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Arabidopsis Mutants Lacking Phenolic Sunscreens Exhibit Enhanced Ultraviolet-B Injury and Oxidative Damage[1]

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We have assessed ultraviolet-B (UV-B)-induced injury in wild-type *Arabidopsis thaliana* and two mutants with altered aromatic secondary product biosynthesis. Arabidopsis mutants defective in the ability to synthesize UV-B-absorbing compounds (flavonoids in *transparent testa 5* [*tt5*] and sinapate esters in *ferulic acid hydroxylase 1* [*fah1*]) are more sensitive to UV-B than is the wild-type Landsberg *erecta*. Despite its ability to accumulate UV-absorptive flavonoid compounds, the ferulic acid hydroxylase mutant *fah1* exhibits more physiological injury (growth inhibition and foliar lesions) than either wild type or *tt5*. The extreme UV-B sensitivity of *fah1* demonstrates the importance of hydroxycinnamate esters as UV-B protectants. Consistent with the whole-plant response, the highest levels of lipid and protein oxidation products were seen in *fah1*. Ascorbate peroxidase enzyme activity was also increased in the leaves of UV-B-treated plants in a dose- and genotype-dependent manner. These results demonstrate that, in *A. thaliana*, hydroxycinnamates are more effective UV-B protectants than flavonoids. The data also indicate that *A. thaliana* responds to UV-B as an oxidative stress, and sunscreen compounds reduce the oxidative damage caused by UV-B.

UV-B (280–320 nm of light) radiation reaching the earth's surface is increasing because of depletion of the stratospheric ozone layer (Frederick et al., 1989; Stolarski et al., 1992). Although UV is damaging to living organisms, the long-term impact of elevated UV-B on plants is unknown (Teramura and Sullivan, 1994). Plants are more resistant to UV-B than are microbes or mammalian cells, in part because they produce a variety of secondary metabolites that effectively absorb UV-B and prevent it from penetrating into the leaf mesophyll cells (Caldwell et al., 1983). For example, flavonoid compounds are synthesized in response to UV-B (Chappell and Hahlbrock, 1984), accumulate in the upper epidermal cells of leaves (Day, 1993; Day et al., 1993), and absorb in the UV range in methanolic extracts (Strid and Porra, 1992). Consistent with this idea, UV irradiation of a flavonoid-deficient maize mutant caused increased accumulation of DNA damage products in leaf tissue (Stapleton and Walbot, 1994). Less attention has been paid to the importance of hydroxycinnamic acids, another widespread and sometimes abundant class of UV-absorptive aromatic secondary metabolites. Although previous studies have shown that soluble phenolics increase in UV-B-treated plants (Liu et al., 1995; Ormrod et al., 1995), none have conclusively established a critical role for these compounds in UV-B resistance.

We are using *Arabidopsis thaliana* mutants to characterize physiological processes that are critical for protecting plants from UV-B stress. This approach has been successfully used for the identification of several UV-sensitive mutants of *A. thaliana*: *uvr1* (Britt et al., 1993) and *uvh1* (Harlow et al., 1994), both deficient in DNA repair, and *tt4*, *tt5* (Li et al., 1993), and *uvs* (Lois and Buchanan, 1994), which are deficient in UV-B sunscreens.

A. thaliana mutants *tt4* and *tt5* are unable to synthesize a normal complement of flavonoid compounds because of defects in flavonoid biosynthetic enzymes and are more sensitive to UV-B light than are wild-type plants (Li et al., 1993). Surprisingly, the chalcone synthase mutant *tt4* is more resistant to UV-B than the chalcone isomerase mutant *tt5*, even though both lack flavonols. The difference in UV-B sensitivity correlates with differences in accumulation of sinapate esters: *tt5* has reduced sinapate esters (hydroxycinnamate esters derived from sinapic acid), whereas *tt4* has elevated levels of sinapate esters (Li et al., 1993). This observation led us to propose that sinapate esters are important UV-B sunscreens. Recently, an *A. thaliana* mutant lacking sinapate esters because of a deficiency in activity of ferulic acid hydroxylase was isolated, allowing us to directly test this hypothesis. The isolation and phytochemical characterization of this mutant, which is now called *fah1*, (previously *sin1*) was described by Chapple et al. (1992).

UV-B causes oxidative stress as a result of secondary free radical formation in bacteria (Peak and Peak, 1983), and it has been proposed that this mechanism of injury also occurs in plants (Krizek et al., 1993; Strid, 1993; Doke et al.,

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Abbreviations: *fah1*, *ferulic acid hydroxylase 1*; *Ler*, Landsberg *erecta*; TBA, thiobarbituric acid; *tt*, *transparent testa*; $UV\text{-}B_{BE}$, biologically effective UV-B.

1994; Foyer et al., 1994). Cellular UV-B chromophores such as aromatic amino acids, NADH, and phenolic compounds can be activated by the absorption of UV-B light and react with oxygen to form singlet molecular oxygen and superoxide radicals (Peak and Peak, 1987). For example, Trp strongly absorbs 290 nm light and is degraded into the photosensitizing compound *N*-formylkynurenine, creating superoxide radicals in the process (Caldwell, 1993). Consistent with the hypothesis that UV-B causes oxidative stress, increased lipid peroxidation occurred in cucumber plants chronically exposed to UV-B (Kramer et al., 1991). Furthermore, it was shown that UV-B-induced expression of the tobacco *PR-1* gene is reversed by treatment with exogenous antioxidants, implying a role for reactive oxygen species in UV-B signal transduction (Green and Fluhr, 1995).

In this study, the relative efficacy of flavonoids and sinapate esters as UV-B protectants was assessed in vivo using wild-type *Ler*, the *tt5* flavonoid-deficient mutant, and the *fah1* sinapate ester-deficient mutant. Several parameters were tested, including effects of chronic or acute dose UV-B on plant growth, accumulation of lipid and protein oxidation products, and ascorbate peroxidase activity. Our results indicate the need for a reevaluation of the relative importance of flavonoids and hydroxycinnamic acids as UV-B protective molecules.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana Ler wild type and two mutants isolated from the *Ler* parental line were used in this study: the chalcone isomerase mutant *tt5* (Shirley et al., 1992) and the ferulic acid hydroxylase mutant *fah1–7*. The *fah1–7* allele was derived by mutagenesis of *Ler*, and the line used was obtained from three backcrosses to *Ler* (see Chapple et al., 1992, for a description of *fah1* mutants, which were originally called *sin1*). Plants for biochemical analysis were grown in sterile Cornell soil-less mix (1 ft^3 of peatmoss, 2 ft^3 of vermiculite, 0.5 lb of Peters Uni-Mix Plus II 10–10–5, and 0.8 lb of limestone) in 6-inch square pots under continuous white light supplied by CW1500 cool-white fluorescent lamps (General Electric) filtered through 0.13-mm-thick Mylar (AIN Plastics, Mt. Vernon, NY) to remove light wavelengths shorter than 310 nm. PPFD varied from 90 to 110 $\mu mol\ m^{-2}\ s^{-1}$, and temperature ranged from 21 to 23°C during the course of this study.

UV-B Treatments and Experimental Design

Supplemental UV light from F40UVB fluorescent lamps (Phillips, Somerset, NJ) was filtered through one or more layers of 0.13-mm-thick cellulose acetate (McMaster-Carr, New Brunswick, NJ) to remove UV-C (wavelengths below 280 nm) and attenuate UV-B (wavelengths between 280 and 320 nm). Transmittance of UV-B through filters was monitored with a spectroradiometer (model OL 752; Optronics Laboratory, Orlando FL) calibrated with an OL 752–150 calibration module and OL 752–10 spectral irradiance standard. The light sensor uses a 180° hemisphere configuration. Cellulose acetate, which photodegrades with UV exposure, was changed as needed to achieve the desired UV-B fluence rates. UV-B doses were transformed using the generalized plant response weighting factors (Caldwell, 1971), normalized to 300 nm using the weighting factor 4.59, and expressed as UV-B_{BE} in $kJ\ m^{-2}\ h^{-1}$ (Li et al., 1993). Figure 1 illustrates the actual fluence spectra, in W/m^2, of the target UV-B_{BE} fluences (0.4, 0.6, and 0.9 kJ UV-B_{BE} $m^{-2}\ h^{-1}$). Control plants were maintained under Mylar filters and received less than 0.002 kJ UV-B_{BE} $m^{-2}\ h^{-1}$.

For all experiments except that shown in Figure 2, UV-B treatments were initiated 14 d after planting. Desired UV-B doses were achieved by varying UV-B fluence rate (0–0.9 kJ $m^{-2}\ h^{-1}$) for total doses ranging from approximately 7 to 22 kJ UV-B_{BE} $m^{-2}\ d^{-1}$. For comparison, the 13-year mean (1979–1991) UV level in the vicinity of Ithaca, NY, for the month of July is approximately 5.4 kJ UV-B_{BE} $m^{-2}\ d^{-1}$ (Total Ozone Mapping Spectrometer data, courtesy of R.D. McPeters and A.J. Krueger of National Aeronautics and Space Agency Goddard Space Flight Center). Pots of the three genotypes were randomized within the growth room. The pots were rotated daily, starting several days prior to the initiation of UV-B treatment and continuing until the end of each experiment. Reproductive shoot elongation did not occur during these experiments.

Plants to be used for dry weight measurement were cut off above the root, weighed, put in paper envelopes, and dried at 45°C for >72 h before re-weighing. Dry weights were measured in quadruplicate, and the means were used to calculate the percentage change in fresh weight to dry weight ratio. Plants harvested for biochemical analysis were immediately frozen on dry ice or in liquid nitrogen and stored at −80°C until extraction. Samples consisted of several plants cut off above the root and pooled to obtain 0.5 to 1.0 g fresh weight.

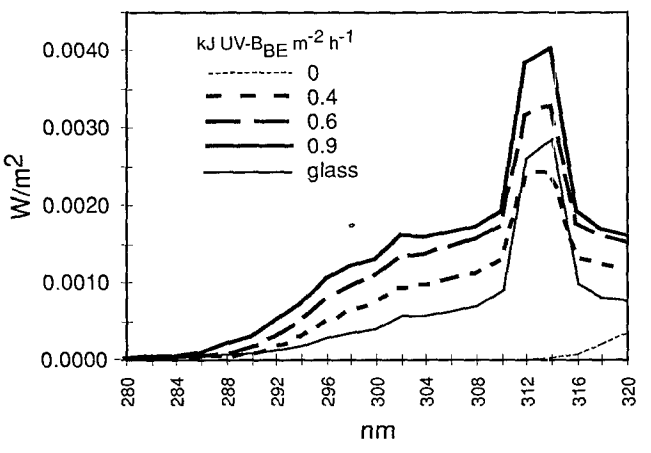

Figure 1. Light spectra of UV-B used. UV-B fluence was measured with a spectroradiometer and converted from W/m^2 (*y* axis) to kJ UV-B_{BE} $m^{-2}\ h^{-1}$ using the generalized plant response weighting factors (Caldwell, 1971) normalized to 280 nm and converted to 300 nm normalization (Li et al., 1993). Fluorescent white light and UV-B were filtered through 0.13-mm-thick Mylar (0.0 kJ $m^{-2}h^{-1}$); one (0.9), two (0.6), or three (0.4) layers of 0.13-mm-thick cellulose acetate; or 3-mm-thick Pyrex glass.

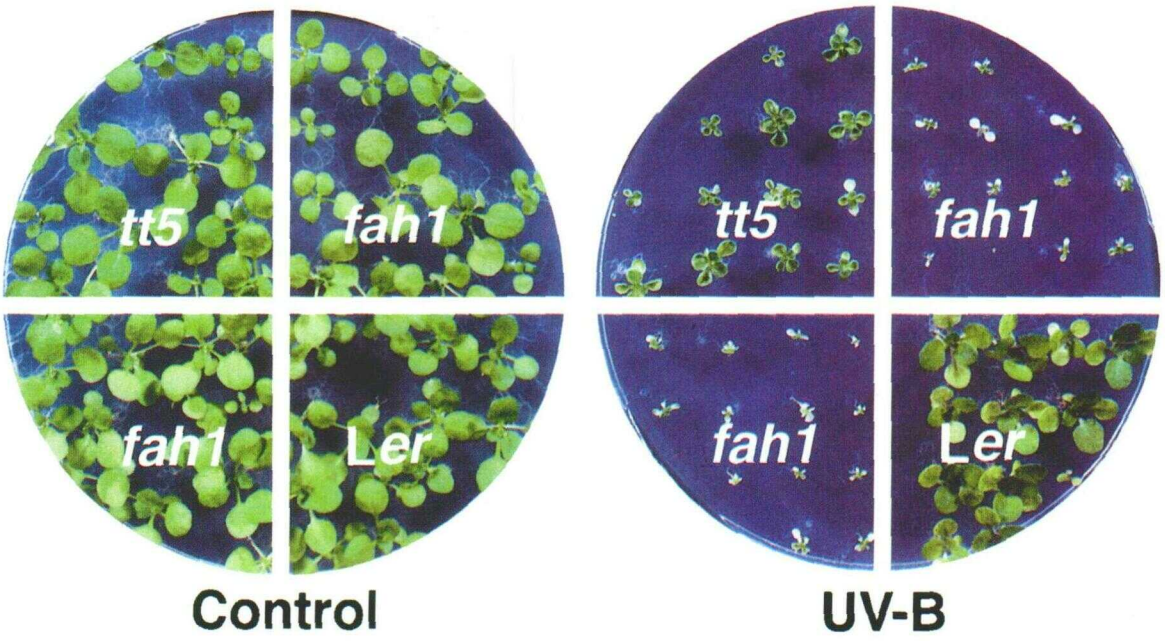

Figure 2. Injury and growth of *A. thaliana* under UV-B exposure. Wild-type L*er*, *tt5*, and *fah1* were grown on nutrient agar and exposed to supplemental UV-B filtered through Pyrex glass (0.3 kJ UV-B_{BE} m^{-2} h^{-1}) initiated 5 d poststratification. Control plants were maintained under Mylar to remove UV-C and UV-B light. Plants are shown on nutrient agar after 13 d with or without UV-B exposure.

To improve the uniformity of plant growth and reduce variability in UV-B light quality caused by cellulose acetate photodegradation during the course of a long-term exposure, we devised an aseptic method for exposing *A. thaliana* to UV-B. For Figure 2, surface-sterilized seeds were placed on nutrient agar medium (Haughn and Somerville, 1986) without Suc in 100- × 20-mm Optilux dishes (Becton Dickinson), stratified for 4 d at 4°C, and germinated under Mylar-filtered continuous white light, as above. Five days poststratification the plastic dish lid was replaced by 3-mm-thick Pyrex glass (Swift Glass, Elmira, NY), and plants were exposed to supplemental UV-B, as above, for 3 weeks. UV-B_{BE} transmittance through the Pyrex filters was 0.3 kJ m^{-2} h^{-1} (Fig. 1); total UV-C was less than 0.0004 kJ m^{-2} h^{-1}.

Tissue Extraction and Assays

Soluble protein for enzyme and carbonyl assays was obtained by homogenizing frozen plant tissue in extraction buffer (50 mM potassium phosphate, pH 7.4, 1 mM EDTA, and 1 mM ascorbic acid). The homogenate was centrifuged at 16,000*g* for 3 min at 4°C, and the resulting supernatant was desalted using a NAP-5 Sephadex G-25 column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with ice-cold extraction buffer. The eluted sample was analyzed immediately or diluted 1:1 with ice-cold 10% (v/v) glycerol in extraction buffer and stored at −80°C before the ascorbate peroxidase assay was performed. Carbonyl, protein concentration, and lipid peroxidation assays were measured using a Beckman model DU-7 spectrophotometer. Ascorbate peroxidase enzyme activity was measured using an Ultraspec III spectrophotometer (Pharmacia). All reagents were purchased from Sigma unless noted otherwise.

Lipid peroxidation in UV-B-treated plants was assessed by reaction with TBA to measure malondialdehyde content (Draper and Hadley, 1990; Esterbauer and Cheeseman, 1990). Assays were performed in duplicate, the mean A was calculated, and the background A_{532} of each sample was subtracted from the sample mean. The concentration of TBA-reactive substances was determined using a 0 to 12 μM malonaldehyde standard curve.

The carbonyl content of leaf extracts was measured by reaction with 2,4-dinitrophenylhydrazine as described by Levine et al. (1994) with the following modifications. Extracts were not treated with streptomycin sulfate to remove nucleic acids because in two test experiments no differences in results were observed between samples prepared with or without this step. Aliquots of 100 μL of extract were reacted with 400 μL of 10 mM 2,4-dinitrophenylhydrazine in 2 M HCl or 400 μL of 2 M HCl (controls) for 1 h without prior precipitation with TCA. Assays were performed in duplicate for each sample and the mean A_{375} minus the A of the control was used to calculate the concentration of hydrazone (extinction coefficient = 22,000 M^{-1} cm^{-1}).

Ascorbate peroxidase activity was measured using a published method (Amako et al., 1994). Reactions were initiated by adding 100 μL of 10 mM H_2O_2 to 50 mM potassium phosphate (pH 7.4), 0.5 mM ascorbic acid, and 100 μL of extract to a final volume of 1 mL. Each sample was assayed in triplicate and the mean rate of decrease in A_{290} at room temperature (approximately 24°C) was calculated. Protein was quantitated using the Bio-Rad protein

dye reagent according to the manufacturer's instructions and BSA as a standard.

RESULTS

fah1 Mutants Are More Sensitive to UV-B than *tt5* or Wild Type

Based on our previous analysis of the flavonoid-deficient *tt4* and *tt5* mutants, sinapate esters were proposed to be UV-B sunscreens in *A. thaliana* (Li et al., 1993). Identification of the *fah1* mutant (Chapple et al., 1992) allowed a critical assessment of the importance of these hydroxycinnamates as UV-B protectants. The effects on plant growth of both short-term acute and longer-term chronic UV-B was compared to determine the relative UV-B sensitivities of the wild-type progenitor L*er*, the flavonoid-deficient *tt5*, and the sinapate ester-deficient *fah1*.

The results of these studies indicate that the relative UV sensitivities of these three lines are *fah1* > *tt5* > L*er*. As shown in Figure 3, the growth of L*er* was not affected by 72-h-duration acute UV-B treatment under any of the three fluences used, although leaf injury was evident under the 0.9-kJ UV-B_{BE} m^{-2} h^{-1} treatment. In contrast, *fah1* showed signs of UV-B stress even at the lowest fluence tested (0.4 kJ UV-B_{BE} m^{-2} h^{-1}), where upward leaf curling was observed. Growth inhibition and uniform tissue discoloration were observed for *fah1* at both 0.6 and 0.9 kJ UV-B_{BE} m^{-2} h^{-1} . Under these growth conditions, the flavonoid-deficient *tt5* mutant did not display upward leaf curling or foliar injury until 0.6 kJ UV-B_{BE} m^{-2} h^{-1}, and the overall rosette size was not noticeably affected under even the highest short-term exposure.

As illustrated in Figure 2, although chronic exposure to 0.3 kJ UV-B_{BE} $m^{-2}h^{-1}$ UV-B treatment for 13 d caused strong upward leaf curling and reduced vigor of *tt5* compared with the wild type, the effects on *fah1* were far more severe. Under these conditions the *fah1* plants exhibited cotyledon bleaching and failed to develop expanded true leaves. Under both chronic and acute treatments, both mutants were as healthy as L*er* when grown under Mylar to remove UV light (Figs. 2, Control, and 3, 0.0). These results support the hypothesis that sinapic acid esters protect *A. thaliana* from UV-B irradiation and suggest that hydroxycinnamic acids are important sunscreens.

Short-term UV-B treatment also caused foliar dehydration, measured as a decreased ratio of fresh weight to dry weight in leaf tissue in two independent experiments (data not shown). Dehydration was extensive in the 0.9-kJ UV-B_{BE} m^{-2} h^{-1} treatment, with a 40% decline in fresh weight to dry weight ratio in *fah1*, a 30% decrease in *tt5*, and a 20% reduction in L*er*. Thus, the amount of dehydration was correlated with the extent of visible injury.

Lipid and Protein Oxidation as a Measure of Enhanced UV-B Damage

Enhanced penetration of UV-B through the epidermis of sunscreen-deficient plants would be expected to lead to increased production of reactive oxygen species, suggesting that the *fah1* mutant should accumulate enhanced oxidative damage products. This hypothesis was tested by assaying products of lipid and protein oxidation. Levels of malondialdehyde, a lipid peroxidation byproduct, were measured in leaves treated with increasing doses of UV-B to determine the minimal amount of UV-B required to increase oxidative damage to lipids in *A. thaliana*. The extent of lipid peroxidation under the highest UV-B treatment correlated with the degree of physiological injury observed for the three genotypes. As indicated in Figure 4, lipid peroxidation increased by 170% in *fah1* and 60% in *tt5* with 0.9 kJ UV-B_{BE} m^{-2} h^{-1}, whereas no significant increase was observed for the wild type. In two additional experiments, UV-B treatment (approximately 0.6 kJ UV-B_{BE} m^{-2} h^{-1} for 72 h) increased lipid peroxidation in *fah1* but not in *tt5* or L*er* (data not shown). This confirmed that the *fah1* mutant was especially susceptible to UV-B-induced membrane damage.

Proteins are also likely targets for UV-B oxidation because aromatic amino acids are chromophores for UV light. Protein oxidation can result in the derivatization of amino acid side chains to form carbonyl groups (Levine et al., 1994). Consistent with the hypothesis that UV-B causes oxidative damage in *A. thaliana*, the carbonyl content relative to total soluble protein was increased by UV-B treatment, as shown in Figure 5. Despite the high concentration of carbonyl compounds in the *fah1* control samples relative to the other two genotypes, irradiation under 0.6 kJ UV-B_{BE} m^{-2} h^{-1} caused increased carbonyl compounds in the sinapate ester-deficient mutant. As was the case for lipid peroxidation, the relative increase in accumulation of carbonyl groups correlated with degree of genotype sensitivity to UV-B. It is surprising that carbonyl concentration in *fah1* did not increase between 0.6 and 0.9 kJ UV-B_{BE} m^{-2} h^{-1}, perhaps because of increased degradation of damaged proteins under the highest UV-B treatment. It should be noted that the trends illustrated in Figure 5 reflect the results obtained in each of the three independent experiments. The large SE values observed when the data from all three experiments are averaged primarily reflect quantitative differences among experiments. These differences could have been be caused by subtle differences in white and UV-B light sources or the properties of the cellulose acetate filters used in these experiments. Taken together, these results are consistent with increased penetration of UV-B in sunscreen-deficient mutants and support the notion that these fluences of UV-B cause enhanced oxidative stress in *tt5* and *fah1* mutants of *A. thaliana*.

UV-B Increases Ascorbate Peroxidase Activity

If UV-B irradiation causes an increased oxidative stress in sunscreen-deficient mutants, as suggested by the results shown in Figures 4 and 5, we would expect the activity of a known antioxidant enzyme to be elevated by UV-B in a dose- and genotype-dependent manner. To test this hypothesis, ascorbate peroxidase activity was measured in leaf extracts of plants treated for 72 h with increasing fluences of UV-B. The results shown in Figure 6 indicate that wild-type L*er* showed increased enzyme activity only under the highest fluence UV treatment. In contrast, the

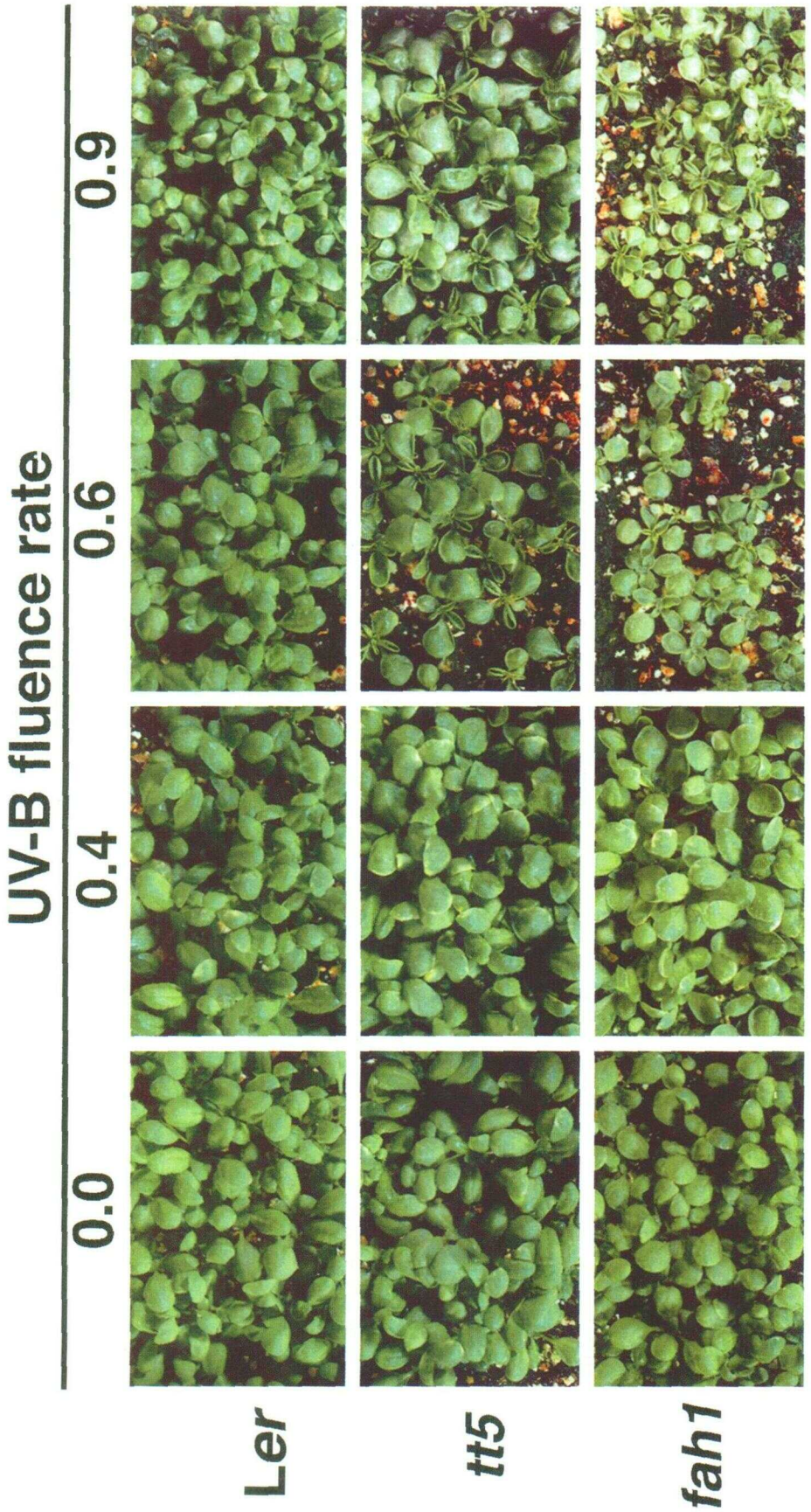

Figure 3. Growth inhibition, leaf cupping, and foliar injury caused by UV-B exposure. Two-week-old plants (L*er*, *tt5*, and *fah1*) were exposed to 0.0, 0.4, 0.6, or 0.9 kJ UV-B_{BE} m^{-2} h^{-1} for 72 h starting 14 d after planting.

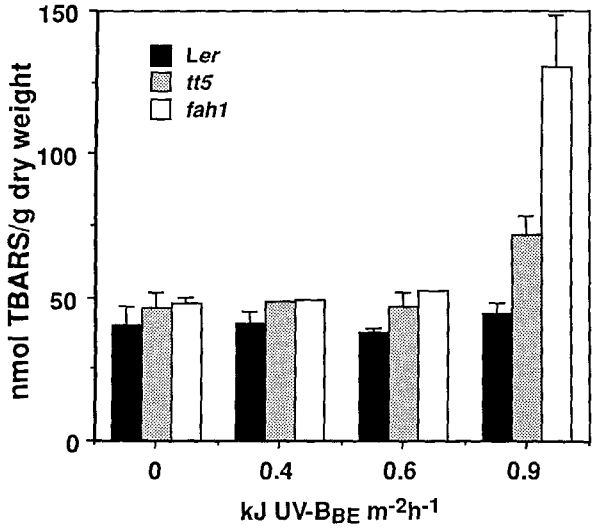

Figure 4. The effect of UV-B on lipid peroxidation in *A. thaliana*. Two-week-old plants (*Ler*, *tt5*, and *fah1*) grown in pots were exposed to UV-B for 72 h, tissue was extracted in TCA, and TBA-reactive substances (TBARS) were quantitated per gram dry weight of tissue. Bars represent the means of two samples and vertical lines show the SES (except for *tt5* at 0.4 kJ m^{-2} h^{-1}, for which only one sample was analyzed).

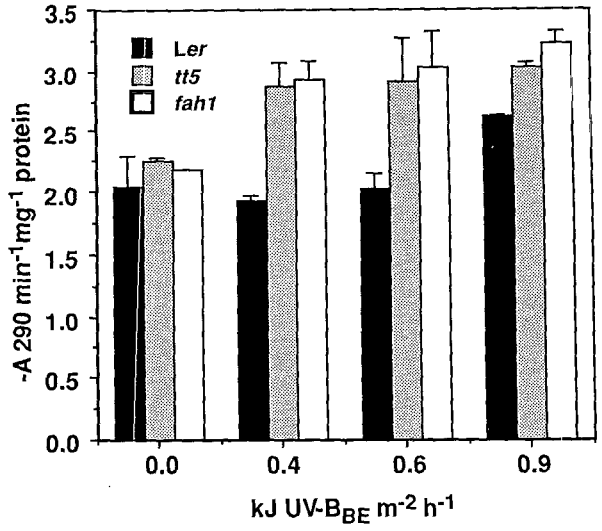

Figure 6. UV-B dose response of ascorbate peroxidase activity. Two-week-old plants grown in pots (*Ler*, *tt5*, and *fah1*) were exposed to 0.0, 0.4, 0.6, or 0.9 kJ $UV\text{-}B_{BE}$ m^{-2} h^{-1} for 72 h. Each bar represents the mean of two samples assayed in triplicate and the SES are shown as vertical lines.

UV-B sunscreen-deficient *tt5* and *fah1* plants exhibited higher levels of ascorbate peroxidase activity than controls at each UV-B fluence tested. These results confirm that the sunscreen mutants experienced an increased oxidative stress due to UV-B.

DISCUSSION

Mutants that were compromised in their ability to produce flavonoid derivatives (*tt5*) or sinapate esters (*fah1*) were more susceptible to UV-B than was wild-type *A. thaliana*. The ferulic acid hydroxylase mutant *fah1* was highly sensitive to both short-term acute and chronic UV-B, the chalcone synthase mutant *tt5* was intermediate in response, and the wild type was the least affected by UV-B. The extreme UV-B sensitivity of *fah1* compared to *tt5* indicates that sinapate esters are more effective in preventing UV-B injury than are flavonol derivatives. These results confirm previously published data showing that the relative UV-B sensitivity of the two flavonoid biosynthetic mutants *tt4* and *tt5* is correlated with sinapate ester levels (Li et al., 1993).

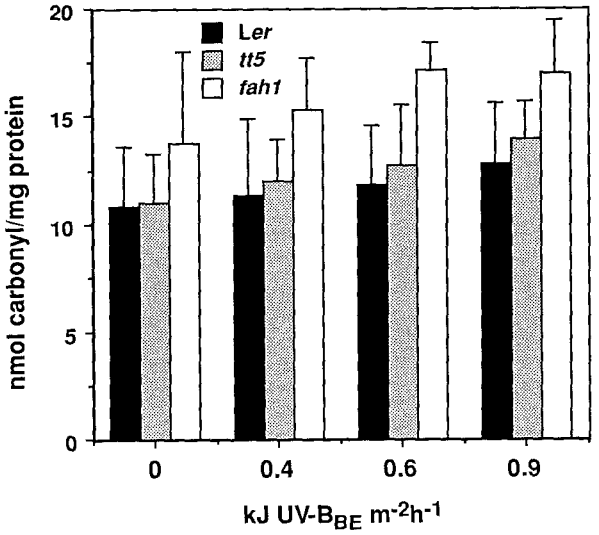

Figure 5. The effect of UV-B on protein oxidation in *A. thaliana*. Soluble protein from leaves of 2-week-old plants grown in pots (*Ler*, *tt5*, and *fah1*) were extracted and carbonyl concentrations were determined. Bars represent the means of six samples (two each from three independent experiments) and vertical lines show the SES.

Upward cupping of leaves, which is a characteristic response of *tt5* to UV-B treatment (Li et al., 1993; Ormrod et al., 1995), was a useful marker for the degree of UV-B stress in the three genotypes tested. When plants were grown under continuous UV-B, leaf cupping was pronounced in the stunted *tt5* plants and could also be seen in the tiny leaves of *fah1* (Fig. 2). During the shorter duration acute UV-B treatment (Fig. 3), cupping apparently occurred only in leaves that grew during the UV-B treatment, suggesting that this phenomenon may be caused by greater inhibition of division or expansion of cells on the upper surface of the leaf than on the lower side.

The differences in UV-B sensitivity between the wild type and sunscreen mutants were further evident in two biochemical estimations of cellular injury: the peroxidation of lipids (Fig. 4) and the oxidation of proteins (Fig. 5). The enhancement in oxidative damage of the sunscreen mutants is likely to be due, at least in part, to enhanced penetration of UV-B photons through the more transparent mutant epidermis. Several studies have shown that UV-B affects plant membrane structure and function (Murphy, 1983); yet it is unclear whether the main cause of membrane damage is due to direct absorption of UV-B by

proteins and lipids or through indirect effects such as free radical formation. Our observation that enhanced lipid peroxidation was observed only with high fluence UV-B (Fig. 4) is consistent with previous reports indicating that lipid peroxidation requires a higher dose of UV-B than other types of membrane injury, such as the disruption of ion channel function and the inactivation of ATPases (Murphy, 1983; Strid et al., 1994).

The greater sensitivity of *fah1* over *tt5* indicates that sinapate esters are more important than flavonoids for preventing UV-B injury in *A. thaliana*. Whereas much discussion in the literature has focused on flavonoid derivatives as UV-B protectants, fewer reports have critically explored the significance of hydroxycinnamate compounds in UV-B protection. Because of the wide distribution of these compounds in plant taxa, it is important to reevaluate the relative importance of flavonoids and monocyclic phenylpropanoid compounds (including hydroxycinnamic acids) in UV-B protection. This is especially crucial considering the differences in UV-B opacity reported for various plant species tested (Day et al., 1992, 1993; Day, 1993). For example, it was shown that the epidermis of conifers is a much more efficient UV-B filter than that of herbaceous dicots. Although anatomical features are likely to contribute to this variability, it is plausible that differences in chemical composition of epidermal cells plays an important role in the relative UV-B penetration and tolerance of various species.

Phytochemical analysis of UV-B absorptive molecules in specific plants under study is essential to critically assess the relative importance of different classes of UV-B protectants. Changes in UV-B absorbance of extracts made from plants exposed to UV cannot be assumed to be due solely to induction of flavonoids, as is common practice in the literature. An example of the importance of this principle was recently demonstrated for two closely related UV-B-absorbing compounds in *Pinus sylvestris* (Scots pine) seedlings (Jungblut et al., 1995). These compounds are flavonol monoglycosides that each contain two *p*-coumaroyl groups attached to the sugar moiety as acyl groups. Upon spectroscopic analysis of the flavonol and coumaroyl constituents, it was demonstrated that the isolated flavonol moiety (quercitin-3-glucoside) has extremely limited absorbance in the UV-B, in contrast to the strong absorbance of the coumaroyl compounds. Thus, although the UV-B-absorbing molecules found in Scots pine seedlings contain a flavonoid, this part of the molecule is unlikely to be the critical UV-B chromophore. Taken together, these results indicate that, in species as diverse as the conifer Scots pine and the herbaceous annual *A. thaliana*, it is likely that nonflavonoid phenylpropanoid molecules such as hydroxycinnamate compounds are important UV-B protectants.

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