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Research article

High impact of seasonal temperature changes on acclimation of photoprotection and radiation-induced damage in field grown *Arabidopsis thaliana*☆

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ABSTRACT

At temperate latitudes environmental factors such as irradiance, including ultraviolet-B radiation (UV-B, 280–315 nm), temperature and day length vary widely over the course of a year in a concerted way. In the present study physiological acclimation of photoprotection, growth and development of the model organism *Arabidopsis thaliana* were correlated to these strongly but gradually changing conditions in a one year field study. Plants were sown in the field avoiding any manipulation (and abrupt change) during their life. Developmental rate was strongly dependent on prevailing temperature. Moderate signs of light stress in form of photoinhibition at photosystem II were significantly related to solar irradiances while amount of DNA damage was low and not correlated to UV-B irradiance. Although all the markers were hypothesized to primarily react to radiation, multiple regression analysis showed at least a similarly strong influence of temperature as that of light. Especially for the classical UV screening compounds a positive correlation to UV-B radiation during the course of the year was absent, whereas there was a significant negative correlation between temperature and quercetin content. The sum of violaxanthin cycle pigments was correlated to both, irradiance and temperature, but with opposite sign. Epidermal UV-B transmittance was also much better related to air temperature than to UV-B irradiance. The data show that under natural conditions temperature has at least a similar importance for photoprotective acclimation and partially also for photosensitivity as solar irradiance.

1. Introduction

In the first years after the discovery of the ozone hole, UV-B has been considered as a threat to plants (Fiscus and Booker, 1995). In recent times, however, it has been recognized that stress effects caused by solar UV-B radiation are rarely seen and the signalling role of this radiation is considered more important (Jansen and Bornman, 2012). Plants growing in the field may be much more resistant to UV-B radiation than laboratory grown plants due to acclimation. This acclimation may be a response to UV-B radiation directly or may be a cross-resistance, caused by signalling cross-talk with other environmental factors.

Reports on UV-B mediated responses in *Arabidopsis thaliana* in the field are confined to short-term exposures or plants transferred form the greenhouse to the field (Morales et al., 2013; Coffey et al., 2017). Effects of artificially increased UV-B radiation in field studies have been presented in a meta-analysis by Searles et al. (2001). One of the few consistent responses to enhanced UV-B radiation was an increase in UV screening pigments. Responses to UV-B have also been implied in shade avoidance reactions of plants (Fraser et al., 2016).

The natural situation is characterized by the simultaneous presence of a multitude of environmental variables which may not only exert their specific physical effects on a plant but will also induce longer lasting acclimatory responses within the plant. The simultaneous presence of the influence of several environmental factors may manifest itself as signalling cross-talk, or may lead to damage of the plant when several unfavourable environmental variables act synergistically (Jansen et al., 2018). Thus, it is possible that during the seasonally varying combinations of environmental conditions at some time points UV-B radiation may cause eustress while at others distress (Jansen et al., 2018). For example, the spectral composition of sunlight may ameliorate UV-B effects on DNA integrity through efficient

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photoreactivation. On the other hand, other factors, such as high or low temperatures, may simultaneously impede photoreactivation and exacerbate damaging UV-B effects.

Acclimation to UV-B radiation includes synthesis of UV absorbing pigments, upregulation of photoreactivation and antioxidant defence as well as photomorphogenic responses (Jansen and Bornman, 2012). Some of the acclimation responses have recently been shown to be regulated by the UVR8 photoreceptor (Morales et al., 2013; Coffey et al., 2017). Under controlled conditions, also other photoreceptors, and even light-independent signalling, have been demonstrated to lead to similar acclimation reactions as observed under natural conditions. One should expect that these mechanisms are at work also in the field. Among those other factors, low temperature has been observed to induce accumulation of polyphenolics (Olsen et al., 2009). Accordingly, Coffey et al. (2017) suggested that concentrations of UV-B absorbing pigments in *A. thaliana* planted outdoors were controlled by seasonal variation of temperature. In addition, these authors saw lower contents of UV-B protective pigments in UV-B irradiated *uvr8* mutants which they interpreted as UV-B induced inhibition of pigment biosynthesis due to DNA damage or oxidative stress. Unfortunately, they did not study DNA damage in these plants. Therefore, it remains unclear if solar UV-B induced DNA damage might affect production of UV-B absorbing compounds.

In addition to the contents of these compounds, cellular UV-B tolerance by repair processes acclimates to irradiance. It was demonstrated that repair of DNA damage by photoreactivation increased under artificial high-light and even stronger under natural solar irradiation in rice (Kang et al., 1998). UV-B dose-dependency deduced from experimental induction of DNA damage predicted between 400 and 800 dimers per megabase at the end of a sunny day without simultaneously ongoing photoreactivation (Dany et al., 2001; Stapleton et al., 1997). Therefore, the absence of such high concentrations of dimers was interpreted as efficient photoreactivation in field grown maize (Stapleton et al., 1997). To our knowledge, seasonal courses of DNA damage or photoreactivation have not been reported yet.

A. thaliana is a highly popular model plant, but very little data exist on its performance under natural conditions, not only with respect to UV-B irradiance, but also to other environmental conditions. When grown in controlled conditions, environmental parameters are usually chosen which are quite far from those encountered naturally (Poorter et al., 2016). The morphological and developmental plasticity together with physiological acclimation under natural seasonally changing conditions have rarely been studied. Furthermore, reactions of plants which have never experienced a manipulative change in their lives may be different from those of plants that have germinated and developed leaves before they were transferred to the field. Since there exists little information on the morphology and physiology of *A. thaliana* in the field, we determined basic data on the growth and development of the plants. It is known that time until flowering, and accordingly maximal leaf number and rosette size, are strongly dependent on the seasonally varying length of photoperiod as well as on vernalisation (Wilczek et al., 2009). Among the large variety of ecotypes of *A. thaliana* we chose Landsberg erecta which is an accession which does not require vernalisation for flowering (Johanson et al., 2000). It has been characterized as an early flowering ecotype with a rapid life cycle with several potential generations per year (Wilczek et al., 2009). Therefore, a completion of the life cycle of this genotype should be possible throughout most of the year, which allowed for comparison of consecutive cohorts over the course of a year.

Due to the relative independency of the development of the used accession of *A. thaliana*, it was possible to study the response of plants of a similar developmental stage to the varying environmental conditions during the annual time course. We hypothesized that properties characterizing the acclimation of plants to solar radiation, such as epidermal UV screening, polyphenolic compounds and photoprotective pigments in the photosynthetic apparatus, would seasonally acclimate to changes in solar irradiance. Accordingly, we expected that parameters commonly used for quantifying damage caused by solar radiation such as maximal PS II quantum yield and the amount of thymine dimers would correlate with light conditions.

2. Material and methods

2.1. Plant material and analysis of phenology

From July 14, 2014 until August 10, 2015 *Arabidopsis thaliana* (L.) Heynh., accession Landsberg erecta, was sown every other week except from November 2014–February 2015. Approximately 15–20 seeds were homogenously sown in 10 round plastic pots (14 cm diameter) filled with peat soil substrate (Einheitserde classic, Einheitserdewerke Werkverband e.V., Sinntal-Altengronau, Germany). The pots were completely dug in the soil leaving the plants on the same level as the surrounding. After germination, plants were removed if necessary to give a homogenous maximum plant density of 10 plants per pot. The surrounding soil and the pots were weeded regularly. Watering was done if natural rainfall was not enough to avoid drought stress. All pots were photographed (Canon, Powershot A 80, Tokyo, Japan) weekly until plants developed fruits. From these pictures several phenological and developmental parameters were derived according to the classification system by Boyes et al. (2001). The date was determined on which the young plants reached growth stage 1.0 with the two cotyledons fully developed. Maximum plant radius was measured on the last pictures of the vegetative stage of each generation (stage 5.10) with ImageJ (National Institute of Health, Bethesda, USA) using the pot diameter as calibration. Further, the date of inflorescence emergence was estimated from the pictures on which the first flower buds appeared. As the photography interval was 7 days, interpolation was used to estimate the date of the different developmental stages with more precision. Mortality was calculated as percentage of plants not surviving until the flowering phase in relation to the number of well-established plants at approximately one week after germination.

2.2. Environmental data

Close to the plants a data logger (CR 1000, Campbell Scientific, Logan, USA) was installed recording several environmental parameters with measurements every second and storage of 10 min means. Approximately 10 cm above the ground UV-B radiation and photon flux density (PFD) were horizontally registered with a UV-B-sensor (UV-S-B-T, Scintec AG, Rottenburg, Germany) and a photodiode (G1118, Hamamatsu Photonics, Hamamatsu-City, Japan), respectively. The photodiode was incorporated into a custom-made housing constructed for cosine-corrected measurements and calibrated against a quantum sensor (LI-190, LI-COR, Lincoln, Nebraska, USA). The UV-B-sensor was calibrated against a double monochromator spectroradiometer (DM 150, Bentham Instruments Ltd, Berkshire, UK). The air temperature was measured with two thermocouples (Type T) in 1 and 5 cm height above the ground. Soil temperature was detected with a temperature probe (107-L, Campbell Scientific) in 3 cm depth. From the data daily sums of PFD and UV-B dose and mean air temperatures were calculated. Matching the sampling days 5 d means of environmental data were determined to be used in regression analysis. For regression analysis of light stress sums of PFD from 7 a.m.–1 p.m. as well as mean air temperature from the same time period was calculated.

2.3. Analysis of physiological parameters

In a two week rhythm plants were sampled for physiological parameters. Since in early November 2014 germination stopped, sowing was interrupted until February 2015 and no plants were available for sampling until mid of March 2015. Sampling was always between 1.00 and 1.30 p.m. local time on mature leaves from plants in the vegetative stage. Sometimes plants were too small to sample single leaves for the measurements and instead whole young plants were sampled without their roots. The youngest fully developed leaf was sampled in 2 ml reaction tubes containing five glass beads (2 and 4 mm diameter; Roth GmbH & Co. KG, Karlsruhe, Germany) and frozen directly in the field in liquid nitrogen for DNA damage determination. The tubes were stored at −86 °C until DNA extraction. The next older leaf was cut from the plant and placed in a petri dish with wet filter paper in a dark box for PSII efficiency measurements. After 60 min dark incubation, F_V/F_M was determined with an Imaging PAM chlorophyll fluorometer (Walz, Effeltrich, Germany). On the same leaves, UV transmittance of the upper epidermis was measured using a Xenon PAM fluorometer (Walz) according to Bilger et al. (1997) with slight modifications. A red reference beam with a wavelength of 650 nm was used as described by Nichelmann and Bilger (2017). As 100% reference values for unprotected samples average fluorescence ratios of isolated chloroplasts from *A. thaliana* were applied. After the transmittance measurements, the leaves were scanned (HP Scanjet 4890, Wilmington, Delaware, USA) and frozen in 2 ml reaction tubes in liquid nitrogen. Until pigment extraction, the tubes were stored at −86 °C. UV absorbing compounds or photosynthetic pigments were analysed from the samples. Polyphenolics were extracted as described in Nichelmann et al. (2016). Compound separation and quantification was done with an HPLC system of the Agilent 1100-series (Agilent Technologies, Santa Clara, USA) equipped with a Licrosphere RP 18-column (4 × 250 mm, 5 µm particle size, Merck KGaA, Darmstadt, Germany). The gradient was as specified in Nichelmann et al. (2016). Hydroxycinnamic acid derivatives (HCAs), as well as kaempferol and quercetin derivatives were identified according to their absorption spectra in comparison to those of reference compounds. The contents of the three classes were analysed separately and the peak areas per class added up. Photosynthetic pigments were analysed as defined in Nichelmann et al. (2016) on the same HPLC system but now equipped with a Hypersil ODS-column (4 × 250 mm, 5 µm particle size, Thermo Fisher Scientific Inc., Waltham, USA.). DNA damage was analysed as concentration of cyclobutane pyrimidine dimers Mb^{-1} DNA (CPDs Mb^{-1}). DNA extraction and subsequent immuno dot-blot assay were run as described in Pescheck et al. (2014). For a better sensitivity at low concentrations of thymine dimers, a higher dilution of the calibrated CPD standard was included in each blot.

2.4. Statistical analysis

Variations of UV transmittance, flavonoid and carotenoid contents, DNA damage and optimal quantum yields were analysed by multiple linear regression analysis using Sigmaplot 11 (Systat Software GmbH, Ekrath, Germany).

3. Results

All measurements in this study were conducted on plants that had germinated and grown in the field. No experimental treatments were applied and no manipulation was made, except for weeding and watering in the case that natural rainfall was not sufficient to avoid drought stress. Therefore, the study allows to derive data on a yearly cycle of several morphological and physiological parameters of *A. thaliana* under close to natural temperature and sunlight conditions.

3.1. Environmental factors

Horizontally detected PFD and UV-B radiation changed in a sinusoidal form over the year closely following day length (Fig. 1). It is noteworthy that there was a factor larger than 10 between daily irradiance during winter and summer. In comparison to the irradiance the temperature curve was shifted by roughly 6 weeks to later dates resulting in cooler days with high light in spring and a period with low light and comparatively warm temperature in fall (see also Poorter et al., 2016). On 67 days during the winter, temperature minima were below the freezing point. Maximal temperature was on Jul 03, 2015 with 30.2 °C when relatively high values were recorded with 25–30 °C for 5 days in a row. During these days minimum temperatures did not drop under 15 °C at night. Daily means of soil temperature in 3 cm soil depth were approximately 3 °C below mean air temperature over the whole period (data not shown).

3.2. Plant phenology

Plant phenology in terms of length of vegetative growth phase and rosette radius as well as mortality varied strongly over the year (Fig. 2). Seeds were sown in the field in intervals of two weeks (circles at top of Fig. 2), except during the period from November 2014 until March 2015 when unfavourable conditions prevailed. Germination paused from November 5, 2014, until April 18, 2015 (vertical marks in Fig. 2, see also Fig. 3a). Rosette size showed two maxima, one in spring and one in fall (Fig. 2, closed diamonds, see also Fig. 3c). The faster induction of flowering might have inhibited further growth of the plants when temperatures rose. These plants had intermediate to small rosettes of less than 1 cm radius. Plants were also partially affected by grazing by flea beetles (*Psylliodes* spec.) and two cohorts in summer 2015 had about 50% loss of leaf area (Fig. 2, open diamonds). Therefore, we cannot exclude that the plants remained small due to herbivory. However, in 2016, when no flea beetles were present, we observed a similar pattern with minimal plant sizes in late June (data not shown).

The overwintering generations remained not only small but also had the highest mortality (Fig. 2, crosses). The few surviving plants synchronously turned red in the end of February 2015, when daily PFD was occasionally above 10 mol m^{-2} d^{-1} (see also Fig. 3b). The high mortality in May and end of June was probably due to herbivory by flea beetles. The radius of the affected plants was measured using the remains of the leaves. Vegetative growth was very slow and lasted for more than 180 days for plants geminated after September 19, 2014. The critical day length for the induction of flowering was in the order of 12 h, as no flowering was observed between October 6 and March 23.

The duration between sowing and germination and between germination and flowering increased in autumn and decreased strongly in spring (Fig. 4). The length of both periods was strongly temperature dependent (inset in Fig. 4). Plants from a summer generation in Jul 2015 needed only 15 days of vegetative growth to reach the reproductive phase. On the other hand, growth virtually ceased during winter.

3.3. Epidermal UV transmittance

Epidermal transmittance for UV-A and UV-B radiation was relatively low around the year with less than 12% and 10%, respectively (Fig. 5). There was a trend for higher transmittance in leaves from plants grown between July to September, in both years, 2014 and 2015, in comparison to leaves harvested in spring 2015. To explain the observed variation of UV-A or UV-B transmittance by the environmental factors UV-B irradiance and temperature, a multiple regression analysis was conducted. Neither type of transmittance was correlated significantly with UV-B radiation or air temperature (Table 1). Mostly, UV-B transmittance was lower than UV-A transmittance, but there was no constant relation between both. This indicates that the relative proportion between hydroxycinnamic acid derivatives, absorbing mainly in the UV-B spectral region, and flavonoids, absorbing mainly in the UV-A region, was not constant.

3.4. UV absorbing pigments

The contents of HCAs, kaempferol and quercetin derivatives were

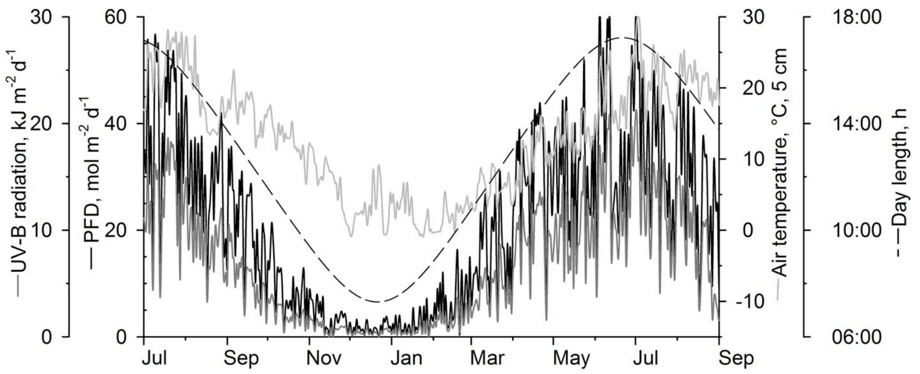

Fig. 1. Environmental data during the year 2014–2015 in Kiel, Germany. Daily sums of PFD (black line) and UV-B dose (dark grey line), mean air temperature 5 cm above ground (light grey line) and day length (civil twilight, downloaded from: https://www.timeanddate.de/sonne/deutschland/kiel, accessed on 17/07/17, dashed line). For details, see Material and Methods.

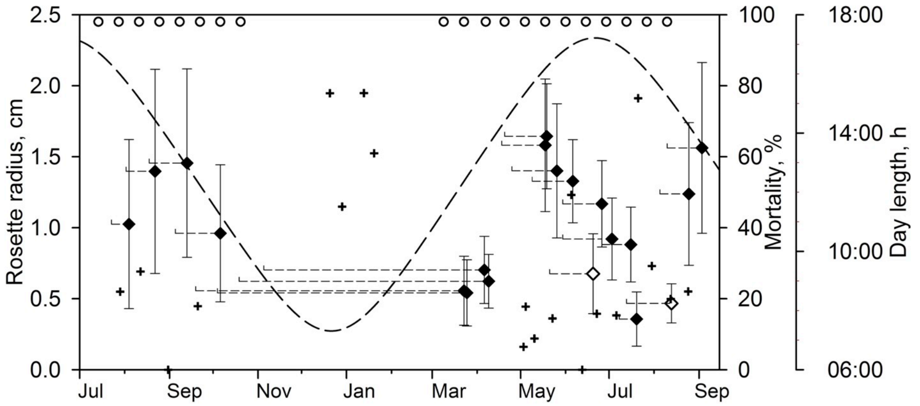

Fig. 2. Rosette radius (diamonds, mean ± std. dev., n > 12), determined when inflorescences emerged, during the course of the year 2014–2015. Completed development of cotyledons (= growth stage 1.0) is marked for each cohort with a short vertical line and connected by a dashed line with the rosette radius of its cohort, thereby indicating the vegetative growth period. Mortality as percent of plants still alive at inflorescence emergence (crosses, mean of 10 pots) is indicated for each cohort in the middle of the respective growth period. The open diamonds in summer 2015 indicate that leaves were strongly affected by herbivory. Sowing dates are indicated at the top of the graph (open circles). Day length (dashed line) is shown again for easier comparison with environmental data presented in Fig. 1.

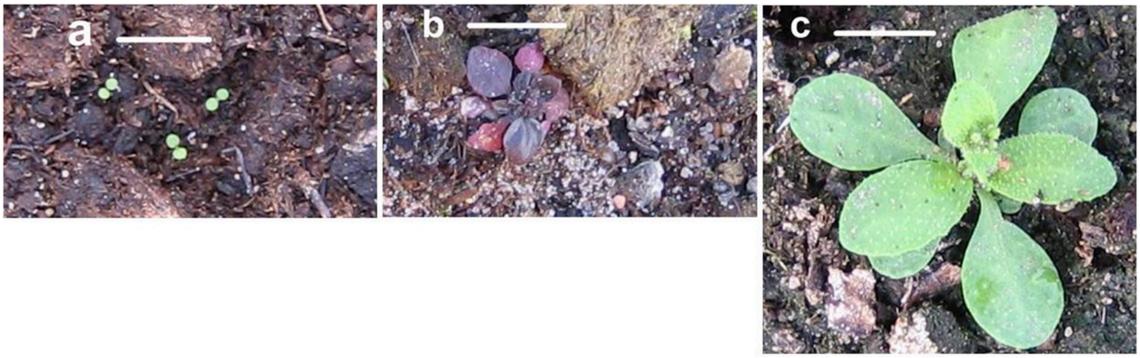

Fig. 3. Examples of phenotypes of *A. thaliana* (a) at growth stage 1.0 in Sep 2014 (sowing on Sep 8, 2014), (b) at emergence of flowers after overwintering (sowing: Sep 22, 2014, inflorescence emergence Mar 23, 2015) and (c) after growth during summer (sowing on Aug 11, 2014, inflorescence emergence Sep 15, 2014). Scale bar = 1 cm.

analysed separately. While we found consistently 4 derivatives of quercetins, and 3–4 derivatives of kaempferol, the number of HCA derivatives varied from 5 to 9 among all samples. All three types of compounds showed indications of an annual time course with a maximum in the cooler time of the year and a minimum in August and September (Fig. 6). Variation was especially pronounced in contents of quercetin derivatives. Multiple regression analysis revealed that only contents of quercetin derivatives could be explained by changes in air temperature (Table 1). At lower temperatures, quercetin derivatives were increased. UV-B irradiance did not explain quercetin derivative accumulation. There was a tendency for a negative correlation between HCA content and UV-B, as the p-value of 0.089 indicates.

3.5. Photosynthetic pigments

It was hypothesized that the violaxanthin cycle pool size (Σ VAZ) and the Chl *a*/*b* ratio would acclimate according to the irradiance. Σ VAZ was comparatively high from spring to summer 2015 (Fig. 7). The mean value over all samples of 136 ± 26 mmol mol^{-1} Chl $a+b$ corresponds with the highest values observed in sun leaves by Demmig-Adams (1998). From summer to fall 2014, Σ VAZ was declining in parallel to the decreasing light as expected. However, from spring to summer 2015 it was from the beginning on the same level as in midsummer, similar to that observed in midsummer 2014. Mean PFD and temperature significantly explained Σ VAZ variations (Table 1).

In spring, lutein/Chl $a+b$ and neoxanthin/Chl $a+b$ were enhanced compared to the rest of the year, whereas beta-carotene showed very constant contents in relation to the sum of Chl (data not shown).

Also the Chl *a*/*b* ratio has been considered to follow the irradiance (Anderson et al., 1995). This ratio fell with values between 3.4 and 4.2 clearly in the range of high light acclimated plants (Fig. 7). There is a tendency recognizable for a decline in fall 2014 and a rise from spring to summer in 2015, but the scatter of the data was very large.

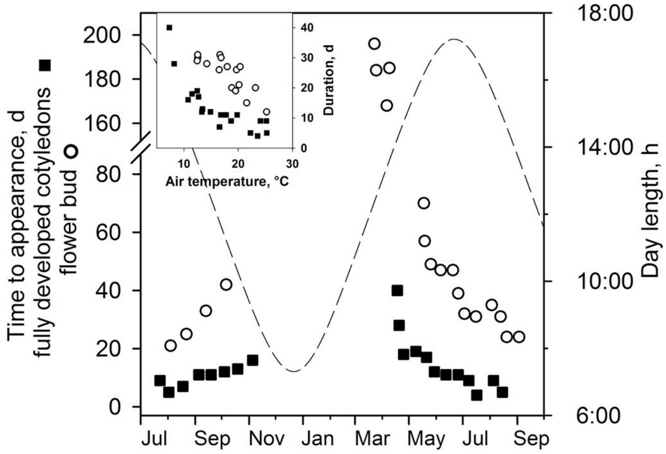

Fig. 4. Time from sowing to fully developed cotyledons (squares) or to appearance of flower buds (circles) plotted against the dates when the plants reached these stages. Day length (dashed line) is shown again for easier comparison with data presented in Figs. 1 and 2. Inset: duration from sowing until cotyledons were fully developed (squares) and length of vegetative phase (emergence of flower buds – fully developed cotyledons, circles) correlated to mean air temperature over the respective phase.

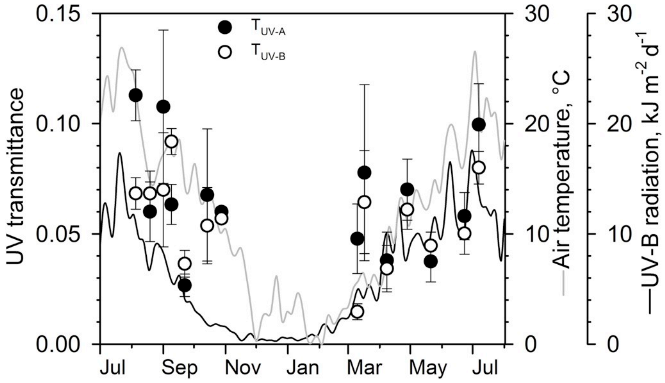

Fig. 5. UV-A (T_{UV-A}) and UV-B (T_{UV-B}) transmittance (mean ± std. dev, n = 2–8) and 5 d mean of air temperature 5 cm above ground (grey line) and 5 d mean of UV-B radiation (black line) during the course of the year 2014–2015.

Table 1
P values and regression coefficients of multiple linear regression analysis for physiological parameters. Significant *p* values are high-lighted in bold.

Parameter	Independent variables *5d mean of Σ PFD*	 *5d mean of UV-B dose*	 *5d mean of air temp.*	*R^2*
UV transmittance				
T_{UVA}		0.880	0.123	0.17
T_{UVB}		0.687	0.055	0.22
Flavonoids				
HCAs		0.089	0.795	0.41
Kaempferols		0.275	0.287	0.44
Quercetin		0.999	**0.050**	0.60
Photosynthetic pigments				
Chl a/b	0.966		0.493	0.00
Σ VAZ-cycle	**0.010**		**0.037**	0.44
	Σ PFD, 7 a.m. – 1 p.m.	*UV-B dose, 7 a.m. – 1 p.m.*	*Mean air temp., 7 a.m. – 1 p.m.*	*R^2*
Light stress				
DNA damage		0.873	0.154	0.15
F_V/F_M	**0.001**		0.061	0.58

3.6. Photodamage to PS II and the DNA

Besides acclimation to stress factors, also selected effects of stress were determined. Optimal quantum yield of PSII was measured at noon in the different cohorts of plants grown over the course of the year (Fig. 8). The obtained PS II efficiencies can be regarded as indicators of light stress for the single sampling days and the prevailing environmental conditions of those days. Lowest F_V/F_M values were observed in August 2014, early spring 2015 and in July 2015. When investigating photoinhibition in *Quercus coccifera*, Werner et al. (2001) related F_V/F_M values to the previously experienced light sum. These authors conducted a detailed analysis of the significant time frame and obtained the best correlations when summing up the preceding 6 h. Therefore, we used the same time interval of 6 h for calculating regression analyses. Multiple regression analysis revealed a significant impact of preceding light sums on the optimal quantum yield (Table 1, Fig. 9a). Together with a minor influence of mean air temperature almost 60% of variation of F_V/F_M was explained by the two factors. The quality of the regression increased substantially (r^2, 0.85) when two measurements from March with temperatures below 12 °C and high solar irradiation were excluded (dashed line in Fig. 9a). Due to the close correspondence between PFD and UV-B radiation (see Fig. 1; the cumulated doses from 7 a.m. to 1 p.m. were highly correlated with $r^2 = 0.95$), we cannot distinguish between an effect of PFD and one of UV-B on F_V/F_M. However, since the leaves were comparatively well protected by screening pigments we expect that the major part of the lowering of F_V/F_M was due to PFD.

While a reduction of PS II efficiency can be caused by both, high PFD or UV-B radiation, cyclobutane-pyrimidine dimer (CPD) formation at the DNA is a UV-B specific damage (Cadet et al., 2012). DNA damage was detectable at 20–100 CPDs Mb^{-1} throughout the year (Fig. 8). In experiments with artificial UV-B exposure of *Arabidopsis* leaves, CPD levels of up to 5000 Mb^{-1} could be observed using the immunoblot assay on the basis of the identical standard (F. Pescheck, unpublished results). However, the observed CPD values were clearly distinguishable from zero level. DNA damage was not significantly correlated to UV-B radiation or temperature (Table 1). In contrast, in 2015 there was a slight trend observable for decreasing values towards the warmer and brighter season. This is even more clearly expressed in Fig. 9b, where a decreasing trend with increasing UV-B doses is shown.

4. Discussion

The annual time course of acclimation to and damage by solar radiation observed in *Arabidopsis* plants can be interpreted as being induced by the interplay of environmental factors with the metabolism of the plant. Since the plants were watered regularly and were planted in well fertilized soil, drought and nutrient deficiency had probably little influence on their responses. Biotic stress due to flea beetles could not be avoided in some cohorts during summer 2015. However, a similar trend in the size of the plants was observed in the year 2016 in the absence of flea beetle infestation. Of the remaining factors radiation, including day length, and temperature were presumably the most decisive. Due to the short generation time of *A. thaliana*, it was possible to observe the influence of the annual variation of these factors on the physiology of plants of the same age throughout the year.

PFD and temperature are strongly interacting in their influence on the rate of photosynthesis and on the balance between absorbed light energy and the utilization of this energy in carbon fixation (Huner et al., 1996). Therefore, changes in photosynthetic pigments, especially photoprotective carotenoids, must be interpreted in relation to both factors simultaneously. On the other hand, formation of CPDs is in the first place caused specifically by UV-B radiation. CPD formation is a pure

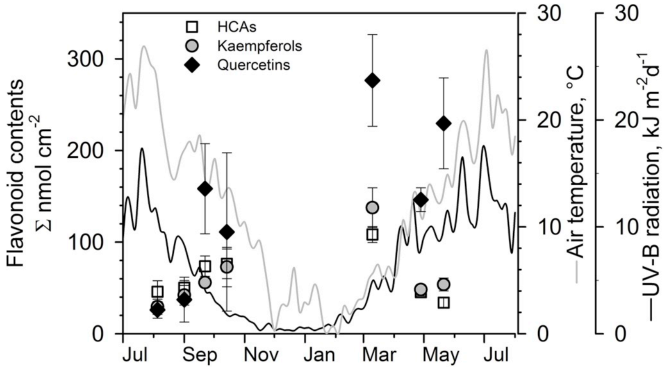

Fig. 6. Concentration of hydroxycinnamic acid (HCA), kaempferol and quercetin derivatives per leaf area (mean of n = 3–8, ± std. dev.) and air temperature 5 cm above ground (grey line) and 5 d mean of UV-B dose (black line) during the course of the year 2014–2015.

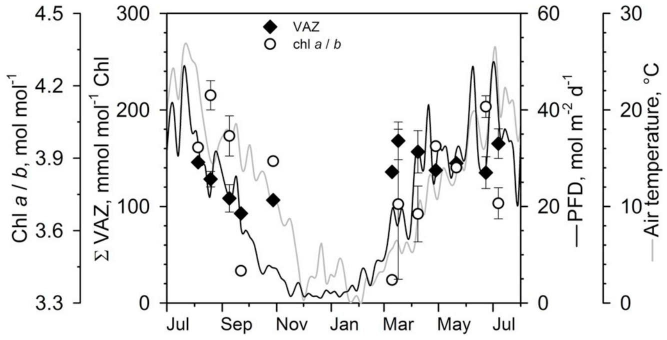

Fig. 7. Chlorophyll *a/b* ratio, pool size of violaxanthin cycle pigments per chlorophyll (ΣVAZ) (mean of n = 1–8, ± std. dev.) and air temperature 5 cm above ground (grey line) and 5 d mean of daily sums of PFD (black line) from Jul 2014–Jul 2015.

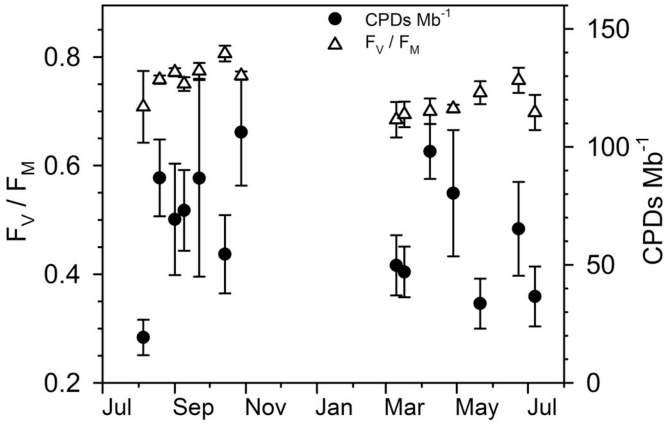

Fig. 8. Optimal quantum yield (F_V/F_M) and DNA damage (cyclobutane pyrimidine dimers, CPDs Mb^{-1}), both measured at 1 p.m. (mean of n = 2–8, ± std. dev.) F_V/F_M was determined after 60 min darkening.

photophysical reaction, and hence, temperature independent within the physiological range of 10–25 °C, as was shown in cucumber seedlings (Takeuchi et al., 1996). However, the ultimate amount of CPDs depends also on the rate of the repair reactions (Kaiser et al., 2009). The latter are enzymatic and, therefore, dependent on temperature (Takeuchi et al., 1996). Accordingly, both, temperature and UV-B radiation, can be expected to modulate DNA integrity. Although UV-B irradiance increased by a factor of more than 10 from winter to summer, CPD concentration was relatively low throughout the year and did not follow the UV-B trend (Fig. 8). Rather, there was a tendency to an opposite response with lower CPD levels during summer than in early spring or late fall. This resulted in decreasing CPD levels with increasing UV-B dose (Fig. 9b), which seems counterintuitive. In field observations CPD levels were shown to increase with rising UV-B irradiance (Ballaré et al., 1996; Stapleton et al., 1997; Rousseaux et al., 1999). Rousseaux et al. (1999) demonstrated that CPD levels in *Gunnera magellanica* increased with short term increases in the UV-B irradiance, caused by passages of the ozone hole over Tierra del Fuego, Argentina. UV-B irradiance explained 68% of the variation in CPDs. However, other field studies showed opposing results. In the Antarctic green macroalga *Prasiola crispa* no diurnal course of DNA damage was detected at ambient UV-B (Lud et al., 2001). Rozema et al. (2006) detected no UV-B-dependent increase of DNA dimers in arctic higher plants over a long-term study with two different UV-B supplementation levels. However, in this study only two time points, after 2 or 6 years, respectively, were investigated and seasonal changes not resolved. Also in Antarctic bryophytes no increase of DNA damage was found after 3 weeks of exposure to enhanced UV-B by supplementation (Boelen et al., 2006). Only a very moderate seasonal increase of DNA damage was observed in the green macroalga *Ulva intestinalis* over 4 weeks in summer (Pescheck et al., 2016). From these reports and the present data it might be hypothesized that photosynthetic organisms can tolerate ambient and moderately enhanced UV-B irradiance without accumulating DNA damage, if no additional stress interferes with repair. The results presented here, where mean day temperature varied between 5 and 20 °C, suggest that temperature may be such an interfering factor, presumably due to inhibition of CPD repair. Photoreactivation has been observed to be dependent on temperature. At 25 °C photoreactivation was increased in cucumber by a factor of 1.6 as compared to 15 °C (Takeuchi et al., 1996). Therefore, our observations could be interpreted as caused by an inhibition of repair processes at lower temperatures, which more than compensated the decreased UV-B irradiance.

A second target of UV-B radiation, which was determined in this study, was maximal quantum yield of PS II, F_V/F_M. This parameter strongly correlated to the sum of PFD experienced during 6 h before its measurement, when two data points from March 2015 were omitted (Fig. 9a). The integration over 6 h was chosen according to Werner et al. (2001), who had shown that this time resulted in the highest correlation between exposure and F_V/F_M. Although PFD is leading to photoinhibition, which is expressed in a reduced F_V/F_M, the observed correlation may also be partially caused by UV-B radiation, since PFD and UV-B were highly correlated.

Although the general relationship between F_V/F_M and PFD showed no interaction with temperature, the two measurements in March clearly show that in some circumstances low temperature may affect PS II integrity. These low values may have been caused by an inhibition of repair of PS II (Allakhverdiev and Murata, 2004), but could also have been caused by the stress accumulated during the winter period. An indication of such stress may have been the anthocyanin accumulation in these leaves (Fig. 3b). One should also not forget that these plants were considerably older than all other plants at the time of measurement (Fig. 4).

A sensitization of plants to UV-B induced damage at low temperature would require additional protection by the plant (Schultze and Bilger, 2018). In this context one may interpret the increased UV protective pigments in winter/early spring. Photoprotective pigments of leaves sampled in the end of October 2014 were higher as in the beginning of August 2014. Similarly, UV-B transmittance was slightly reduced in October 2014 compared to August 2014. It is well known that flavonoid accumulation is induced at low temperature (Bilger et al., 2007; Schulz et al., 2016) as well as at increased UV-B irradiance (Bornman et al., 1997; Ryan et al., 2002; Barnes et al., 2017). On the other hand, a negative influence of UV-B radiation was observed on the accumulation of HCAs in grape and barley leaves by Kolb et al. (2001) and Schmitz-Hoerner and Weissenböck (2003), respectively. The relative effect of the two factors UV-B and temperature under natural

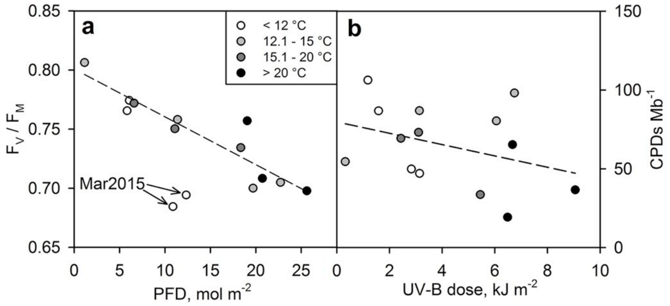

Fig. 9. (a) Optimal quantum yield (F_V/F_M) related to sum of PFD and (b) DNA damage concentration (CPDs Mb^{-1}) related to UV-B dose. Sum of PFD and UV-B dose were integrated from 7 a.m.–1 p.m. on the sampling day. Data were grouped in 4 classes of mean air temperature between 7 a.m.–1 p.m. The regression in (a) was calculated without 2 measurements from Mar 2015 with low air temperature.

conditions is difficult to predict. Our observations suggest that the temperature dependent process may override the UV-B dependent induction. Similarly, Coffey et al. (2017) show that seasonal variation in temperature explained concentration of UV absorbing pigments much better than changes of UV-B irradiance in *A. thaliana* grown outdoors. With higher flavonoid contents in winter, the increased ratio of damage induction and repair at low temperatures might be counterbalanced, although we still observed a slight enhancement of DNA damage at low temperature. Flavonoids may also have additional roles at low temperature. It was shown that flavonoid accumulation increases freezing tolerance in *A. thaliana* (Schulz et al., 2016). However, during the time when samples were taken for pigment determination, no freezing occurred.

Whereas UV screening pigments should be related to UV-B irradiance, photosynthetic pigment contents need to be considered with respect to irradiance in the visible spectral range. Σ VAZ has been shown many times to be correlated to irradiance (Demmig-Adams, 1998; Nichelmann et al., 2016). Accordingly, we observed a highly significant correlation between both parameters (Table 1). But, we also found a significant correlation to temperature (Table 1). It has been shown that low temperature induces an increased Σ VAZ (Somersalo and Krause, 1990). In natural populations of *Mahonia repens*, Logan et al. (1998) observed higher Σ VAZ in winter than in summer, attributing this to the enhanced excessive PFD at low temperature (Bilger et al., 1997). Low temperature causes a decrease in Calvin cycle activity, leading to excessive absorbed light (Huner et al., 1996). When in spring 2015 temperature and PFD rose in parallel, Σ VAZ remained more or less constant. During the period from March to July the rise in irradiance may have been compensated by a rise in Calvin cycle activity due to increased temperature, leading to almost constant excessive PFD. In contrast, in fall 2014 the temperatures tended to remain higher which presumably led to less limitation of the Calvin cycle. This may have caused Σ VAZ to decline in parallel to irradiance. Hence, with respect to Σ VAZ, the hysteresis in the relationship between light and temperature led to different reactions between spring and fall. Also, the peak contents of quercetin were found when high irradiances in spring coincided with low temperatures (Fig. 6). Both compounds, zeaxanthin and quercetin, are regarded as antioxidants (Havaux et al., 2007; Agati et al., 2009). Chl a/b ratios did not show a significant acclimation over the year, indicating that the low light levels in winter had no major influence on the structure of the pigment complexes.

While some physiological parameters showed a relative constancy, morphology of the plants was varying widely over the year. This variability contrasts strongly with the uniformity of plants grown in climate chambers and used for physiological experiments. One may expect that larger plants should also produce more seeds (e.g. Külheim et al., 2002), and physiological mechanisms should be coined by evolution to maximize reproduction.

However, explaining rosette size as affected by environmental parameters is difficult. In the field, temperature and daily light sums are to some extent correlated, although a strong hysteresis for the relationship between both factors can be observed (Poorter et al., 2016). Smaller rosette sizes combined with slower development in fall may be interpreted as a cumulative effect of low temperatures and low irradiance on the rates of growth and development (Figs. 2 and 4). On the other hand, despite enhanced rates of growth and development a second minimum of rosette size was observed during midsummer. This paradoxical situation could be explained by a stronger stimulation of the developmental timing as compared to the growth rate (Wilczek et al., 2009). In *A. thaliana* rosette growth ceases as soon as the reproductive phase with flower bud emergence begins (Boyes et al., 2001). The used accession Landsberg erecta was described as a facultative long day plant which does not to require vernalisation to flower (Johanson et al., 2000). In accordance to this, no acceleration of development until flowering was observed in the overwintering cohorts or in plants germinated in early spring in our experiment. However, when the critical day length of 12 h was exceeded (Wilczek et al., 2009), faster development correlated with increasing mean temperatures. This observation agrees with results of Hasdai et al. (2006) who showed in *A. thaliana* that time until flowering was shortened by a factor of 3 by an increase of air temperature from 6 to 22 °C, which restricted the time of growth to a short period. On the other hand, the small plant sizes at midsummer may also be caused by other factors. Minimal time requirement for development until flowering was observed in late August in both years, but at that time the plants were among the largest. Potentially, some stress factors caused the reduced growth in July.

To conclude, *A. thaliana* proved to be able to acclimate to the local environmental conditions and to grow and survive over the complete course of the year. Although sowing was interrupted during winter, overwintering by cohorts that had been sown in fall was observed. The acclimatory responses to abiotic factors observed in the present study when the plants were exposed to gradual changes might be different from what can be observed under controlled experimental conditions when conditions change abruptly. Therefore, studies when plants are exposed to natural conditions are highly needed.

Temperature proved to be a very important environmental parameter. E.g., developmental timing was strongly dependent on temperature (Fig. 3). But temperature influenced also parameters, which were expected to be mainly dependent on short wave radiation, such as UV-B-induced DNA damage and contents of photoprotective pigments. It is important to consider temperature as a fundamental environmental variable in physiological investigations of *A. thaliana* much more than is done so far.

Declarations of interest

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