Convergent Responses to Stress. Solar Ultraviolet-B Radiation and *Manduca sexta* Herbivory Elicit Overlapping Transcriptional Responses in Field-Grown Plants of *Nicotiana longiflora*[1[w]]

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The effects of solar ultraviolet (UV)-B (280–315 nm) on plants have been studied intensively over the last 2 decades in connection with research on the biological impacts of stratospheric ozone depletion. However, the molecular mechanisms that mediate plant responses to solar (ambient) UV-B and their interactions with response mechanisms activated by other stressors remain for the most part unclear. Using a microarray enriched in wound- and insect-responsive sequences, we examined expression responses of 241 genes to ambient UV-B in field-grown plants of *Nicotiana longiflora* Cav. Approximately 20% of the sequences represented on the array showed differential expression in response to solar UV-B. The expression responses to UV-B had parallels with those elicited by simulated *Manduca sexta* herbivory. The most obvious similarities were: (a) down-regulation of several photosynthesis-related genes, and (b) up-regulation of genes involved in fatty acid metabolism and oxylipin biosynthesis such as *HPL* (hydroperoxide lyase), *α-DIOX* (alpha-dioxygenase), *LOX* (13-lipoxygenase), and *AOS* (allene oxide synthase). Genes encoding a WRKY transcription factor, a ferredoxin-dependent glutamate-synthase, and several other insect-responsive genes of unknown function were also similarly regulated by UV-B and insect herbivory treatments. Our results suggest that UV-B and caterpillar herbivory activate common regulatory elements and provide a platform for understanding the mechanisms of UV-B impacts on insect herbivory that have been documented in recent field studies.

UV-B induces multiple responses in terrestrial plants. These responses have been studied using a variety of experimental approaches, from controlled-environment experiments to large-scale field trials, and covering a broad spectrum of scales, from molecular to ecosystem level processes. Controlled-environment experiments have contributed most of the information presently available on responses at the molecular level. However, this type of experiment, particularly those that are based on the use of heavily unbalanced light sources (i.e. with unnaturally high ratios of UV-B to photosynthetically active radiation [PAR] or UV-B to UV-A [315–400 nm]), are frequently criticized on the grounds that they lack functional and ecological realism (Fiscus and Booker, 1995; Caldwell and Flint, 1997). It has been shown clearly that treatments with high UV-B to PAR ratios, which are the norm in controlled-environment exposures, tend to result in greatly exaggerated effects of UV-B on photosynthesis and growth inhibition. Because these effects have been hard to duplicate under field conditions, it is unclear to what extent the molecular and mechanistic data that have been produced under spectrally unbalanced conditions (which commonly involve PAR levels lower than 1:10 of full sunlight) contribute to our understanding of the normal physiological responses of plants to ambient UV-B stress. In fact, it has been clearly demonstrated that the level of PAR strongly modulates the effect of UV-B on the transcription of various photosynthetic genes: the higher the PAR, the smaller the UV-B-induced alteration in message abundance, which in several cases could be completely eliminated (Jordan et al., 1992; Strid et al., 1996).

Under field conditions, the inhibitory effect of ambient UV-B on the growth of herbaceous terrestrial plants seldom exceeds 20% (Ballaré et al., 1996, 2001; Krizek et al., 1998; Rousseaux et al., 2001). A much larger impact of solar UV-B has been documented for plant-herbivore interactions. Field experiments in a

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broad spectrum of ecosystems show that the intensity of insect herbivory frequently increases when the UV-B component of solar radiation is experimentally attenuated using filters (Bothwell et al., 1994; Ballaré et al., 1996; Rousseaux et al., 1998; Mazza et al., 1999b; Zavala et al., 2001). In some cases, at least part of this effect of solar UV-B could be attributed to direct behavioral responses of insects to UV-B photons (Mazza et al., 2002). However, insect growth and choice bioassays have demonstrated that UV-B effects on herbivory are in many cases indirect; i.e. mediated by UV-B-induced changes in plant tissues, which in turn affect insect feeding behavior and growth performance (Ballaré et al., 1996; Rousseaux et al., 1998; Mazza et al., 1999b; Zavala et al., 2001). The nature of these changes is unknown. Functional bioassays have detected significant effects of UV-B on plant tissue quality in the absence of any effect of UV-B on overall plant growth, total leaf nitrogen (N), tissue toughness, or lignin content (Ballaré et al., 1999; Zavala et al., 2001).

Plants often activate a battery of defense mechanisms in response to herbivore attack, which improve their chances of surviving further attacks by the same species or other species of herbivores (Karban and Baldwin, 1997). The molecules used in plant defense vary greatly across plant taxa but frequently include secondary compounds such as phenylpropanoids, alkaloids, and terpenes, and defense-related proteins such as proteinase inhibitors (PIs; for review, see Gatehouse, 2002). Oxylipins, including jasmonic acid (JA), produced through the octadecanoid pathway (Weber, 2002), are known to play a central role as signaling molecules mediating plant responses to insect herbivores, including production of PI and defense-related secondary metabolites (Ryan, 2000; Walling, 2000; Gatehouse, 2002). Interestingly, controlled-environment studies with Arabidopsis have shown that UV-B, delivered against a background of low PAR (approximately 1:10 of full sunlight), can trigger JA accumulation (A.-H.-Mackerness et al., 1999). In tomato (*Lycopersicon esculentum*), UV-B treatments did not result in increased JA levels (Stratmann et al., 2000b), but accumulation of PI in response to treatment with germicidal UV-C has been reported (Conconi et al., 1996). Stratmann et al. (2000b) concluded that UV-B/A treatments do not induce PI by themselves in tomato but can enhance the PI response induced by mechanical damage.

We wanted to determine whether the effects of solar UV-B on insect resistance in field-grown plants are mediated by the elicitation of defense responses similar to those induced by insect attack. As an initial step in this direction, we attempted to identify genes involved in the responses of field-grown plants of *Nicotiana longiflora* to solar UV-B using a custom-made cDNA microarray that contained approximately 250 *Nicotiana attenuata* genes known to be regulated in response to attack by chewing larvae of *Manduca sexta* (Halitschke et al., 2003). We first studied transcriptional differences between plants exposed to ambient UV-B and plants grown under filters that selectively attenuated the UV-B component of sunlight. We then compared the transcriptional responses to UV-B with those elicited by a simulated *M. sexta* herbivory treatment. Our results demonstrate a significant convergence in the transcriptional responses elicited by solar UV-B and insect attack and suggest that this convergence plays a functional role mediating the negative effects of ambient UV-B on herbivory intensity.

RESULTS

Experimental Design and Application of a cDNA Microarray to Study UV-B Impacts on Defense-Related Genes

The method used to array PCR fragments of insect-responsive *N. attenuata* genes onto epoxy-coated slides has been described (Halitschke et al., 2003). The arrays contained *N. attenuata* genes found to be differentially expressed in response to: (a) *M. sexta* herbivory (*M. sexta*-attacked plants versus intact controls, sampled 24 h after initiation of feeding) by differential display reverse transcription-PCR and subtractive hybridization techniques (Hermsmeier et al., 2001; Hui et al., 2003), and (b) *M. sexta* oral secretions and regurgitates (wounds treated with regurgitates versus wounded control, sampled 24 h after wounding) by cDNA-amplified fragment length polymorphism analysis (Halitschke et al., 2003). In addition, the array contained probes for well-characterized *N. attenuata* control genes implicated in plant defense: *PI* (proteinase [trypsin] inhibitor), *HPL* (hydroperoxide lyase), *PMT1* (putrescine methyl transferase), *AOS* (allene oxide synthase), *XET* (xyloglucan endo-transglycosylase B1), and *WRKY-2* (WRKY transcription factor; Halitschke et al., 2003). Each gene was represented on the microarray by two independent PCR fragments that, in turn, were spotted in quadruplicate (Halitschke et al., 2003). Independent tests of this microarray comparing wounded versus wounded +regurgitate-treated leaves (Halitschke et al., 2003) and *M. sexta*-attacked versus intact plants (C. Voelckel and I.T. Baldwin, unpublished data) yielded gene expression patterns that were highly consistent with those detected using other differential expression techniques, thereby establishing the reliability of the system.

For our field experiments, we used 5-week-old plants of *N. longiflora*, a wild species native to the central region of Argentina that is closely related to *N. attenuata*. The plants were grown in large, replicated plots for 3 weeks in a field site in Córdoba (Central Argentina) under near-ambient or attenuated solar UV-B radiation (see "Materials and Methods" for a description of the experimental layout and the growth conditions). At the time of harvest, plants

were still at the rosette stage, and there were no obvious differences between UV-B treatments in overall plant growth or morphology, which is consistent with the results obtained in the field for other Solanaceous species exposed for short periods to similar UV-B treatments (Ballaré et al., 1996). One-half of the plants were exposed to a simulated herbivory treatment, in which leaves were gently wounded and the wounds treated with *M. sexta* regurgitate. This treatment (see "Materials and Methods" for details) is known to elicit transcriptional responses that are similar to those induced by *M. sexta* folivory (Hermsmeier et al., 2001; Schittko et al., 2001). Leaf samples were taken 24 h after the simulated herbivory treatment.

Solar UV-B Down-Regulates Several Photosynthetic Genes and Up-Regulates Genes Implicated in Biotic Defense Responses

Of the 241 *M. sexta*-responsive, regurgitate-responsive, and control genes included in the array, 48 revealed differential expression in response to solar UV-B in the field-grown plants of *N. longiflora* (Fig. 1; Table I; the complete data set is available as Supplemental Table I at http://www.plantphysiol.org). The genes included in the array were clustered into functional groups based on known or inferred function of the gene product. For many of the sequences, no functional information is available other than their transcript abundance is regulated in response to *M. sexta* herbivory or regurgitate treatment; therefore, they are likely to play some role in metabolic reconfiguration after insect attack. Those genes were included in two separate categories: *M. sexta* regulated and regurgitate responsive (see "Materials and Methods" for details).

The most consistent expression responses to solar UV-B were found in the clusters of photosynthesis- and fatty acid metabolism-related genes (Fig. 1; Table I). Most of the probes included in the array for genes that encode components of the photosynthetic apparatus indicated lower transcript abundance in plants chronically exposed to solar UV-B than in the control plants. Among these were genes for Calvin cycle enzymes, such as the small subunit of Rubisco, and genes encoding for PSII polypeptides (e.g. genes with similarity to the tomato *lhbC1* gene for LHCII type III, spinach 6.1-kD polypeptide, and NtPII10). Interestingly, one photosynthesis-related gene, encoding a PSII-O_2-evolving complex 23-kD polypeptide, was up-regulated in plants exposed to ambient UV-B (Table I).

In contrast to the response of photosynthesis-related transcripts, most of the probes for genes that encode enzymes involved in fatty acid metabolism and oxylipin synthesis showed increased transcript abundance in plants exposed to solar UV-B. Among the up-regulated genes were *α-DIOX*, *HPL*, *LOX*, and *AOS* (Fig. 1; Table I).

A similar UV-B-induced increase in expression level was found for genes that encode enzymes directly or indirectly involved in N metabolism (such as the ferredoxin-dependent Glu-synthase [FDGS]

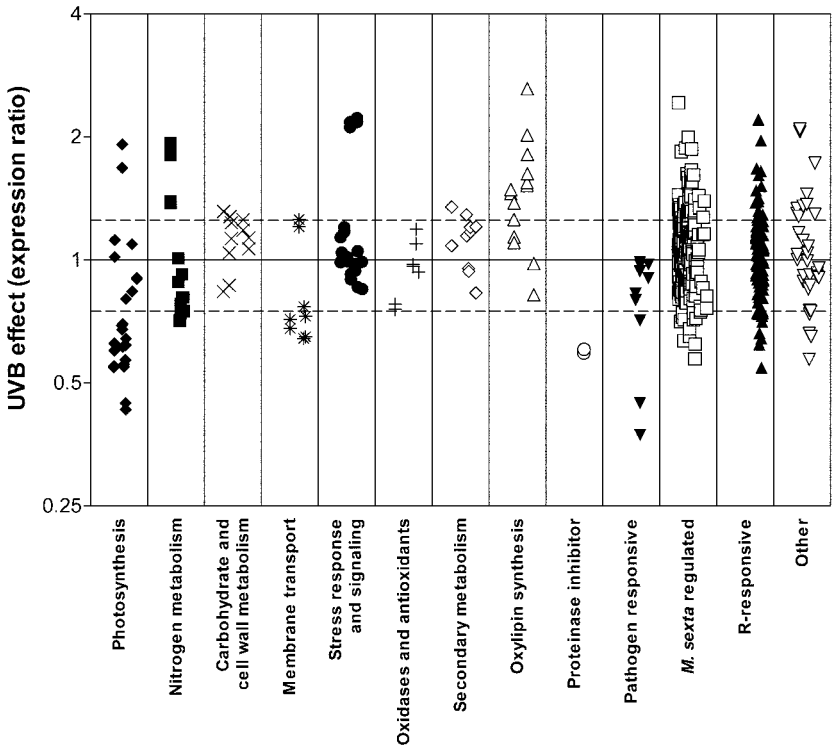

Figure 1. Effect of chronic exposure to ambient UV-B on the abundance of transcripts of insect-responsive genes in field-grown *N. longiflora* plants. Plants were grown in the field for 3 weeks in replicated plots under filters that were fully transparent to the solar spectrum (near-ambient UV-B treatment) or selectively attenuated the UV-B component of sunlight (attenuated UV-B treatment; see "Materials and Methods" for details). The expression ratio (ER) indicates transcript abundance in the near-ambient UV-B treatment relative the attenuated UV-B treatment. The arrayed genes were clustered into 13 functional groups according to the predicted function of the gene product. For a list of UV-B-responsive genes, see Table I. The complete list of the responses of all arrayed genes is available as Supplemental Table I (http://www.plantphysiol.org).

Table I. *Summary of the significant effects of solar UV-B exposure on gene expression in N. longiflora (see "Materials and Methods" for explanation of the criteria used to assess the significance of the expression changes)*

For brevity, the average of the expression ratios obtained with the two probes used for each gene is shown in the table. The complete list is available as Supplemental Table I (see http://www.plantphysiol.org).

Identification No.	Description (Name or Sequence Similarity)	Clone	Accession No.	Mean
Photosynthesis-related genes				
1406/7	Tomato lhbC1 gene for LHCII type III	61_1	AW191826	0.55
1414/5	*Nicotiana sylvestris* mRNA for the small subunit of Rubisco	64_7	AW191829	0.61
1548/9	*Hevea brasiliensis* latex plastidic aldolase-like protein mRNA	RE234	CA591767	0.68
1552/3	Tobacco (*Nicotiana tabacum*) PSII oxygen-evolving complex 23-kD polypeptide	RE283	CA591769	1.80
1562/3	Spinach (*Spinacia oleracea*) mRNA for 6.1-kD polypeptide of PSII	RN032	CA591774	0.65
1584/5	Tobacco Rubisco small subunit pseudogene	RF071	CA591784	0.55
1608/9	Tobacco PSII (NtPII10/11)	DH108	CA591793	0.51
1612/3	Tobacco Rubisco small subunit pseudogene	DH120	CA591795	0.52
1615	Tobacco GapC mRNA-encoding cytosolic glyceraldehyde-3-phosphate dehydrogenase	DH123	CA591796	0.64
Nitrogen metabolism				
2400/1	Spinach ferredoxin-dependent glutamate synthase precursor	55_2	AW191824	1.87
2403	*Nicotiana plumbaginifolia* molybdopterin synthase sulfurylase gene	55_7	AW191825	1.38
2560/1	Tomato mRNA for beta-alanine synthase	RN021	CA591773	0.71
Carbohydrate and cell wall metabolism				
3446/7	Tomato xyloglucan endo-transglycosylase B1 mRNA (XET)	RB271	CA591719	1.28
Membrane transport				
4774/5	Pea (*Pisum sativum*) mRNA for pore protein	DH47	CA591865	0.60
Stress response and signaling				
5556/7	Tobacco mRNA for transcription factor NtWRKY2	RE322	CA591771	1.90
5678/9	*N. attenuata* WRKY (NtWRKY2)	WRKY	CA591771	2.20
Oxylipin synthesis				
8382	*N. attenuata* (DIOX_NICAT) pathogen-inducible alpha-dioxygenase	DH54	CA591872	1.45
8668/9	*N. attenuata* HPL	HPL	AJ414400	1.53
8672/3	*N. attenuata* LOX	LOX	AY184822	2.32
8674/5	*N. attenuata* AOS	AOS	AJ295274	1.72
PIs				
9666/7	*N. attenuata* PI	PI	AY184823	0.60
Pathogen-responsive genes				
10652/3	Tobacco gene for basic form of pathogenesis-related protein	DH099	CA591812	0.41
M. sexta-responsive genes with unknown function				
11330/1	Pepper (*Capsicum annuum* Yolo Wonder) Sn-1 gene	6_1	AW191805	1.39
11340/1	Unknown	11_1/5	AW191807	1.33
11398/9	Unknown	54_4		0.64
11426/7	Unknown	75_7		1.32
11450/1	Unknown	RB332		1.63
11496/7	Unknown	RC131		1.57
11500/1	Unknown	RC173		1.77
11518/9	Unknown	RD105		1.33
11524/5	Unknown	RD151		0.66
11534/5	Unknown	RE065		1.32
11544/5	Unknown	RE214		1.47
11590/1	Unknown	RF101		0.73
11616/7	Unknown	DH135		1.36
11620/1	Arabidopsis clone RAFL04–10-H14 (R09662) putative 60S ribosomal protein (At3g49910) mRNA	DH138		0.60

(*Table continues on following page.*)

Table I. (*Continued from previous page.*)

Identification No.	Description (Name or Sequence Similarity)	Clone	Accession No.	Mean
R-responsive genes with unknown function				
12730/1	Tobacco mRNA C-7	DH25	CA591843	1.35
12736/7	Unknown	DH28		1.91
12750/1	Unknown	DH35		0.64
12776/7	Tobacco chloroplast genome DNA	DH48	CA591866	1.81
12786/7	Unknown	DH53		0.59
12798/9	Unknown	DH59		0.72
Other				
13412/3	*Manilkava zapota* 18S ribosomal RNA gene	64_4	AW191828	1.32
13464/5	*Novosphingobium aromaticivorans* Saro_115	RB493	CA591728	2.07
13582/3	*Smilax bona-nox* internal transcribed spacer 2 and 26S large subunit ribosomal RNA gene	RF064	CA591783	1.41
13626/7	Tobacco RNA-binding Gly-rich protein (RGP-1a) mRNA	DH162	CA591801	0.62
13656/7	Tobacco GTP-binding protein (Ran-A1) mRNA	DH124	CA591814	0.70
13728/9	Tobacco chloroplast genome DNA	DH24	CA591842	1.51

and a molybdopterin synthase sulfurylase), and a gene encoding a WRKY-type transcription factor (*WRKY-2*). In contrast, exposure to solar UV-B reduced the abundance of *PI* transcripts and transcripts with similarity to the basic-type pathogenesis-related protein PR1 of tobacco. Solar UV-B also affected the abundance of several transcripts of unknown function that have been previously shown to be regulated by natural or simulated *M. sexta* herbivory and/or wounding + regurgitate treatment (Fig. 1; Table I).

Reproducibility of the UV-B Effect

We tested the generality of the UV-B impact by comparing the transcriptional responses shown in Figure 1 with those elicited in *N. attenuata* by realistic levels of UV-B provided against high PAR in a completely independent experiment under greenhouse conditions (see "Materials and Methods").

The two experiments showed a similar pattern of UV-B response (Fig. 2). The most obvious parallels were: (a) very similar effects on the abundance of photosynthesis-related transcripts, and (b) similar up-regulation of genes involved in fatty acid metabolism and oxylipin synthesis and consistent effects on *FDGS* (up-regulation) and *PR1* (down-regulation). The only remarkable difference between the expression patterns detected in these experiments was the positive effect of artificial UV-B on *PI* expression in *N. attenuata*, which contrasts with the reduction in *PI* transcript abundance in *N. longiflora* plants exposed to ambient UV-B (Fig. 2; see Supplemental Table I at http://www.plantphysiol.org). Analysis of the data presented in Figure 2 shows a highly significant correlation between the data sets ($P < 0.0001$ for a linear model; $R^2 = 0.29$; or $R^2 = 0.50$ if the *PI* data points are removed). This similar regulation in two *Nicotiana* spp., tested in completely independent experiments, provides strong evidence that the results presented in Figures 1 and 2 reflect a general pattern of transcriptional response to ambient or near-ambient UV-B doses.

UV-B and Simulated *M. sexta* Herbivory Have Similar Effects on Various Functional Groups of Genes

To compare the effects of solar UV-B and insect herbivory, we applied a simulated-herbivory treatment to field-grown *N. longiflora* plants. For the treatment, we wounded the leaves with a fabric pattern wheel and applied to the wounds oral secretions collected from fourth instar *M. sexta* caterpillars. Twenty-four hours after elicitation, the leaves were harvested, and RNA was extracted for analysis.

The observed expression responses for well-characterized genes (small subunit of Rubisco gene and pseudogene, *AOS*, *HPL*, *α-DIOX*, *PI*, and *WRKY-2*; see Supplemental Table I at http://www.plantphysiol.org), which had been previously evaluated by extensive northern-blot analysis and poly-Lys microarrays (Hermsmeier et al., 2001; Ziegler et al., 2001; Hui et al., 2003; Voelckel and Baldwin 2003), were consistent with the previous results. The expression changes that we observed were also consistent with the responses detected with the poly-epoxide microarray used in this study in *N. attenuata* plants exposed to 24-h *M. sexta* feeding (C. Voelckel and I.T. Baldwin, unpublished data). These similarities indicate that the expression responses to simulated herbivory measured in our experiments can be regarded as general and reproducible for *Nicotiana* spp.

Figure 3 shows direct comparisons of gene expression responses to solar UV-B and simulated herbivory in *N. longiflora*. The treatments had similar effects on various functional groups of genes (Fig. 3A). In the photosynthesis-related group, the same genes encoding for PSII polypeptides that were down-regulated by UV-B were also down-regulated by simulated herbivory. Probes for Rubisco also

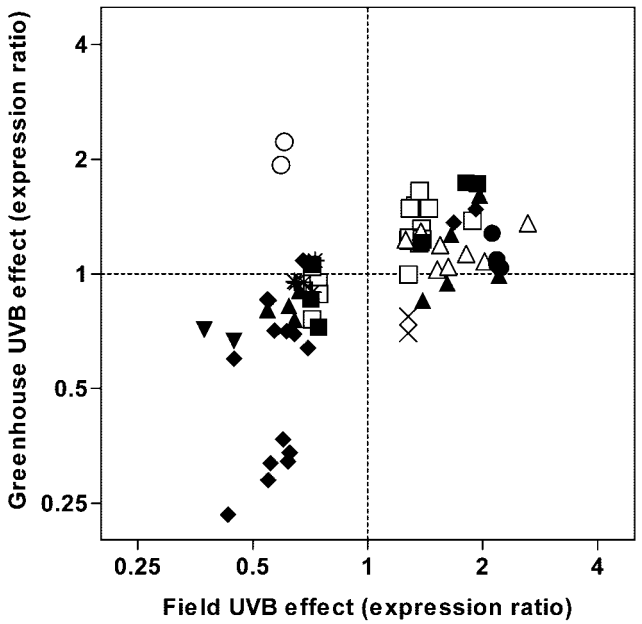

Figure 2. Comparison between the effects of solar UV-B in *N. longiflora* and simulated ambient UV-B in *N. attenuata* on the expression of insect-responsive genes. The data for *N. longiflora* were obtained from Figure 1. The data for *N. attenuata* were obtained in a completely independent greenhouse experiment under simulated-ambient UV-B (see "Materials and Methods" for details). The graph shows the responses to UV-B in *N. attenuata* of all those genes that were significantly affected by UV-B in *N. longiflora*: photosynthesis-related genes (♦), N metabolism (■), carbohydrate and cell wall metabolism (×), membrane transport (*), stress response and signaling (●), oxylipin synthesis (△), PI (○), pathogen-responsive (▼), *M. sexta*-responsive genes of unknown function (□), and regurgitate-responsive genes of unknown function (▲). The complete list of the responses of all arrayed genes is available as Supplemental Table I (http://www.plantphysiol.org).

showed reduced transcript abundance in both treatments. Both treatments also resulted in increased abundance of transcripts of genes involved in fatty acid metabolism (*α-DIOX*, *HPL*, and *AOS*), although the simulated herbivory treatment had a stronger effect on the first two genes than UV-B exposure.

Simulated herbivory, like solar UV-B, increased the abundance of *WRKY-2* transcripts. Also, as expected from previous herbivore-challenged experiments (Hermsmeier et al., 2001) and consistent with the effect of UV-B, our simulated herbivory treatment increased the abundance of transcripts for FDGS and molybdopterin synthase sulfurylase. The effects of UV-B and simulated herbivory were also similar regarding the abundance of *PR1* transcripts, which was reduced by both treatments (Fig. 3A; see Supplemental Table I at http://www.plantphysiol.org). Analysis of the data shown in Figure 3A shows a significant correlation between the responses to solar UV-B and simulated herbivory ($P < 0.001$ for a linear model; $R^2 = 0.22$ when the *PI* data points are removed). There was also correspondence between the effects of solar UV-B and simulated herbivory on the class of genes

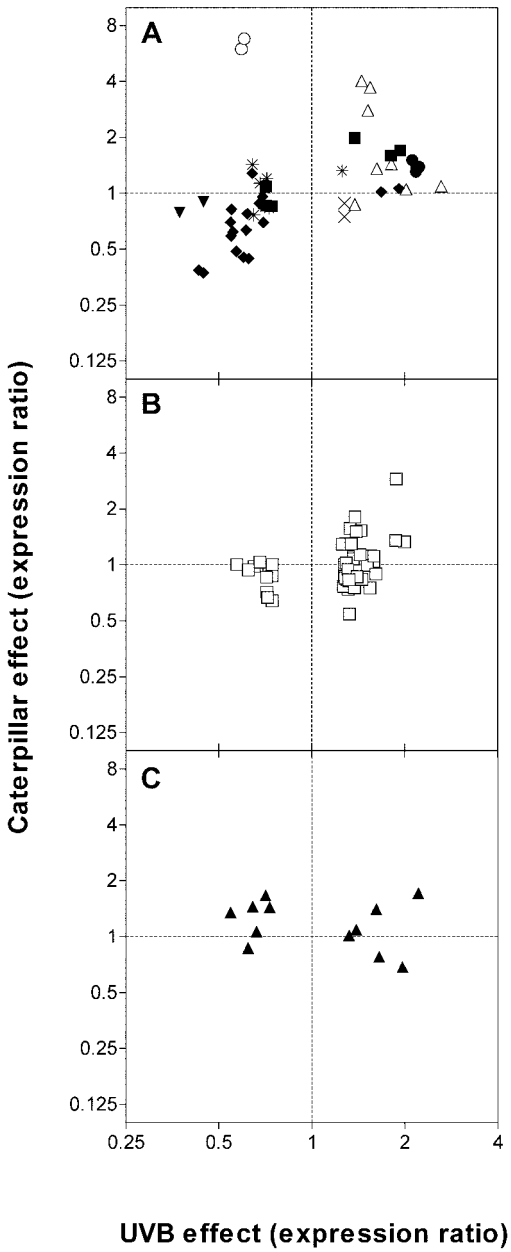

Figure 3. Comparison between the effects of solar UV-B and a simulated caterpillar treatment in *N. longiflora* (for details, see "Materials and Methods"). The graph shows the responses to the simulated caterpillar treatment (wounding + *M. sexta* regurgitate relative to the intact control, both under attenuated UV-B) of genes that were significantly affected by UV-B in field-grown plants. A, Responses in functional categories 1 to 10: photosynthesis-related genes (♦), N metabolism (■), carbohydrate and cell wall metabolism (*XET*; ×), membrane transport (*), stress response and signaling (*WRKY-2*; ●), oxylipin synthesis (△), PI (○), and pathogen responsive (*PR1*; ▼). B, Functional category 11, i.e. *M. sexta*-regulated genes of unknown function (□). C, Functional category 12, i.e. regurgitate-responsive genes of unknown function (▲). The complete list of the responses of all arrayed genes is available as Supplemental Table I (http://www.plantphysiol.org).

defined as unknown function/*M. sexta* regulated (Fig. 3B; $P = 0.004$ for the linear model).

Few genes were affected in opposite directions by solar UV-B and simulated herbivory. This is indicated by the paucity of data points in the northwest and southeast quadrants of Figure 3, A and B. A noteworthy case of opposite response was *PI*, which was strongly up-regulated by simulated herbivory (as expected) and down-regulated by solar UV-B in *N. longiflora*. More cases of opposite responses were found among the genes known to be specifically responsive to components of *M. sexta* oral secretions and regurgitates (regurgitate-responsive genes; Fig. 3C).

Functional Effects of UV-B on Plant Defenses

Insect elicitation commonly results in increased plant resistance to subsequent herbivore attacks. This increased resistance can be measured using standardized insect growth bioassays, and in the particular case of *M. sexta* and *N. attenuata*, induction of plant defenses by caterpillar attack has been detected with bioassays within 24 h after the elicitation with JA (Pohlon and Baldwin, 2001) and feeding insects (A. Kessler and I.T. Baldwin, unpublished data). Because we had detected similarities in the transcriptional responses elicited by UV-B and simulated *M. sexta* herbivory (Fig. 3), we wondered whether the transcriptional reorganization induced by UV-B had detectable consequences at the functional level. We exposed *N. longiflora* and *N. attenuata* plants to simulated ambient UV-B in a greenhouse and measured the impacts of UV-B on: (a) the kinetics of PI induction by a wound + regurgitate treatment, and (b) the performance of first instar *M. sexta* caterpillars using a no-choice feeding bioassay.

Simulated herbivory induced higher PI activity in *N. attenuata*, as expected (Pohlon and Baldwin, 2001; van Dam et al., 2001). Interestingly, this induction was significantly enhanced by plant exposure to UV-B (Fig. 4). This is consistent with the fact that both UV-B and simulated herbivory up-regulated the *PI* gene in this species (Fig. 1). In *N. longiflora*, PI activity was approximately 10 times lower than in *N. attenuata*, and although detailed analysis revealed that they were slightly increased by the wound + regurgitate treatment (data not shown), we were not able to detect a significant effect of UV-B-promoting PI activity levels (Fig. 4). In fact, a (nonsignificant) reduction in PI levels was apparent in UV-B-exposed plants. Again, this was consistent with the microarray data, which showed down-regulation of *PI* in response to ambient UV-B in *N. longiflora* (Fig. 1).

In the bioassay with both *Nicotiana* spp., insects fed on plants that were exposed to simulated-ambient UV-B before and during the experiment grew less rapidly than their counterparts fed on control plants (Fig. 5). Because the insects themselves were not exposed to UV-B during the feeding trials, it is clear that the growth differences reflected changes in plant tissue quality induced by UV-B.

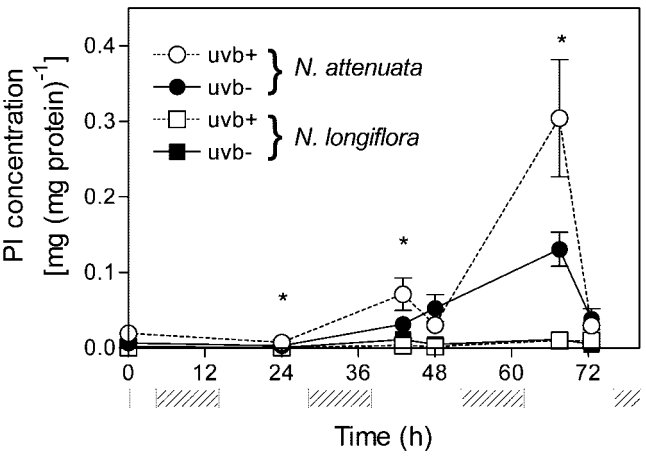

Figure 4. Effects of simulated ambient UV-B (greenhouse experiment) on the kinetics of PI activity changes in response to a simulated caterpillar treatment (wounding + *M. sexta* regurgitate) applied at time = 0 to *N. longiflora* and *N. attenuata* plants. PI activity levels in the non-wounded controls were always below 0.008 mg per milligram of protein and are not shown for clarity. A significant UV-B effect on PI levels (in *N. attenuata*) is denoted by asterisks ($P < 0.05$); bars indicate ±1 SE ($n = 3$ plants per sampling). *N. longiflora* produced very low PI levels, and there was a trend for reduced PI activities in UV-B-exposed plants ($P = 0.3$ at time = 48 h). The white and dashed segments below the *x* axis represent day and night, respectively.

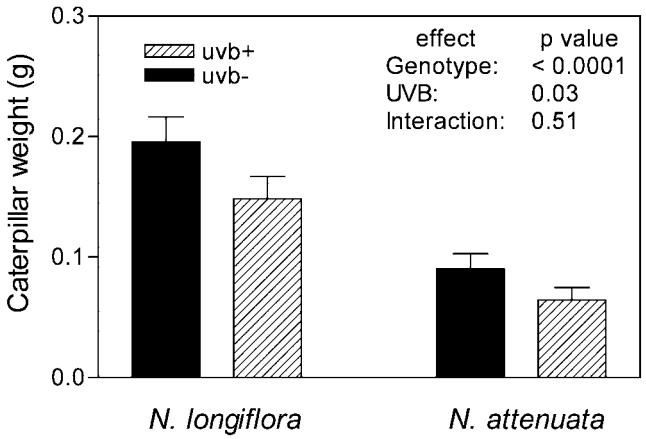

Figure 5. Effects of simulated ambient UV-B (greenhouse experiment) on the growth (weight gain) of *M. sexta* caterpillars. The experiment had a duration of 1 week. Bars indicate ±1 SE ($n = 10$ [*N. attenuata*] and 8 [*N. longiflora*] replicate plants, each with two caterpillars).

DISCUSSION

Our data provide a first glimpse into the transcriptional changes activated by solar UV-B in field-grown plants. The comparison in Figure 3 indicates that ambient UV-B induces a transcriptional reorga-

nization in *N. longiflora* that has parallels with the response induced by caterpillar herbivory. This result suggests that UV-B and chewing insects activate common regulatory elements and that this signaling convergence ultimately leads to increased plant resistance to herbivore attack in plants exposed to solar UV-B radiation (Fig. 5).

Signaling Interactions between Solar UV-B- and Herbivore-Induced Responses

Where do the UV-B- and caterpillar-induced signaling cascades converge? A major obstacle in the quest for answers to this question is our very limited understanding of the mechanisms of UV-B perception and signaling in plants. Dedicated photoreceptors for red, far-red, blue, and UV-A radiation are well characterized in plants; in contrast, no specific UV-B receptors have yet been identified. Based on evidence from studies in other organisms, some plant responses to UV-B are thought to be initiated by signals derived from UV-B-induced DNA damage (Beggs and Wellmann, 1994). However, spectral considerations (Ballaré et al., 1995; A.-H.-Mackerness et al., 1996; Frohnmeyer et al., 1999), acclimation experiments (Kalbin et al., 2001), and studies with DNA repair mutants (Boccalandro et al., 2001) have suggested that at least some responses to UV-B are triggered by activation of other molecular targets besides DNA. In animal cells, activation of membrane receptors by UV-B is thought to be the initial step in the chain of molecular events that leads to the generation of some cellular responses to UV-B, such as apoptosis induced by exposures to high UV-B doses. Yalamanchili and Stratmann (2002) used suspension-cultured *Lycopersicon peruvianum* cells to test the possibility that UV-B activates membrane receptors for systemin, a small peptide involved in the initial steps of the wound response in tomato. Their experiments showed that pulses of UV-B are effective in eliciting two early components of the wound response: medium alkalization and phosphorylation of a mitogen-activated protein kinase (Stratmann et al., 2000a). Further pharmacological experiments indicated that the UV-B effect on these responses could be abrogated by treatment of the cells with suramin, an inhibitor of the systemin receptor, and Ala-17-systemin, a systemin analog that competitively inhibits the binding of systemin to its receptor. This evidence, coupled with reciprocal desensitization experiments that involved UV-B and systemin, led Yalamanchili and Stratmann (2002) to suggest that the systemin receptor and/or other systemin-responsive signaling elements are the initial phototargets in the response to pulses of UV-B in *L. peruvianum* cells. In principle, this model may be compatible with our gene expression results in *Nicotiana* spp., which revealed a consistent effect of physiological levels of UV-B up-regulating genes that are known to be up-regulated in response to wounding (and systemin in the tomato model), such as those that encode enzymes involved in oxylipin synthesis (Ryan, 2000). Interestingly, the systemin receptor (SR 160) has been shown recently to be identical to the tBRI1 receptor (Montoya et al., 2002), suggesting that there also might be similarities to explore between UV-B and steroid hormone signaling.

Another potential point of overlap between UV-B- and herbivore-induced defense responses may occur at the level of the generation of reactive oxygen species (ROS). Work in tomato seedlings has shown that hydrogen peroxide (H_2O_2) is generated systemically in response to wounding and systemin, and this response is absent in *def-1*, a mutant impaired in the octadecanoid pathway (Orozco-Cárdenas and Ryan, 1999). Feeding experiments using H_2O_2, free radical generators, and an NAPDH-oxidase inhibitor have suggested that H_2O_2 production by an NADPH-oxidase plays a key role in signaling events that lead to activation of defense genes, such as *PI* and *PPO* (polyphenol oxidase; Orozco-Cárdenas et al., 2001). UV-B can generate ROS in plant tissues (Malanga and Puntarulo, 1995; Hideg et al., 2000; Hideg et al., 2002), and it is tempting to speculate that this increased ROS production activates elements of the herbivore-induced response. In fact, convergence in the transcriptional responses elicited by artificial UV-B and oxidant agents (such as ozone), antioxidant inhibitors, and artificial ROS generators has been noted in controlled-environment experiments with other plant species (Willekens et al., 1994; Green and Fluhr, 1995; Rao et al., 1996; Surplus et al., 1998; A.-H.-Mackerness et al., 1999, 2001; Sävenstrand et al., 2002). However, there are caveats with applying this interpretation to the *N. longiflora* case. First, it has not been established that physiological levels of UV-B induce elevated ROS levels in field-grown plants, although increases in activity of H_2O_2-scavenging enzymes (catalase and ascorbate peroxidase) due to solar UV-B exposure have been reported (Mazza et al., 1999a). Second, gene expression studies in the tomato model indicate that only the expression of "late" genes (i.e. *PI* and *PPO*) is H_2O_2 dependent, whereas the wound and systemin induction of the "early" genes (i.e. genes associated with the octadecanoid pathway, such as *AOS* and *LOX*) is H_2O_2 independent (Orozco-Cárdenas et al., 2001). This pattern of control appears to be opposite to the one detected in *N. longiflora*, where solar UV-B up-regulated the "early" signal pathway genes but not the defense product, *PI* (Fig. 1).

As noted earlier, work on the Arabidopsis model also has provided evidence for interactions between UV-B and oxylipin signaling. UV-B, provided against backgrounds of low PAR, has been shown to induce the expression of the defensin gene (*PDF1.2*), a gene known to be up-regulated by wounding and JA (A.-H.-Mackerness et al., 1999, 2001). The effects of UV-B

on *PDF1.2* could be cancelled by pretreatment of the plants with an antioxidant, ascorbic acid. Moreover, increased *PDF1.2* expression could also be elicited by feeding the plants with 3-amino-1,2,4-triazole (an oxidant) in wild-type (Col4) plants, but not in the *jar-1* mutant, which is impaired in jasmonate sensing. Based on this evidence, A.-H.-Mackerness et al. (1999) concluded that the UV-B effect on *PDF1.2* expression was mediated by UV-B-induced ROS production, which, at variance with the current model for tomato (Orozco-Cárdenas et al., 2001), appeared to play a signaling role upstream of JA. It is also noteworthy that UV-B promotes JA accumulation in Arabidopsis (A.-H.-Mackerness et al., 1999), whereas no effects of UV-B on JA levels could be detected in tomato (Stratmann et al., 2000b). A family of calmodulin-binding transcription factors (*AtSR1–6* for Arabidopsis "signal-responsive" genes) that are induced by multiple internal regulators (ethylene and methyl jasmonate) and external factors (heat shock, cold, UV, NaCl, and wounding) has been characterized recently in Arabidopsis (Yang and Poovaiah, 2002). This family might be involved in the interactions between signaling pathways initiated by herbivory and ambient UV-B; however, none of the differential display techniques used to detect transcript regulated by *M. sexta* herbivory in *N. attenuata* revealed sequences with similarity to the *SR* Arabidopsis family.

Regardless of the precise way of interaction, our data suggest that one of the mechanisms whereby solar UV-B makes *Nicotiana* plants less suitable for insect consumers (Fig. 5) may be by supercharging the octadecanoid pathway, increasing the abundance of transcripts of oxylipin biosynthetic genes (Fig. 1). This effect of UV-B appears to result in higher PI accumulation upon herbivore attack in *N. attenuata* but not in *N. longiflora* (Fig. 4). *N. longiflora* produces very low PI levels, which suggests that other defense products, and not PI, are involved in UV-B- (and insect-) induced defenses in this species.

Responses of Other Functional Groups of Genes

Many of the effects of solar UV-B on transcript abundance detected in our experiments involved genes that have not been investigated before; therefore, comparisons with other studies are difficult to establish. Transcriptional responses to UV-B have been studied for several photosynthesis-related genes in controlled-environment experiments. In all cases, previous results with pea (A.-H.-Mackerness et al., 1996, 1998; Brosché et al., 1999, 2002; Sävenstrand et al., 2002) and Arabidopsis (Surplus et al., 1998; A.-H.-Mackerness et al., 1999, 2001) show down-regulation of this functional group, which is broadly consistent with our results for both *Nicotiana* spp. Notice, however, that in our experiments at least one of the arrayed probes for photosynthetic genes indicated up-regulation by exposure to solar UV-B (PSII-O_2-evolving complex 23-kD polypeptide). Because photosynthetic gas exchange is normally not affected by chronic UV-B exposure in field-grown terrestrial plants (Allen et al., 1998), one has to interpret the transcriptional changes that we detected in *Nicotiana* spp. as part of an underlying acclimation response, which makes the photosynthetic apparatus more tolerant to UV-B-induced damage or frees up resources that are required for the activation of UV-B defenses.

There were also some apparent contrasts between the transcriptional responses to UV-B that we detected and those obtained in previous studies. In *N. longiflora*, we found down-regulation of *PI* in plants exposed to solar UV-B (Fig. 1). In tomato, *PI* expression increased in response to treatment with germicidal UV-C (Conconi et al., 1996) but not in response to UV-B (Stratmann et al., 2000b). Although these observations are not readily comparable, because different species and irradiation protocols have been used, it appears that up-regulation of *PI* cannot be regarded as a general response to physiological doses of UV-B. In fact, we obtained contrasting responses to similar UV-B levels in the two *Nicotiana* spp. used in our experiments (Fig. 2). In the same vein, the down-regulation of *PR1* by UV-B in *N. longiflora* and *N. attenuata* (Figs. 1 and 2) was to some extent unexpected, given previous results showing up-regulation of pathogenesis-related genes in tobacco and Arabidopsis plants exposed to: (a) artificial UV-B under low PAR backgrounds (Green and Fluhr, 1995; Thalmair et al., 1996; A.-H.-Mackerness et al., 1998, 2001; Surplus et al., 1998), (b) treatments with germicidal UV-C (Yalpani et al., 1994), or (c) ozone fumigation (Ernst et al., 1992, 1996; Yalpani et al., 1994). UV-B-induced up-regulation of *PR* genes appears to be mediated by ROS (A.-H.-Mackerness et al., 1999, 2001); therefore, we suspect that increased *PR* expression is less likely to occur under field conditions than under experimental protocols that combine plants with a low antioxidant capacity (e.g. plants grown under low PAR) with treatments that generate large quantities of ROS. However, differential regulation of PR genes (e.g. differential induction of acidic and basic PR proteins by stress treatments) has been demonstrated (Brederode et al., 1991), and this may also explain the differences between our results and those reported for other systems.

Ecophysiological Considerations

From an ecophysiological point of view, it is to some extent puzzling that the effects of UV-B and caterpillar herbivory on certain functional categories of genes are so similar (Fig. 3). This is particularly true in the case of photosynthetic genes because the parallels between the challenges imposed by UV-B photons and herbivore attack on the photosynthetic machinery are far from obvious. The similar regula-

tion by UV-B and simulated herbivory on genes for WRKY, FDGS, molybdopterin synthase sulfurylase, PR1, and on various transcripts of unknown function (Fig. 3) also resists facile explanations. One possibility is that this similarity is only apparent and that subtle differences in timing and relative changes in expression levels of key genes are of central importance to define the functional specificity of the transcriptional reorganization triggered by each particular stimulus (for discussion, see Bowler and Fluhr, 2000). However, a look at the end product of the UV-B response in *Nicotiana* spp., which is in fact an increased resistance to herbivore attack (Fig. 5), suggests that the functional specificity may be actually rather limited. It can be argued that the plant has only a limited number of signaling circuits and molecules available, and, because of that, redundancy or overlap in the responses initiated by different signals is, to some extent, unavoidable. On the other hand, it is also possible that the observed transcriptional convergence represents an evolutionary compromise between highly "stimulus-specific" responses and "multipurpose" responses that help the plant to cope with stress factors that, from the "plant's point of view," are more similar than they appear from our reductionist perspective. For example, under natural conditions, exposure to full solar UV-B (e.g. in sunny canopy gaps) and exposure to folivore insects might pose some similar limitations to plant growth, which in turn demand similar re-accommodations of plant function (e.g. toward a more conservative use of resources). Examples of morphological and biochemical responses that might increase the resistance to high UV-B, and at the same time reduce the damage imposed by folivores, may include reduced investment in leaf area, production of thicker leaves, increased antioxidant levels, and increased levels of phenolic compounds. Last, it is possible that both UV-B and herbivore attack tend to co-occur in the habitats in which these species evolved and, hence, have selected for shared signaling pathways.

MATERIALS AND METHODS

Seed Sources and Germination

Seeds of the native plant *Nicotiana longiflora* Cav. were collected from wild specimens growing near the La Falda lake, in the Punilla Valley (Córdoba, central Argentina) and sown directly on agar plates for germination. *Nicotiana attenuata* Torr. Ex W. (seven times inbred line of seeds collected from the DI Ranch, Section T40S R19W, Utah) seeds were soaked for 1 h in a GA_3 (100 mM) + smoke solution (House of Herbs, Passaic, NJ; Baldwin et al., 1994) and germinated in agar plates. Seedlings were transplanted to individual 0.5-L pots filled with standard topsoil.

Plant Growth in Field Experiments

Our field experiments were located at the Dr. Miguel Culaciati Botanical Garden in Huerta Grande (31° south, Córdoba, central Argentina, within 5 km of the *N. longiflora* seed collection site). Seedlings were grown for 1 week in a greenhouse after transplanting, then transferred to the field plots and assigned to the experimental UV-B treatments. The individual pots were sunk into the recently cultivated soil of the plots to facilitate watering and avoid overheating of the root zone. There were three independent blocks of plants (replicates). During the period in the field (3 weeks), the plants were fertilized twice with 0.5 g of N:P:K per pot and watered twice daily to maintain the soil near field capacity. The experimental plants of *N. longiflora* were very similar in appearance to their wild relatives that occurred in moist areas near river banks or abandoned fields.

Plant Growth in Greenhouse Experiments

Seedlings (in individual 0.5-L pots) were grown for 4 weeks in a greenhouse in Buenos Aires (34° S), without supplemental lighting, and then transferred to the experimental UV-B treatments within the same greenhouse. There were three independent blocks of plants (replicates). During the period under the UV-B lamps (3 weeks), the plants were fertilized once with 0.5 g of N:P:K and watered daily to maintain the soil near field capacity. Peak PAR in the greenhouse was 1,400 μmol m^{-2} s^{-1}; natural photoperiod varied between 12.5 and 13.5 h.

UV-B Treatments

In the field, the plants were grown in 1- × 1.4-m plots covered with clear plastic filters. The filters were of two types: clear polyester (Oeste Aislante, Buenos Aires; 0.1 mm thick), which filtered out more than 90% of the UV-B component of solar radiation (attenuated UV-B treatment); and "Stretch" film (Bemis Co., Minneapolis; 0.025 mm thick), which has very high transmittance in the UV-B region (near-ambient UV-B treatment). Both films have very high transmittance in the UV-A and PAR regions of the spectrum (for spectral scans and details, see Mazza et al., 2000). There were three filters for each experimental treatment. The field exposures were carried out during the summer season (January to February), which coincides with the period of peak growth in natural populations. To minimize natural herbivory on the experimental plants, the plots were surrounded with mosquito nets.

In the greenhouse, the plants were randomly assigned to one of two treatments: simulated ambient UV-B and no UV-B. UV-B radiation was obtained with three UVB 313 fluorescent lamps (Q-Panel) filtered through one 0.13-mm-thick layer of cellulose di-acetate (JVC Plastics, Lemirada, CA) to remove UV-C photons. In the no-UV-B treatment, the cellulose di-acetate film was replaced by a 0.1-mm clear polyester sheet (which filtered out both UV-C and UV-B). The cellulose di-acetate film was replaced every 4 d. The plants were irradiated for 6 h each day, with the irradiation period centered at solar noon. The biologically effective daily UV dose, calculated using the generalized plant action spectrum of Caldwell (1971) normalized at 300 nm, was 9.9 kJ m^{-2}. This dose is comparable with the natural UV-B doses that *N. longiflora* and *N. attenuata* plants experience during the summer solstice in their natural areas of distribution in Central Argentina and the southwestern United States, respectively. However, a direct comparison is difficult to establish because the output of our lamps was not modulated to follow the variation in natural PAR in the greenhouse; therefore, the UV-B to PAR ratios are likely to have exceeded the natural values during cloudy days and/or at the extremes of the 6-h daily irradiation periods. Inspection of the plants at the end of the irradiation period indicated that the treatment was effective in inducing significant accumulation of UV-absorbing sunscreens in the epidermis without causing marked growth inhibition (18% reduction in plant height; no obvious differences in leaf area; data not shown). Therefore, the simulated ambient UV-B treatment can be regarded as a reasonable approximation to natural UV-B exposure.

Simulated Herbivory Treatment

To simulate herbivore damage (microarray and PI determination experiments), three (third to fifth) fully expanded leaves of rosette stage plants of the attenuated UV-B plots were treated in all cases. Three rows of puncture wounds were created with a fabric pattern wheel on each side of the mid-vein and 20 μL per leaf of a 1:6 (v/v) dilution of *Manduca sexta* regurgitate collected from fourth instar caterpillars were applied to the fresh wounds (simulated caterpillar treatment).

RNA Isolation

Approximately 0.5 g of frozen tissue was ground in liquid N using a pestle and mortar. This was followed by the addition of 5 mL of extraction

buffer (0.18 M Tris-HCl [pH 8.2], 0.09 M LiCl, 4.5 mM EDTA, and 1% [w/v] SDS) and 1.7 mL of phenol acid:chloroform (5:1 [v/v]). The frozen slurry was ground and then centrifuged (12,000*g* for 20 min at 4°C). The aqueous phase was then removed and nucleic acids precipitated by the addition of LiCl (final concentration 2 M). Samples were well mixed and nucleic acids precipitated overnight at 4°C. After the precipitation step, the samples were centrifuged (12,000*g* for 20 min at 4°C). The pellet was redissolved in 2 mL of water and 200 μL of AcONa followed by the addition of 5.5 mL of 100% (v/v) ethanol and precipitated at −20°C for 2 h. The samples were then centrifuged (12,000*g* for 30 min at 4°C), and the pellets were washed in 2 mL of 70% (v/v) ethanol and centrifuged (12000*g* for 20 min at 4°C). The pellets were dried and resuspended in water.

cDNA Microarray

The method used to spot *M. sexta*- and regurgitate-responsive genes and seven well-characterized *N. attenuata* control genes (*PI*, *HPL*, *PMT1*, *AOS*, *XET*, *WRKY*, 3′ region of *LOX*, and 5′ region of *LOX*) onto epoxy coated slides has been described by Halitschke et al. (2003). For each cDNA, two PCR fragments, with 5′-Aminolink C6 modification (Sigma-ARK, Darmstadt, Germany) on either strand, were synthesized. Even numbered fragments (see Supplemental Table I at http://www.plantphysiol.org) carry the Aminolink modification at primers TOP9–22, SMA4–23, or ASV6–22, whereas odd numbered fragments carry the modification at primers TOP10–24, SMA3–22, or ASV5–21. All cDNA samples, including the seven well-characterized *M. sexta*-induced genes as controls, were commercially spotted four times by Quantifoil Micro Tools GmbH according to their procedure on the slides using a robot equipped with six printing tips (Biorobotics MicroGrid II Microarrayer: Genemachine, Apogent Discoveries, Hudson, NH). Hence, each gene was represented on the microarray by two independent PCR fragments that, in turn, were spotted in quadruplicate. A complete list of identities and positions of spotted PCR products on the microarray can be found in Halitschke et al. (2003).

Sampling, Microarray Hybridization, Quantification, and Analysis

The three treated leaves from each plant of the simulated caterpillar treatment, three leaves per plant of equivalent nodal positions from unwounded plants of the UV-B treatments, and three leaves per plant of unwounded plants of the attenuated or no UV-B treatment were harvested 24 h after the simulated caterpillar folivory and flash frozen in liquid N. In each experiment, there were 12 individual plants for each treatment (four from each replicate block), and leaves from the same treatment were pooled together for RNA extraction. The UV-B- and simulated caterpillar-treated samples served as treatment (Cy3), and the unperturbed, non-UV-B-exposed samples were labeled and hybridized as controls (Cy5). mRNA isolation, labeling, determination of labeling efficiency, and microarray hybridization were carried out as described (Halitschke et al., 2003). The hybridized microarrays were scanned with an Affymetrix 428 Array Scanner (Affymetrix, Inc., Santa Clara, CA). Scanning, image analysis, background correction, and slide-specific normalization were all carried out as explained by Halitschke et al. (2003). The ratios of normalized fluorescence values for Cy3 and Cy5 of each individual spot (ER) and the mean of the four replicate spots for each cDNA (two for each gene, ER1 and ER2) were calculated. Log-transformed ERs were subjected to a one-tailed Student's *t* test against the hypothesis of no treatment effect (i.e. ER = 1).

Our criteria to assess the significance of the expression changes for a given gene were: (a) a significant Student's *t* test value in the comparison against ER = 1 ($n = 4$ replicate spots; $P < 0.05$), (b) a deviation of at least ± 0.25 from ER = 1, and (c) verification that criteria (a) and (b) were fulfilled by the ERs obtained for both PCR fragments (i.e. ER1 and ER2). An arbitrary threshold was utilized for two reasons: first, to account for normalization errors; and second, to account for the fact that replicate data did not result from repeated hybridizations with the same RNAs but from repeated probe spotting. An ER calculated as a mean of replicate ERs and the use of statistically rigorous criteria to evaluate within-array variance allowed us to use lower thresholds with this polyepoxide microarray in comparison with the poly-Lys microarray used in a previous study (Hui et al., 2003). To evaluate our criteria, two arrays were hybridized with the same two cDNA pools (R. Halitschke and I.T. Baldwin, unpublished data). It was found that 210 of 241 genes (87%) had the same regulation identified by the criteria described above. Of the 31 genes that did not show consistent regulation between the two repeated hybridizations, 24 had the same direction in mean ER, but did not meet the statistical requirements for a significant change.

Determination of PI Activity

The UV-B and simulated caterpillar treatments and the leaf harvest procedures were carried out as described above for the microarray experiments. The simulated caterpillar treatment was applied in the early afternoon, and leaves were harvested before and several times after elicitation and flash frozen in liquid N. There were three replicates (three individual plants) per treatment and time point. PI activity in leaf extracts were determined using the method of Jongsma et al. (1993, 1994; van Dam et al., 2001). Protein levels were determined following Bradford (1976).

Insect Growth Bioassays

M. sexta eggs were obtained from the Entomology Department of North Carolina State University (Raleigh). Two neonate larvae were placed on each of 16 plants of *N. longiflora*, and 20 plants of *N. attenuata* were grown under greenhouse conditions (see above). The two caterpillars were placed on the same leaf (fourth–fifth fully expanded leaves of rosette stage plants). One-half of the plants of each species had received UV-B supplementation for at least 2 weeks before the caterpillars were placed on them, and one-half belonged to the no-UV-B control. The UV-B treatments continued during the feeding period (1 week). To prevent direct exposure of the caterpillars to UV-B, the host leaf in each plant was enclosed in a cotton gauze bag that had its upper side covered with aluminum foil. These bags were applied to UV-B-exposed and control plants. Caterpillars were weighted at the end of the experiment.

Distribution of Materials

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.

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