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UVR8 Mediates UV-B-Induced *Arabidopsis* Defense Responses against *Botrytis cinerea* by Controlling Sinapate Accumulation

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ABSTRACT Light is emerging as a central regulator of plant immune responses against herbivores and pathogens. Solar UV-B radiation plays an important role as a positive modulator of plant defense. However, since UV-B photons can interact with a wide spectrum of molecular targets in plant tissues, the mechanisms that mediate their effects on plant defense have remained elusive. Here, we show that ecologically meaningful doses of UV-B radiation increase *Arabidopsis* resistance to the necrotrophic fungus *Botrytis cinerea* and that this effect is mediated by the photoreceptor UVR8. The UV-B effect on plant resistance was conserved in mutants impaired in jasmonate (JA) signaling (*jar1-1* and *P35S:JAZ10.4*) or metabolism of tryptophan-derived defense compounds (*pen2-1*, *pad3-1*, *pen2 pad3*), suggesting that neither regulation of the JA pathway nor changes in levels of indolic glucosinolates (iGS) or camalexin are involved in this response. UV-B radiation, acting through UVR8, increased the levels of flavonoids and sinapates in leaf tissue. The UV-B effect on pathogen resistance was still detectable in *tt4-1*, a mutant deficient in chalcone synthase and therefore impaired in the synthesis of flavonoids, but was absent in *fah1-7*, a mutant deficient in ferulic acid 5-hydroxylase, which is essential for sinapate biosynthesis. Collectively, these results indicate that UVR8 plays an important role in mediating the effects of UV-B radiation on pathogen resistance by controlling the expression of the sinapate biosynthetic pathway.

Key words: defense; jasmonate; phenolics; phenylpropanoids; photomorphogenesis; photoreceptor; plant–microbe interactions.

INTRODUCTION

Light is an important regulator of the interactions between plants and consumer organisms. Studies in natural and managed ecosystems have shown that shade and high plant density increase infection by a range of pathogens (reviewed in Alexander and Holt, 1998; Burdon and Chilvers, 1982; Gilbert, 2002; Roberts and Paul, 2006). These effects of shade and high density are often attributed to factors such as humidity and leaf surface wetness, which are clearly important for pathogen success. However, a number of studies have shown that plant density and shading can also affect host resistance to pathogen infection (Gilbert, 2002; Roberts and Paul, 2006). The mechanisms that mediate these effects of light on plant immunity are not well understood, but significant evidence has emerged in the last few years indicating that light, acting through specific photoreceptors, can be an important modulator of hormonal signaling pathways involved in the orchestration of plant defense (Ballaré, 2011; Kazan and Manners, 2011).

Solar UV-B radiation (280–315 nm) has well-documented effects increasing plant resistance to herbivorous insects under field conditions (Ballaré et al., 2011; Kuhlmann and Müller, 2011). Part of these effects of solar UV-B can be attributed to changes in plant tissue quality induced by UV-B radiation (Ballaré et al., 1996; Rousseaux et al., 1998; Mazza et al., 1999), which include accumulation of protective phenolic compounds and enhancement of JA-dependent defense responses (Stratmann et al., 2000; Zavala et al., 2001; Izaguirre et al., 2003; Caputo et al., 2006; Foggo et al., 2007; Izaguirre et al., 2007; Kuhlmann and Müller, 2009; Demkura et al., 2010). UV radiation has also been reported to increase plant resistance to leaf pathogens (Gunasekera et al., 1997; Gunasekera and Paul, 2007; Kunz et al., 2008), and can affect the composition of

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microbial communities in the phyllosphere (Kadivar and Stapleton, 2003; Balint-Kurti et al., 2010). However, the effects of solar UV-B radiation on microbial diseases have not been investigated in great detail.

Our understanding of the mechanisms of plant response to UV-B radiation has been greatly advanced by the identification of UVR8 (UV RESISTANCE LOCUS 8) as a primary UV-B photoreceptor (Rizzini et al., 2011). UVR8 controls the expression of numerous genes involved in acclimation to and protection against UV-B radiation (Jenkins, 2009). The genes regulated by UVR8 include genes involved in the biosynthesis of flavonoids (protective phenolic sunscreens), the gene encoding a cyclobutane pyrimidine dimer photolyase (*UVR2*, which is essential for repair of UV-B-induced DNA damage), and genes connected with protection against oxidative stress and photooxidative damage (Brown et al., 2005; Favory et al., 2009). Absorption of UV-B induces instant monomerization of the photoreceptor and interaction of UVR8 with the protein COP1 (Rizzini et al., 2011), which is required for UV-B-stimulated gene activation in light-grown seedlings (Oravecz et al., 2006). Many of the effects of UVR8 on plant photomorphogenesis, including the regulation of genes involved in flavonoid metabolism, are mediated by activation of the bZIP transcription factors ELONGATED HYPOCOTYL 5 (HY5) (Ulm et al., 2004; Brown et al., 2005) and HY5 HOMOLOG (HYH) (Brown and Jenkins, 2008). However, HY5-independent UVR8 effects (Fehér et al., 2011) and UVR8-independent responses to UV-B radiation (Brown and Jenkins, 2008; Besteiro et al., 2011) have also been reported.

None of the well-documented effects of solar UV-B radiation on plant resistance to pathogens and herbivorous organisms has been yet linked with UVR8-mediated responses (Ballaré et al., 2011). In fact, the effects of UV-B on plant defense might result from pleiotropic consequences of UV-induced cellular damage and activation of a 'generalized' stress response (Brown and Jenkins, 2008; Besteiro et al., 2011). Nevertheless, it is important to note that, under field conditions, functional defense responses are induced by relatively low UV-B irradiances (less than 0.1% of the total shortwave quanta), which do not cause stress symptoms or visible plant damage (discussed in Demkura et al., 2010).

In this paper, we show that UV-B radiation increases plant resistance to the necrotrophic fungus *Botrytis cinerea* and that this effect is mediated by the photoreceptor UVR8. The UV-B effect on plant resistance was conserved in mutants affected in JA signaling and metabolism of tryptophan-derived defense compounds, suggesting that neither JA regulation nor the production of typical antifungal compounds such as iGS and camalexin is required for this response. UV-B radiation, acting through UVR8, increased the levels of flavonoids and sinapates in leaf tissue. The UV-B effect on pathogen resistance was conserved in *tt4-1*, a mutant deficient in chalcone synthase and therefore impaired in the synthesis of flavonoids, but it was absent in *fah1-7*, a mutant deficient in ferulic acid 5-hydroxylase, which is essential for sinapate biosynthesis. Collectively, these results indicate that UVR8 plays an important role in mediating the effects of solar UV-B radiation on pathogen resistance by controlling the expression of the sinapate biosynthetic pathway.

RESULTS

Low Levels of UV-B Radiation Increase Plant Resistance to *B. cinerea* Acting via UVR8

Arabidopsis plants were grown to the rosette stage in a glasshouse under natural levels of photosynthetically active radiation (PAR, control treatment, C) or PAR supplemented with low levels of UV-B radiation (UV-B treatment, UV; see Supplemental Figure 1). This UV-B treatment did not cause any visible symptoms of leaf damage; only a slight growth inhibition was detected in wild-type plants (Figure 1A), which is a typical response of field-grown *Arabidopsis* plants to natural doses of UV-B radiation (e.g. Caputo et al., 2006). The *uvr8-6* mutant was, if anything, less inhibited than the wild-type; this observation is consistent with the hypothesis that growth inhibition is a photoreceptor-mediated, adaptive response to ecologically meaningful levels of UV-B radiation (Ballaré et al., 1995). After the light treatment, plants were inoculated in the same glasshouse with a spore suspension of *B. cinerea* and kept in plastic chambers to prevent desiccation. The UV-B source was turned off at that point, to avoid any direct effects of the UV-B treatment on the fungus (e.g. Ensminger, 1993). Measurements of the spread of the leaf lesions in the infection bioassays revealed that plant exposure to UV-B radiation resulted in increased resistance to *B. cinerea*, both in the Col-0 (Figure 1B) and L*er*-0 (Supplemental Figure 2) wild-types. This protective effect of UV-B was not detectable in the *uvr8* mutants, indicating a requirement for activation of the UVR8 photoreceptor (Figure 1B and Supplemental Figure 2).

The UV-B Effect on *Arabidopsis* Resistance to *B. cinerea* Is Not Mediated by Changes in JA Response

Plants defend against necrotrophic pathogens, such as *B. cinerea*, by activating the JA response pathway, and previous work in other species has shown that pulses of UV-A/UV-B radiation (Stratmann et al., 2000) and natural doses of UV-B radiation (Demkura et al., 2010) can enhance defense responses by increasing plant sensitivity to JA. We tested this possibility in *Arabidopsis* by treating control and UV plants with different doses of methyl-JA (MeJA) and measuring the expression levels of *PDF1.2* (a plant defensin), *SUR2*/*CYP83B1* (a cytochrome P450 monooxygenase involved in indolic GS biosynthesis), and the well-characterized JA-responsive gene *VEGETATIVE STORAGE PROTEIN 1* (*VSP1*). None of these JA response markers showed a significant response to UV-B radiation (Figure 2).

To further investigate the potential role of JA in the UV-B effect on plant resistance to *B. cinerea*, we used two genotypes impaired in JA sensitivity: *jar1-1*, which is impaired in the synthesis of the bioactive JA-Ile conjugate (Staswick and Tiryaki,

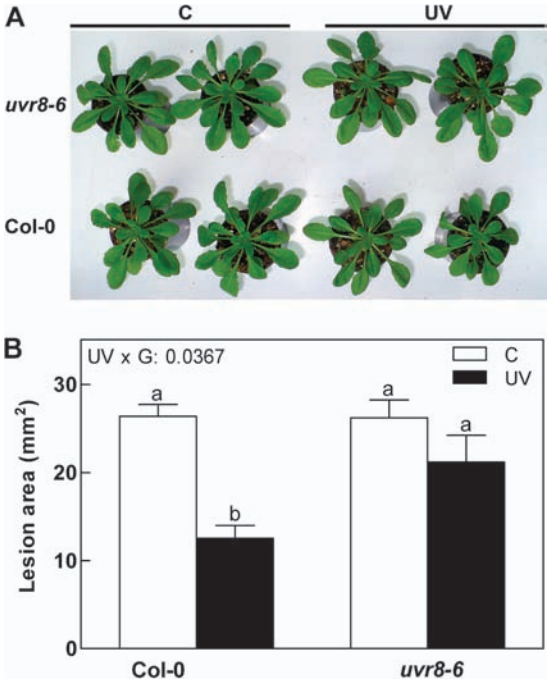

Figure 1. Supplemental UV-B Radiation Increases *Arabidopsis* Resistance to *B. cinerea* Infection in Wild-Type Plants but Not in *uvr8* Mutants.

(A) The UV-B radiation treatment used in these experiments did not cause visible damage or strong growth inhibition.

(B) UV-B radiation reduces the size of the lesions produced by *B. cinerea* in leaves of wild-type (Col-0) plants, but does not reduce disease symptoms in the *uvr8-6* mutant. Lesion areas were measured 48 h post inoculation (see the 'Methods' section for details). Each bar represents the mean +1 SE of eight infected plants (lesion area of each plant is the mean of five infected leaves). C, PAR; UV, PAR supplemented with UV-B radiation (see the 'Methods' section for details). The *P*-value for the UV × G interaction term of the ANOVA is shown; different letters indicate significant differences between means ($P < 0.05$, Tukey test).

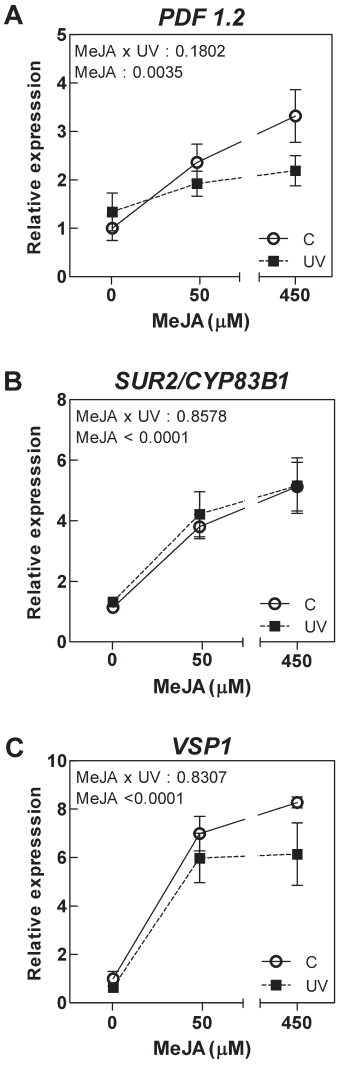

Figure 2. UV-B Radiation Does Not Affect the Expression Levels of Typical JA Response Marker Genes.

(A) *PDF1.2*.

(B) *SUR2/CYP83B1*.

(C) *VSP1*.

The experimental treatments resulted from a factorial combination of UV-B and MeJA applications: C, PAR; UV, PAR supplemented with UV-B radiation (for details, see the 'Methods' section). Samples for qPCR analysis were obtained 3 h after MeJA application. Expression data are normalized to the expression level detected in the control × 0 μM MeJA combination. Thin bars indicate ±1 SE ($n = 3$; each biological replicate is a pool of three individual plants). The *P*-value for the MeJA term and UV × MeJA interaction of the ANOVA are indicated (data for *SUR2* expression were log transformed to meet the assumptions of the test).

2004) and is more sensitive to *B. cinerea* (Ferrari et al., 2003), and *P35S:JAZ10.4*, a transgenic line that overexpresses a splice variant of the JAZ10 protein that is highly resistant to JA-induced degradation (JAZ10.4) and, as a consequence, is insensitive to JA (Chung and Howe, 2009). Complementary experiments carried out in our laboratory fully confirmed the lack of sensitivity to JA of the *P35S:JAZ10.4* line under our growth conditions, as indicated by male sterility and complete lack of response to MeJA in several typical JA response markers, including *MYC2*, *VSP1*, and *PDF1.2* (Supplemental Figure 3), among others. Plants of *jar1-1* and *P35S:JAZ10.4* were more susceptible to *B. cinerea* than those of the Col-0 wild-type, as expected, but UV-B radiation had a similar effect, increasing plant resistance to the fungus in all three genotypes (Figure 3). From these experiments (Figures 2 and 3), we conclude that the effect of UV-B radiation increasing *Arabidopsis* resistance to *B. cinerea* is unlikely to be mediated by UV-B-induced changes in JA signaling.

The UV-B Effect on Resistance to *B. cinerea* Is Not Mediated by Changes in GS or Camalexin

An important determinant of resistance to pathogen infection is the production of GS (mainly iGS) (Lipka et al., 2005; Halkier and Gershenzon, 2006; Bednarek et al., 2009; Schlaeppi et al., 2010). A related trp-derived defense metabolite, camalexin (Glawischnig, 2007), also plays an important role in pathogen

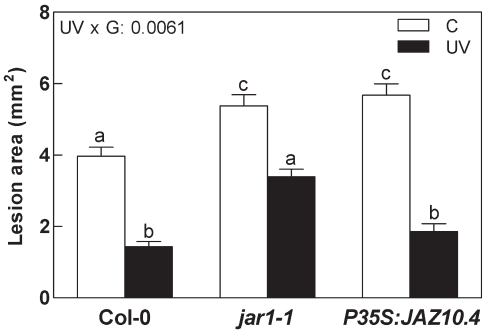

Figure 3. The Effect of UV-B Radiation Increasing Plant Resistance to *B. cinerea* Does Not Require JA Signaling.

Lesion areas were measured 48 h post inoculation. Each bar represent the mean +1 SE of five to eight infected plants (lesion area of each plant is the mean of five infected leaves). C, PAR; UV, PAR supplemented with UV-B radiation. The *P*-value for the UV × G interaction term of the ANOVA is shown. Different letters indicate significant differences between means ($P < 0.05$, Tukey test).

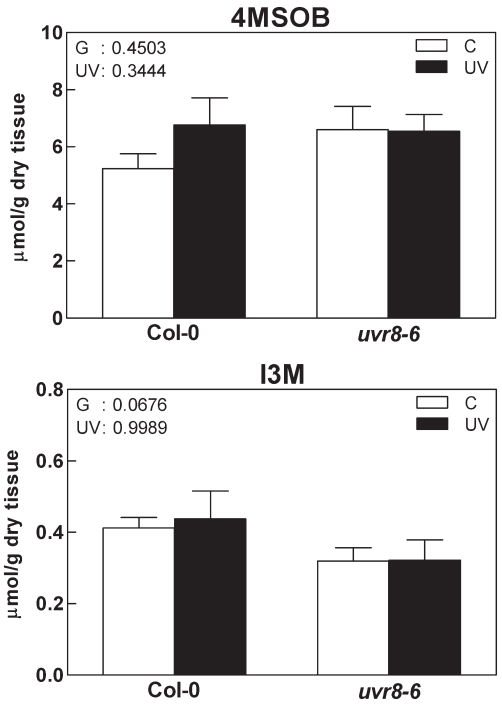

Figure 4. UV-B Irradiation Does Not Affect GS Content.

Major aliphatic (4MSOB) and indolic (I3M) desulphoglucosinolates in the Col-0 background and *uvr8-6* mutants were quantified by HPLC after 4 d of UV-B irradiation. Each bar represents the mean +1 SE of four biological replicates (each replicate is a pool of three individual plants). C, PAR; UV, PAR supplemented with UV-B radiation. The *P*-values for the main terms of the ANOVA are indicated on the graph (data for I3M levels were log transformed to meet assumptions of the test).

defense in *Arabidopsis* (Ferrari et al., 2003; Kliebenstein, 2004; Kliebenstein et al., 2005; Schlaeppi et al., 2010), and previous work has indicated that camalexin levels can increase in response to UV-C ($\lambda < 280$ nm) treatments or long exposures to UV-B radiation (Mert-Turk et al., 2003; Glawischnig, 2007). Our gene expression experiments did not reveal any effects of the UV-B radiation treatment used in this study on transcript levels of *SUR2* (*CYP8391*) or *PAD3* (*CYP71A13*), two key genes of the iGS and camalexin biosynthetic pathways (Figure 2 and Supplemental Figure 4). Furthermore, the levels of the major aliphatic (4-methylsulfinylbutyl (4MSOB)) and iGS (indol-3-ylmethyl (I3M)) in the *Arabidopsis* Col-0 background were not affected by our UV-B treatment, and did not differ between Col-0 and *uvr8-6* plants (Figure 4). In order to directly test the role of trp-related metabolites (indolic GS and camalexin) in the protective effect of UV-B, we carried out infection bioassays with mutants deficient in the production of the bioactive hydrolysis products of iGS (*pen2-1*), camalexin biosynthesis (*pad3-1*), and the *pen2 pad3* double mutant. These mutants were more sensitive than the Col-0 wild-type to *B. cinerea* infection; however, the expression of the UV-B effect on plant defense was completely conserved in all of them (Figure 5).

The UV-B Effect on Plant Resistance to *B. cinerea* Is Likely Mediated by Increased Sinapate Production

Well-known targets of UVR8 regulation are the genes encoding enzymes of the flavonoid pathway (Jenkins, 2009). Chalcone synthase (CHS), the enzyme that catalyzes the first committed step of this pathway, has been extensively characterized in terms of its regulation by UVR8 at the transcriptional level (Kliebenstein et al., 2002; Brown et al., 2005; Favory et al., 2009). Imaging of UV-induced chlorophyll fluorescence (Mazza et al., 2000) demonstrated a strong, UVR8-dependent decrease in epidermal UV transmittance (Figure 6A). This decrease in UV-induced chlorophyll fluorescence is caused by the accumulation of photoprotective, UV-absorbing phenolic compounds, which reduces UV penetration to the chloroplasts (Bilger et al., 1997; Barnes et al., 2000; Mazza et al., 2000).

Our metabolite analysis confirmed that quercetin and kaempferol glycosides (the major *Arabidopsis* flavonoids) are strongly induced by UV-B both in the Col-0 and L*er*-0 genetic backgrounds, and that this induction is mediated by UVR8 (Figure 6B–6F and Supplemental Figure 5 for the L*er*-0 phenolic profile). In addition, we found that the accumulation of sinapates (sinapoyl malate and sinapoyl glucose) in response to UV-B radiation was completely dependent on UVR8 in both genetic backgrounds (Figure 6G, 6H and Supplemental Figure 5).

To investigate the role of these phenolic compounds in UV-B-induced resistance to *B. cinerea*, we used a genetic approach. The flavonoid-deficient mutant *tt4-1*, impaired in *CHS* expression (Figure 7A; see Supplemental Figure 6 for a representative chromatogram of the phenolic profile of the mutant) still showed a significant UV-B effect halting *B. cinerea* infection (Figure 7B, left panel). However, this effect was entirely absent in *fah1-7* (Figure 7B, right panel), a mutant

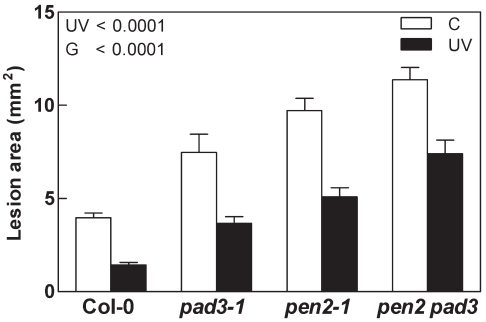

Figure 5. The Effect of UV-B Radiation Increasing Resistance to *B. cinerea* Was Conserved in Mutants Deficient in the Production of the Bioactive Hydrolysis Products of iGS or Camalexin Biosynthesis.

Lesion areas were measured 48 h post inoculation. Each bar represent the mean +1 SE of five to eight infected plants (lesion area of each plant is the mean of five infected leaves). C, PAR; UV, PAR supplemented with UV-B radiation. The *P*-value for the UV × G interaction term of the ANOVA is shown. Different letters indicate significant differences between means ($P < 0.05$, Tukey test).

deficient in ferulic acid 5-hydroxylase (Figure 7A and Supplemental Figure 6), which is essential for sinapate biosynthesis (Meyer et al., 1996; Ruegger et al., 1999). These results suggest that the effects of physiological doses of UV-B increasing *Arabidopsis* resistance to *B. cinerea* are the result of UVR8-mediated sinapate production.

DISCUSSION

There is growing evidence that the light environment is an important modulator of plant defense against pathogens and herbivores (Ballaré, 2009, 2011; Kazan and Manners, 2011; Morker and Roberts, 2011; Kangasjärvi et al., 2012). Previous work has connected phytochromes and cryptochromes (photoreceptors of red:far-red and blue light, respectively) with plant resistance to pathogen infection (Genoud et al., 2002; Griebel and Zeier, 2008; Wu and Yang, 2010; Kazan and Manners, 2011; Cerrudo et al., 2012). Our experiments add UVR8 to the list of photomorphogenic photoreceptors that regulate the expression of antimicrobial defenses, and provide a functional explanation for the effects of solar UV-B radiation protecting plants against infection by the necrotrophic leaf pathogen *B. cinerea*.

Plant resistance to necrotrophic pathogens depends on activation of JA signaling (Glazebrook, 2005; Pieterse et al., 2009), and JA responses are known to be regulated by informational photoreceptors (Moreno et al., 2009; Robson et al., 2010; Cerrudo et al., 2012). This regulation of the JA response by light signals is thought to play an important role under natural conditions, modulating the resource allocation balance between growth and defense as a function of the ecological context of the plant (Ballaré, 2009, 2011). Previous experiments have shown that UV-B radiation can modulate JA responses, such as the accumulation of defensive proteinase inhibitors (Stratmann et al., 2000; Demkura et al., 2010),

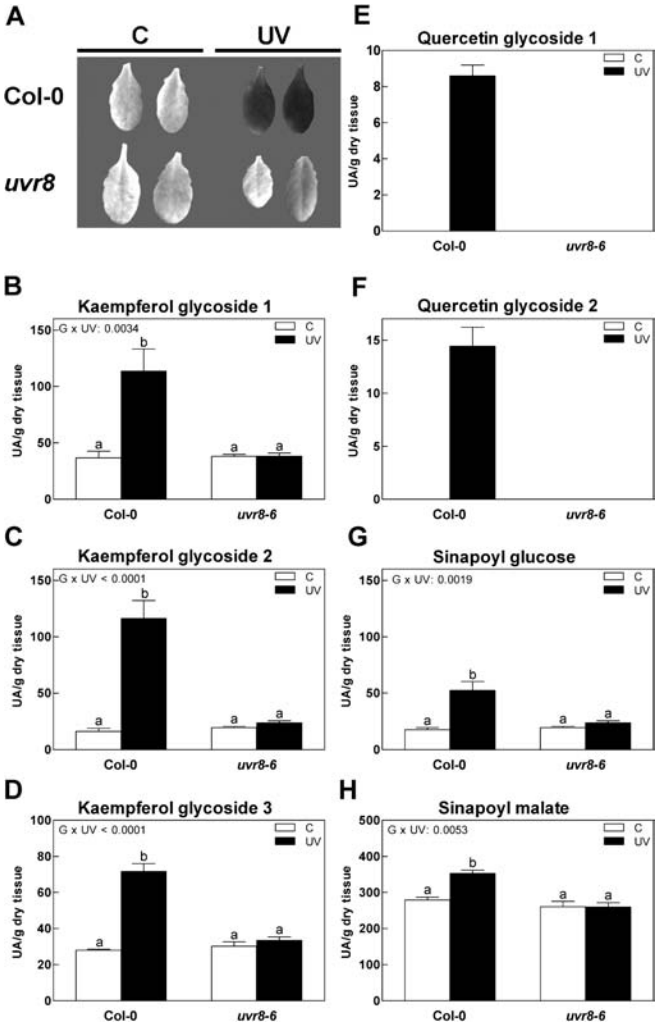

Figure 6. UV-B Radiation Induces the Accumulation of Leaf Phenolics in a UVR8-Dependent Manner.

UV-B radiation induced the accumulation of phenolic compounds in the leaf epidermis, resulting in decreased UV-induced chlorophyll fluorescence in Col-0 but not *uvr8-6* leaves **(A)** (darker leaves indicate lower intensities of chlorophyll fluorescence under UV irradiation; see Mazza et al. (2000) for details). Three kaempferol glycosides **(B–D)**, two quercetin glycosides **(E, F)**, sinapoyl glucose **(G)**, and sinapoyl malate **(H)** were quantified from leaf tissue by HPLC after 4 d of UV-B irradiation. Each bar represents the mean +1 SE of four biological replicates (each replicate is a pool of three individual plants). C, PAR; UV, PAR supplemented with UV-B radiation. The P-values for the UV × G interaction term of the ANOVA for each compound are shown (data for kaempferol glycosides were log transformed to meet assumptions of the test; data for quercetin glycosides were not statistically analyzed because these compounds were detectable only in Col-0 plants exposed to UV-B radiation). Different letters indicate significant differences between means ($P < 0.05$, Tukey test).

and that JA signaling is required for the activation of some antiherbivore effects of UV-B radiation (Caputo et al., 2006; Demkura et al., 2010). Our present experiments confirm the important role of JA in plant defense against *B. cinerea*, by showing that plants impaired in the biosynthesis of bioactive

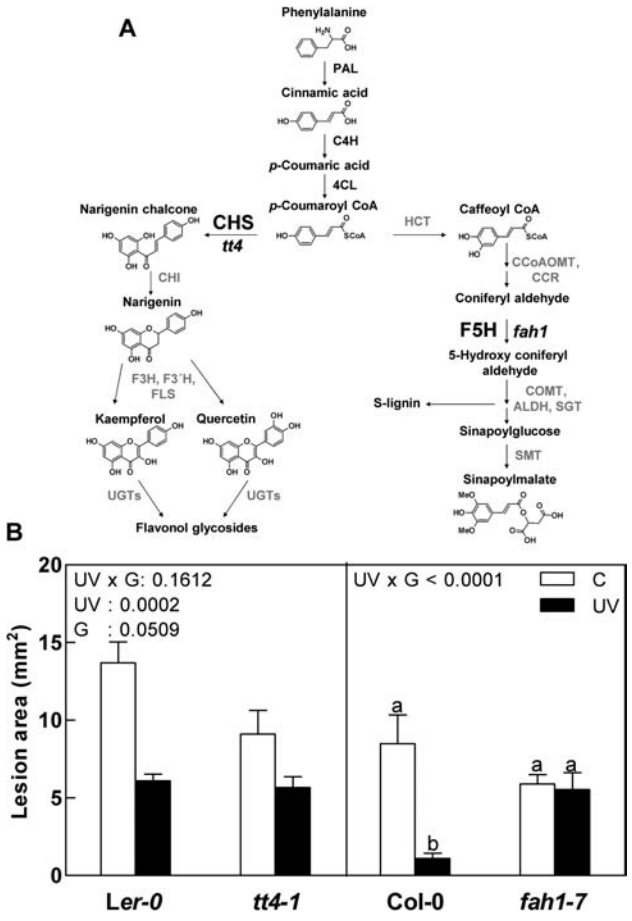

Figure 7. The Effect of UV-B Radiation Increasing Resistance to *B. cinerea* Requires Sinapate but Not Flavonoid Production.

(A) Schematic representation of the main steps and enzymes involved in the phenylpropanoid pathway leading to the synthesis of C15 (left), and C3–C6 (right) derivatives in *Arabidopsis* (modified from Besseau et al. (2007) and AraCyc (http://pmn.plantcyc.org/ARA/server.html)). Key enzymes affected in the *tt4-1* and *fah1-7* mutants are highlighted in bold face. PAL, phenylalanine ammonia lyase; C4H, C4-hydroxylase; 4CL, 4-coumaroyl-CoA ligase; HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase; CHS, chalcone synthase; CCoAOMT, caffeoyl-CoA O-methyltransferase; CCR, cinnamoyl-CoA reductase; COMT, caffeic acid O-methyltransferase; ALDH, aldehyde dehydrogenase; F5H, ferulate 5-hydroxylase; SGT, sinapate UDP-glucose sinapoyltransferase; SMT, sinapoylglucose malate sinapoyltransferase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; FLS, flavonol synthase; UGTs, UDP sugar glycosyltransferases.

(B) Effect of UV-B radiation on *Arabidopsis* resistance to *B. cinerea* in flavonoid-deficient (*tt4-1*, left panel) and sinapate-deficient (*fah1-7*, right panel) mutants. Lesion areas were measured 48 h post inoculation. Each bar represent the mean +1 SE of five to eight infected plants (lesion area of each plant is the mean of five infected leaves). C, PAR; UV, PAR supplemented with UV-B radiation. Each mutant was analyzed against its respective background, as shown in the graph. The *P*-value for main factors and the UV × G interaction term of the ANOVA are shown (data were log transformed to meet assumptions of the test). Different letters indicate significant differences between means ($P < 0.05$, Tukey test).

JA (*jar1-1*), or insensitive to JA (*35S::JAZ10.4*), are less resistant to fungal infection (Figure 3). However, UV-B failed to increase transcript levels of several JA-responsive genes involved in plant defense (*PDF1.2*, *SUR2*/*CYP83B1*, *VSP1*) (Figure 2), and the bioassays with JA signaling mutants (Figure 3) indicate that JA sensitivity is not required for the expression of the effect of physiological doses of UV-B on plant resistance to *B. cinerea*.

Arabidopsis resistance against *B. cinerea* has been shown to depend on a variety of defense-related proteins, such as plant defensins (Penninckx et al., 1996, 2003), and secondary metabolites, including GSs, camalexin, and phenolic compounds (Ferrari et al., 2003; Kliebenstein, 2004; Kliebenstein et al., 2005; Lipka et al., 2005; Halkier and Gershenzon, 2006; Bednarek et al., 2009; Schlaeppi et al., 2010). Our experiments confirm the important role of trp-derived compounds, by showing that mutants deficient in the key enzymes involved in the synthesis or processing of these metabolites are hypersensitive to fungal infection (Figure 5). However, they also demonstrate that these metabolites are neither induced by natural levels of UV-B radiation (Figure 4) nor required for the expression of the protective effect of UV-B radiation on plant defense (Figure 5).

Phenolic compounds (phenylpropanoids and polyketides) (Figure 7A), derived from phenylalanine through the phenylpropanoid biosynthetic pathway, can be effective defenses against several stressors (D'Auria and Gershenzon, 2005). Phenolic compounds are known to contribute to fungal resistance in several species of cultivated plants, and have been extensively studied, particularly in connection with resistance to postharvest diseases (Goetz et al., 1999; Wurms et al., 2003; Terry et al., 2004; Guetsky et al., 2005). However, the role of these secondary metabolites in antifungal defense has not been investigated in great detail in *Arabidopsis* plants (Ferrari et al., 2003; Kliebenstein et al., 2005). A particular group of phenolic compounds, the flavonoids, which are derived from a phenylpropanoid unit by sequential decarboxylative addition of three units of malonyl-CoA catalyzed by CHS (Figure 7A), are strongly induced by UV-B radiation as a protective UV-absorbing sunscreen (Li et al., 1993; Burchard et al., 2000). In our experiments with natural doses of UV-B, this induction was completely dependent on UVR8 activation (Figure 6 and Supplemental Figure 5). This UVR8 dependency for flavonoid accumulation is consistent with the well-established role of UVR8 mediating the effect of UV-B radiation on *CHS* gene expression (Brown et al., 2005; Favory et al., 2009). However, the results of our bioassays with the *tt4-1* mutant (deficient in CHS, and impaired in the production of all flavonoids (Li et al., 1993)) suggest that these compounds do not mediate the UV-B effect increasing *Arabidopsis* resistance to *B. cinerea* (Figure 7B, left panel).

Besides flavonoids, C3–C6 phenylpropanoids (Figure 7A) are important UV-B-induced phenolic compounds involved in UV photoprotection in *Arabidopsis* (Li et al., 1993; Landry et al., 1995; Sheahan, 1996; Mazza et al., 2000) and other

plant species (Burchard et al., 2000). C3–C6 phenylpropanoids, such as caffeic acid, coumaric acid, and other hydroxycinnamic acids, are thought to contribute to constitutive and induced resistance against *B. cinerea* (Wurms et al., 2003) and other fungal diseases in various species of cultivated plants (Daayf et al., 2000). Sinapic acid is an important hydroxycinnamic acid required for the biosynthesis of syringyl lignin in angiosperms and, in some species, as a precursor for soluble compounds such as sinapate esters (Figure 7A). Sinapate deficiency in the *fah1-7* mutant (which does not convert ferulic acid into 5-hydroxyferulic acid (Meyer et al., 1996)) did not correlate with increased susceptibility to *B. cinerea* in previous bioassays carried out in growth cabinets under UV-free conditions (Kliebenstein et al., 2005). Sinapates are induced by UV-B radiation in *Arabidopsis* (Li et al., 1993), and our results in Col-0 and L*er*-0 plants demonstrate that this induction is mediated by UVR8 (Figure 6 and Supplemental Figure 5). This result is consistent with the observation that *FAH1* transcription is rapidly up-regulated by UV-B radiation and that this up-regulation requires UVR8 (Favory et al., 2009). In our *B. cinerea* infection bioassays, *fah1-7* was as resistant as the Col-0 wild-type under UV-B-free conditions (Figure 7B, right panel), confirming previous results (Kliebenstein et al., 2005). However, this mutant was distinctly different from the wild-type in that it did not express the resistance phenotype induced by UV-B radiation (Figure 7B, right panel). We conclude from these experiments that the effect of physiological doses of UV-B radiation boosting *Arabidopsis* resistance to fungal infection is caused by induction of increased sinapate production through an UVR8-dependent mechanism. Sinapates may contribute to *Arabidopsis* resistance to *B. cinerea* by serving as precursors for the synthesis of syringyl-type ('defense') lignin (Figure 7A), which is involved in cell wall fortification and could prevent penetration of fungal hyphae into plant cells (Kishimoto et al., 2006; Quentin et al., 2009; Lloyd et al., 2011). In agreement with this interpretation, preliminary observations of *Arabidopsis* leaves stained with phloroglucinol-HCl (Mohr and Cahill, 2007) showed increased lignin deposition in response to UV-B in Col-0 plants, which was not observed in the *uvr8-6* mutant (Supplemental Figure 7). Further analysis will be necessary to test the functional connection between UV-B-induced sinapate accumulation, lignification of cell walls, and resistance to fungal infection.

In conclusion, this study demonstrates that variations in UV-B levels, perceived by UVR8, should be added to the list of environmental signals that regulate the expression of plant defense in canopies. We suggest that the well-documented reduction of plant resistance to disease associated with shading and increased canopy density (Burdon and Chilvers, 1982; Alexander and Holt, 1998; Gilbert, 2002; Roberts and Paul, 2006) could be caused, at least in part, by the loss of the protective effect of UV-B radiation under conditions of reduced sunlight exposure. UV-B-activated defense mechanisms might be interesting targets for utilization in agricultural systems that allow manipulation of the light environment (e.g. Wargent et al., 2006; Vänninen et al., 2010).

METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana seeds were germinated as described previously (Moreno et al., 2009). Seven days after germination, seedlings were transferred to individual pots (0.11 L) with a vermiculite:perlite:peat mixture and watered every 2 d with Hakaphos Rojo solution 18–18–18 (Compo). Plants were grown in a glasshouse under short-day conditions (10/14-h light/dark cycles). Daily temperatures varied between 9 and 25°C. Peak levels of PAR in the glasshouse varied between 600 and 1000 μmol m^{-2} s^{-1}. Rosette-stage plants of similar age and size (typically 3–4 weeks old) were selected for experiments and randomly assigned to the treatments. The *uvr8-6* mutant in the Columbia (Col-0) background was obtained from Roman Ulm (University of Geneva, Switzerland); the *uvr8* mutant in the Landsberg *erecta* (L*er*-0) background was provided by Gareth Jenkins (University of Glasgow, Glasgow, UK); phenolic-deficient mutants *fah1-7* (CS8604), *tt4-1* (CS85), and the JA signaling mutant *jar1-1* (CS8072) were obtained from the ABRC (www.Arabidopsis.org); *pad3-1*, *pen2-1*, and the *pad3 pen2* double mutant were kindly provided by Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Cologne, Germany); the *P35S:JAZ10.4* transgenic line was provided by Gregg Howe (Michigan State University, Michigan, USA).

Light Treatments

The experimental setup was similar to that described previously (Izaguirre et al., 2007). Plants growing on a glasshouse bench (as described in the previous section) received ecologically realistic levels of UV-B radiation from fluorescent lamps (UVB TL100W/01, Phillips). Radiation from the lamps was filtered through a clear polyester film (Oeste Aislante, Argentina, 100 μm), to remove the UV-B photons provided by the lamps (control treatment, C), or a clear polyethylene film (Rolopac, Argentina, 20 μm), which is highly transparent throughout the UV spectrum (UV-B treatment, UV; see Supplemental Figure 1 for spectral details). Plants were irradiated for 4 h each day, with the irradiation period centered at solar noon; pots were randomly rotated within the irradiation area every 2 d to minimize position effects. The biologically effective UV-B dose (BE-UV-B) in all the experiments was 5.5 kJ m^{-2} (calculated using the plant action spectrum normalized at 300 nm (Caldwell, 1971)). Plants were randomly assigned to the elicitation treatments (MeJA) or inoculated with *B. cinerea* (for the bioassays) after 4 d of irradiation.

Fungal Culture and *B. cinerea* Bioassays

Botrytis cinerea was grown and maintained on potato dextrose agar (1.5% agar, 2% potato extract, 2% dextrose).

Conidia were collected from agar plates with distilled water and a glass rod, filtered, and re-suspended in a 0.1-M sucrose/0.07-M KH_2PO_4 solution to induce germination (Elad, 1991). Five leaves of 4-week-old rosettes were inoculated on the adaxial surface with a 5-μl droplet of spore suspension (5×10^5 conidia ml^{-1}). Plants were kept in cylindrical chambers made of clear polyester to prevent desiccation. After 48 h, infected leaves were collected and scanned with a HP Scanjett 4500c (Hewlett-Packard). Lesion areas were measured using Adobe Photoshop software (version 7.0; Adobe Systems).

MeJA Treatments

The effect of UV-B radiation on plant response to JA was assessed by spraying 4-week-old soil-grown rosettes with MeJA (Sigma-Aldrich) solutions; plants not assigned to the JA treatment were sprayed with distilled water, which was supplemented with ethanol in the same proportion (0.04‰) as that used to dissolve MeJA in the solutions used for the JA treatment. Rosettes were harvested 3 h after the elicitation treatment, and immediately frozen in liquid nitrogen for analysis of gene expression.

Leaf Phenolics

Chlorophyll fluorescence imaging was used to assess the accumulation of UV-absorbing compounds in the leaf epidermis, essentially as described previously (Mazza et al., 2000). Leaf phenolics were determined following established protocols (Demkura et al., 2010), with minor modifications. Briefly, freeze-dried tissue without the midvein (between 10 and 15 mg) was ground in a mortar and transferred to an Eppendorf with 1.5 ml of a methanol:0.25% acetic acid mixture (2:3, v/v). Samples were vortexed for 45 s and centrifuged at 12 000 rpm for 20 min. The supernatant was filtered through a 45-μm syringe filter and kept at −20°C until use. Phenolics were separated by HPLC (Knauer Euroline) on a Restek Pinnacle II C18 (5.0 μm, 4.6 × 150 mm) column with solvents A (0.25% aqueous H_3PO_4) and B (acetonitrile), eluted with a gradient of 5% B at 0 min, 50% B at 22 min, 5% B at 25 min, with an equilibration time of 10 min and a flow rate of 1 ml min^{-1}. The injection volume was 20 μl, and elution was monitored with a diode array detector at 230, 305, and 320 nm. Sinapoyl malate, kaempferol, and quercetin glycosides were identified comparing their UV spectrum and their relative retention times with previously published data (Besseau et al., 2007).

Leaf Glucosinolates

GS were extracted and quantified using previously described protocols (Brown et al., 2003), with minor modifications. Freeze-dried tissue without the midvein (between 10 and 15 mg) was ground in a mortar and transferred to an Eppendorf tube. Each tube was filled with 1.2 ml of 70% methanol containing 0.125 μmol of sinigrin (2-propenyl-glucosinolate, Sigma-Aldrich) as internal standard. Samples were incubated for 1 min at 70°C in a water bath and centrifuged at 12 000 rpm for 2 min; two more aliquots of 70% methanol were added to repeat the extraction procedure and supernatants were combined. After extraction, supernatants were transferred into columns filled with 600 μl (75 mg) of DEAE-Sephadex A-25 (previously equilibrated with 800 μl of MilliQ water); loaded columns were washed with 600 μl 70% methanol and 600 μl of MilliQ water. To de-sulfate GSs retained in the column, 25 μl of arylsulphatase solution (Sigma-Aldrich; H-1 from *Helix pomatia*, prepared as described (Graser et al., 2001)) was re-suspended in 600 μl of 0.02 M sodium acetate buffer pH 4.0 were added, and capped columns were incubated overnight at room temperature. After incubation, desulphoglucosinolates were eluted with 600 μl of MilliQ water.

Desulphoglucosinolates were analyzed by HPLC (Knauer Euroline) on a Restek Pinnacle II C18 (5.0 μm, 4.6 × 150 mm) column with solvents A (water) and B (20% acetonitrile), eluted with a gradient of 1% B at 0 min, 10% B at 10 min, 75% B at 22–24 min, with an equilibration time of 10 min and a flow rate of 1 ml min^{-1}. The injection volume was 20 μl, and elution was monitored with a diode array detector at 229 nm. The major GS in the Col-0 background 4-methylsulfinylbutyl (4MSOB) and indol-3-ylmethyl (I3M) were identified on the basis of their relative retention times and UV spectra. To calculate molar concentrations of individual GS, relative response factors (Brown et al., 2003) were used to correct for absorbance difference between the internal standard and other compounds. Solvents used for determination of leaf phenolics and GS were purchased from Sintorgan.

Gene Expression

Total RNA was extracted from 100 mg of frozen tissue using the LiCl-phenol/chloroform method (Izaguirre et al., 2003). Purified fractions of total RNA were subjected to RQ1 (RNase-free) DNase treatment (Promega) to avoid contamination with genomic DNA. For cDNA synthesis, fractions of 1 μg of RNA were reverse transcribed using oligo(dT) as primer and M-MLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. The obtained cDNA samples were diluted 1:10 before use. Quantitative real-time PCR (qPCR) was performed in a 7500 Real Time PCR System (Applied Biosystems) following the manufacturer's standard method for absolute quantification using FastStart Universal SYBR Green Master Mix (Roche Applied Science) and primers at a final concentration of 500 nM. *A. thaliana UBIQUITIN* (*UBC*) gene was used to normalize for differences in concentrations of cDNA samples. Primer sequences were the following: 5′-CTGCGACTCAGGGAATCTTCTA-3′ and 5′-TTGTGCCATTGAATTGAACCC-3′ (*UBC*); 5′-TTGCTGCTTTCGACGCA-3′ and 5′-TGTCCCACTTGGCTTCTCG-3′ (*PDF1.2*); 5′-TCACGCCATATCTACCAGC-3′ and 5′-TGGACGTCATGACTGGAC-3′ (*SUR2*); 5′-TCCCCAAGTGTTGTCCGAATCTCGT-3′ and 5′-GGATTGGTGGAGTCGCTGGCA-3′ (*PAD3*); 5′-CCGAAAACCCGAATCTGGAT-3′ and 5′-GGGTCTGAGAATGAACCGGAC-3′ (*MYC2*); 5′-GGGCGTACTGGTCGTGGTTA-3′ and 5′-CCGGGAGTCCTGGAGTTGAT-3′ (*VSP1*).

Statistical Analysis

Statistical analyses were carried out using INFOSTAT software (professional version 1.1). Bioassays were analyzed using a two-way ANOVA with UV-B and genotype as factors. When the genotypes used in the experiments differed in their genetic background (i.e. Col-0 or L*er*-0), each mutant or set of mutants was analyzed separately against their respective wild-type, as indicated in the figures. Data on soluble phenolic compounds and GS accumulation were analyzed using a two-way ANOVA with UV-B, and MeJA as factors; each genotype was analyzed separately. Quercetin glycosides, which were only detected in the Col-0 and L*er*-0 plants exposed to UV-B, were not subjected to statistical analysis. Gene expression data were analyzed using a two-way ANOVA with UV-B and MeJA dose as factors. When interaction terms were significant, differences between means were analyzed using Tukey comparisons. Appropriate transformations of the primary data were used when needed to meet the assumptions of the analysis.

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant Online*.

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