 25 plant UV-responses (Rozema et al., 1997; Jansen et al., 1998; Neugart et al., 2014). However, in 26 this study no UV-B-mediated changes in the concentrations of UV-absorbing pigments were 27 noted, notwithstanding significant effects of UV-radiation on biomass, leaf area and rosette 28 diameter. Rather, temperature was identified as the primary driver behind the seasonal changes 29 in UV absorbing pigments. Previous studies also reported that the strong effect of temperature 30 on concentrations of UV absorbing pigments can mask smaller changes induced by UV (Leyva et 31 al., 1995; Bilger et al., 2007). Outdoor studies on lichens and mosses revealed that seasonal 32 variations in environmental conditions elicited much larger changes in UV-absorbing pigments 33 than UV-B (Gehrke, 1999; Bjerke et al., 2005). Long term field studies in Tierra del Fuego 34 (southern Argentina) show that UV-B induced increases in UV-B absorbing pigments can be 35 observed in some years, but not in many others. The lack of UV-induced changes in total UV-36 absorbing pigment concentration does trigger questions about the importance of UV-sensing by 37 plants. This is an intriguing question. Yet, it should be recognized that although this study failed 38 to reveal a UV-induced change in the total concentration of UV screening pigments, this does not 39 necessarily mean that no changes in UV-B absorbing pigments occur. For example, it cannot be 40 excluded that some redistribution of flavonoids between epidermis and underlying mesophyll 41 cells takes place, such a redistribution might be visualized using Dualex and other optimal 42 measurements. Furthermore, not just the total amounts of flavonoids but also the flavonoid 43 profile can be modified by environmental variables. Studies using supplemental UV-B have shown 44 UV-induced changes in the ratio between quercetins and kampferols, as well as in the specific 45 flavonoid-glycosylation pattern (Hectors et al., 2014; Neugart et al., 2014). For example, kale 46 (Brassica oleracea var. sabellica) exposed to a low temperature of 5°C accumulated almost twice 47 as much of the polyphenol, kaempferol-3-0-sophoroside-7-0-glucoside, as plants at 15°C.

1 However, kale plants at 15°C accumulated ca 25% more kaempferol-3-O-caffeoyl-sophoroside-7-2 O-glucoside (Neugart et al., 2014). These subtle shifts in flavonoids with different hydroxylation 3 and/or glycosylation status can be visualized through HPLC analysis, or as alteration in 4 antioxidant activity measured with, for example, the Folin-Ciocalteu assay. Outdoor studies on 5 birch trees have also revealed changes in concentrations of individual phenolic compounds 6 change in response to UV-B radiation rather than the size of the total phenolic pool (Kotilainen et 7 al., 2009; Morales et al., 2010). At present, the function of these subtle changes in phenolic profile 8 is not clear. Thus, an important quest will be to characterize specific UV and/or temperature

- 9 induced changes in the phenolic profile, and to analyse the scope for cross-protection.
- 10
- 11 <u>Genotypes</u> 12

13 Genotypic differences between accession can be significant and have the potential to enhance our 14 understanding of the ecological role of specific adaptations. Cooley et al., (2001) compared the 15 responses of seven accessions exposed to supplementary UV-A and UV-A/B under outdoor 16 conditions in the period May to June. Several morphological parameters were measured and 17 compared. Plant responses were found to range from UV insensitive, promotive to inhibitory, and 18 results varied with treatment, accession and the parameter measured (Cooley et al., 2001). No 19 such accession specific responses were observed in this paper. In the study by Cooley et al. (2001) 20 Ler and Col-4 responded to supplemental UV-A/B, but not to UV-A, by reducing leaf area, width 21 and length and petiole length significantly. These data on inhibitory UV effects during the summer 22 period, are similar to those reported in this paper. However, the current study goes one step 23 further, and shows that the UV-responses are highly dependent on seasonal factors, and that 24 distinct results will be obtained depending on the time of year. It is concluded that a fixed time

- 25 point approach does not necessarily show the full scope of variation in plant UV-responses.
- 26

27

28 <u>In conclusion</u>

29 30 Arabidopsis is widely used for mechanistic studies of plant responses but is seldom used in 31 outdoor trials. This study highlights the potential discrepancies between findings obtained under 32 controlled conditions and in the outdoors. Responses routinely observed under laboratory 33 conditions, including UVR8 mediated changes in total UV-absorbing pigments, and plant 34 morphology, were not observed in this outdoor study. This study finds a clear UV induced 35 morphological effect, though only in the summer, and possibly associated with plant stress. 36 Conversely, low temperatures were identified as a major regulator of the accumulation of UV-37 absorbing pigments. This study shows that UV-effects need to be analysed in the context of 38 weather, and other co-occurring natural factors, and emphasizes the importance of a holistic, 39 multifactorial approach for the investigation of environmentally relevant UV-effects

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44

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 \mathbb{R}^2 values from the multiple regression model, using data from all seven months. Dependent variable = Constant + (B1 x Temp) + (B2 x uv-a/b) + (B3 x uv-a), asterisks are used to indicate significance of the R^2 value (* = p ≤ 0.05, **=p≤0.001, *** = p≤ 0.0001).

	Temperature			Hours of sunshine			Global solar radiation			UV-B		
	Ler	Col-0	Bur-0	Ler	Col-0	Bur-0	Ler	Col-0	Bur-0	Ler	Col-0	Bur-0
Rosette	0.73	0.61	0.74	0.30	0.30	0.3	0.36	0.39	0.39	0.22	0.31	0.26
	***	***	***	***	***	***	***	***	***	***	***	***
Leaf	0.74	0.65	0.63	0.36	0.41	0.31	0.43	0.49	0.43	0.28	0.37	0.34
Area	***	***	***	***	***	***	***	***	***	***	***	***
Total	0.7	0.72	0.7	0.17	0.21	0.27	0.2	0.22	0.27	0.09	0.11	0.16
Phenolics	***	***	***	**	***	***	***	***	***		*	*
Fv/Fm	0.49 ***	0.55 ***	0.61 ***	0.07	0.12 **	0.16 **	0.15 ***	0.26 ***	0.26 ***	0.09	0.18 *	0.17 *

Table 2. Slopes (beta coefficient), their significance and the Part Nos. Squared from a
multiple linear regression model (Dependent variable = Constant + (B1 x Temp) + (B2
x uv-a/b) + (B3 x uv-a)) including temperature and the 3 filters as independent
variables. The slope informs if a particular variable is making a statistically significant
and unique contribution to the equation. The Part No. Squared describes the unique
contribution that independent variable makes to the total ${\ensuremath{R}}^2$ and thus to the variation
in the dependent variable.

	Ler			Col-0			Bur-0			
		Slope	Sig	Part No. Squared	Slope	Sig	Part No. Squared	Slope	Sig	Part No. Squared
Leaf	Temp	0.82	***	0.67	0.78	***	0.6	0.77	***	0.6
area	UV-A/B	-0.30	***	0.07	-0.26	**	0.05	-0.21	**	0.03
	UV-A	-0.17	*	0.02	-0.10	ns	7.7x10-3	-0.07	ns	4.23x10-3
Rosette	Temp	0.82	***	0.67	0.73	***	0.53	0.82	***	0.69
Diamete	UV-A/B	-0.27	***	0.06	-0.33	***	0.08	-0.24	**	0.04
	UV-A	-0.13	ns	0.01	-0.16	ns	0.02	-0.04	ns	1.37x10-3
Fv/Fm	Temp	0.69	***	0.47	0.74	***	0.54	0.77	***	0.6
	UV-A/B	-0.10	ns	8.1x10-3	0.001	ns	1x10-6	-0.10	ns	6.89x10-3
	UV-A	-0.14	ns	0.01	-0.07	ns	3.14x10-3	-0.16	ns	0.02
Total Phenolic	Temp	-0.84	***	0.72	-0.84	***	0.72	-0.84	***	0.7
	UV-A/B	-0.03	ns	5.29x10-4	-0.08	ns	4.6x10-3	-0.01	ns	8.1x10-5
	UV-A	0.07	ns	3.6x10-3	0.003	ns	9x10-6	-0.67	ns	3.36x10-3

				January						
Main Effects		-	Biomass (mg)	Rosette Diameter(mm)	Leaf Area(mm ²)	Total Phenolics	Biomass (mg)	Rosette Diameter(mm)	Leaf Area (mm ²)	Total Phenolics
Accession	on Ler		11.26 a	12.06 a	8.75 a	0.0152 a	137.54 a	31.05 a	73.01 a	0.0054 a
	Col-0		11.39 a	15.23 b	10.10 a	0.10 a 0.0161 a		31.40 a	78.31 a	0.0068 a
	Bur	-0	12. 18 a	14.29 b	11.56 a	0.0160 a	150.77 a	37.69 b	78.55 a	0.0068 a
Filter	UV-	A/B	11.14 a	13.21 a	9.0824 a	0.014 a	105.21 a	25.90 a	54.28 a	0.0058 a
	UV	-A	11.06 a	13.92 a	9.8979 a	0.018 b	126.60 a	33.38 b	68.89 a	0.0058 a
	UV	-0	12.63 a	14.46 a	11.4301 a	0.016 ab	217.71b	40.85 c	102.77 b	0.0073 a
Df						ANOVA	•			
F value F	Ecotype	2	0.556	22.613	2.736	0.772	0.213	167.933	0.226	2.903
	Sig		ns	***	ns	ns	ns	*	ns	ns
F value	Filter	2	1.761	2.856	1.961	9.505	12.137	670.466	19.929	2.837
	Sig		ns	ns	ns	**	***	***	***	ns
Genotype	x Filter	4	0.482	0.406	0.558	0.83	0.187	57.349	0.956	0.863
	Sig		ns	ns	ns	ns	ns	ns	ns	ns
	Total Jan July	27 18								

Table 3. Summary of two-way ANOVAs on the effects of accession and filter type on biomass (mg), rosette diameter(mm), leaf area (mm²) and total UV-absorbing pigments in Arabisdopsis grown outdoors for 10 days in January 2013 and July 2013.

ns= not significant, * = p ≤0.05, **=p≤0.001, *** = p≤ 0.0001, according to two-way ANOVA. Comparisons to be made within columns Means in the

same column and same main effect with the same letter are not significantly different, p>0.05 according to Tukey tests.

Legends to the figures

Figure 1. Preparation of plant material used in exposure studies.

Figure 2. The biomass (mg), panel (a), rosette diameter (mm) panel (b) and leaf area (mm²) panel (c) of rosettes of Arabidopsis accessions Landsberg erecta (Ler), Columbia-0 (Col-0) and Burren-0 (Bur-0) grown for 10 days outdoors at 7 different time-points throughout the year. Biomass and leaf area data represents data for leaf 4 of the rosettes. Error bars represent the standard deviation from the mean of 4 replicates. Starting rosette diameter, biomass and leaf area of the whole rosettes was 9 mm or less, no more than 5 mg and less than 50 mm² respectively. Filter specifications: UV-A/B (visible + UVA and UVB), UV-A (visible + UVA) and UV-0 (visible).

Figure 3. Total UV-absorbing pigments in leaf 4, extracted with a 1% acidified methanol solution and normalized using leaf area. Data show pigment content for Arabidopsis accessions Landsberg erecta (Ler), Columbia-0 (Col-0) and Burren-0 (Bur-0) grown for 10 days outdoors, at 7 different time-points throughout the year. Error bars represent the standard deviation from the mean of 4 replicates.

Figure 4. Pearsons correlation between rosette diameter and 2 independent variables temperature and UV-B for 3 Arabidopsis accessions, Ler (a, d), Col-0 (b, e) and Bur-0 (c, f). \blacklozenge = UV-A/B == UV-A \blacktriangle =UV-0.

Figure 5. Pearsons correlation between accumulated UV-absorbing pigments, and the 2 independent variables temperature and UVB for 3 Arabidopsis accessions, Ler (a, d), Col-0 (b, e) and Bur-0 (c, f). \blacklozenge = UV-A/B = UV-A \blacktriangle =UV-0.



Figure 1



Figure 2.



Figure 3.



Figure 4.



Figure 5.