

Solar Ultraviolet-B Radiation and Insect Herbivory Trigger Partially Overlapping Phenolic Responses in *Nicotiana attenuata* and *Nicotiana longiflora*

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• **Background and Aims** Plants exposed to solar ultraviolet-B radiation (UV-B, 280–315 nm) frequently suffer less insect herbivory than do plants that receive attenuated levels of UV-B. This anti-herbivore effect of solar UV-B exposure, which has been documented in several ecosystems, is in part mediated by changes in plant tissue quality. Exposure to UV-B can modify the abundance of a number of secondary metabolites, including phenolic compounds with potential impacts on insect herbivores. The aim of this study is to assess the potential anti-herbivore role of UV-B-induced phenolic compounds by comparing the phenolic profiles induced by UV-B and simulated insect herbivory in two wild species of the genus *Nicotiana*.

• **Methods** Plants grown under field and glasshouse conditions were exposed to contrasting levels of UV-B. Half of the plants of the attenuated UV-B treatment were given a simulated herbivory treatment, where leaves were mechanically damaged and immediately treated with oral secretions of *Manduca sexta* caterpillars. This treatment is known to mimic the impact of real herbivory on the expression of plant defences in *Nicotiana*. Phenolic profiles induced by UV-B and simulated herbivory were characterized using high-performance liquid chromatography–mass spectrometry (HPLC–MS).

• **Key Results** UV-B induced the accumulation of several UV-absorbing phenolic compounds that are known to play a significant role in UV-B screening. Interestingly, there was a significant convergence in the phenolic profiles induced by UV-B and simulated herbivory: chlorogenic acid and dicaffeoylspermidine isomers, in particular, displayed a similar pattern of response to these stimuli. In contrast, rutin, the only flavonoid that accumulated in significant quantities in the experiments, was only induced by UV-B.

• **Conclusions** The results suggest that the anti-herbivory effect induced by UV-B may be mediated at least in part by the accumulation of phenylpropanoid derivatives that are similar to those induced by the plant in response to insect herbivory.

Key words: Chlorogenic acid, flavonoids, phenolic compounds, rutin, UV-B, *Nicotiana*.

INTRODUCTION

The effects of ultraviolet B radiation (UV-B) on the growth and ecological relationships of terrestrial plants have been studied intensively during the last three decades. This body of research has shown that solar UV-B is a significant modulator of the interactions between plants and phytophagous insects in terrestrial ecosystems. Exposure to solar UV-B frequently reduces the quality of plant tissues for herbivores (for reviews, see Ballaré *et al.*, 2001; Caldwell *et al.*, 2003; Roberts and Paul, 2006). This effect has been attributed to a variety of potential causes, including UV-induced variations in leaf nitrogen (Hatcher and Paul, 1994), specific phenolic derivatives (McCloud and Berenbaum, 1994), cyanogenic compounds (Lindroth *et al.*, 2000) and defence-related proteins such as proteinase inhibitors (Stratmann *et al.*, 2000, 2003).

One of the most consistent responses of plants to solar (Mazza *et al.*, 2000) and enhanced (Searles *et al.*, 2001) UV-B is the accumulation of increased levels of leaf phenolics (but see Rousseaux *et al.*, 2001). These compounds

act as selective sunscreens that reduce the penetration of UV-B through the epidermis and protect sensitive targets in the mesophyll cells. Besides this photo-protective function, several phenolic compounds are known to play a role in plant–herbivore interactions. Unfortunately, most of the field studies concerned with UV-induced photo-protection only report the change in the pool of phenolics that are extracted with a particular solvent, without identifying the individual compounds. This is a significant limitation if the goal is to assess the potential significance of UV-B-induced changes in phenolic chemistry on trophic relationships (Bassman, 2004; Roberts and Paul, 2006).

Detailed analytical information is available from studies carried out in growth-chamber and glasshouse conditions. Several of these studies were focused on woody perennials, and the majority of them show a significant impact of UV-B increasing the foliar content of compounds of the flavonoid fraction, which are effective UV-B filters. In one of the species that has been studied in greatest detail, *Betula pendula*, UV-B has been shown consistently to increase the contents of flavonoids with quercetin and kaempferol backbones (Lavola *et al.*, 1998; de la Rosa

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et al., 2001; Tegelberg et al., 2004). Field studies with several species of trees confirmed a general tendency towards increased levels of flavonoids in response to UV-B (Tegelberg et al., 2001; Warren et al., 2002; Rousseaux et al., 2004). The impacts of UV-B on compounds belonging to the group of phenolic acids (such as chlorogenic acid; CHA), which may play important roles in plant–herbivore interactions, have received less attention. In *B. pendula*, there are results showing increased levels of CHA in response to UV-B (Tegelberg et al., 2004), and results showing no significant effects (Lavola et al., 1998; de la Rosa et al., 2001). In a multiyear field study, Tegelberg et al. (2001) found that the effects of UV-B supplementation on the various fractions of phenolic compounds in *B. pendula* varied between seasons.

The analytical information on changes in phenolic chemistry induced by solar UV-B (or ecologically meaningful UV-B treatments) is more limited in the case of herbaceous plants. Studies carried out under growth-chamber and glasshouse conditions have also focused on the flavonoid fraction and, in agreement with the results in woody perennials, most of the studies show a clear effect of UV-B increasing the levels of quercetin- and kaempferol-based flavonoids (e.g. Ballaré et al., 1995; Ryan et al., 1998; Wilson et al., 1998; Olsson et al., 1999; Hofmann et al., 2003).

In the experiments reported herein, the phenolic profile induced by solar and simulated solar UV-B was characterized in adult plants of two annual species of the genus *Nicotiana*, and this profile was compared with the changes induced in response to simulated insect herbivory. The purpose of this characterization was to assess the potential significance of phenolic compounds induced by solar UV-B on the interactions between plants and herbivorous insects.

MATERIALS AND METHODS

Two experiments were carried out, one in a glasshouse, using *Nicotiana attenuata*, and the other under field conditions, using *Nicotiana longiflora*. In each experiment, plants were exposed to two UV-B treatments (near-ambient and low UV-B) for 3 weeks, after which plants in the low UV-B treatment were either given a simulated herbivory treatment (see below) or left undamaged. This design allowed the effects of UV-B and insect herbivory on the abundance of phenolic compounds to be compared. The methods used for plant cultivation, UV-B manipulation and herbivory treatments have been described in detail by Izaguirre et al. (2003), and are only briefly outlined below.

Glasshouse experiment

Seedlings of *N. attenuata* were grown in individual 0.5 L pots in a glasshouse in Buenos Aires (34°S), without supplemental lighting. After 4 weeks of growth, the plants were transferred to the experimental UV-B treatments within the same glasshouse. There were two treatments: no-UV-B and simulated ambient UV-B, with three

replicates per treatment. UV radiation was obtained with three UVB 313 fluorescent lamps (Q-Panel). The output of these lamps was filtered through a 0.1 mm clear polyester sheet [which filtered out both UV-C (<280 nm) and UV-B; no-UV-B treatment] or a 0.13 mm thick layer of cellulose diacetate (which filtered out the UV-C component; simulated ambient UV-B treatment). The cellulose diacetate film was replaced every 4 d. The plants were irradiated for 6 h each day, with the irradiation period centred at solar noon. The biologically effective daily UV doses, calculated using the generalized plant action spectrum (Caldwell, 1971) normalized at 300 nm, were 0 and 9.9 kJ m⁻² for the no-UV-B and simulated ambient UV-B treatments, respectively (for details, see Izaguirre et al., 2003). The plants remained under the treatments until they were harvested to determine leaf phenolics.

Field experiment

The experiment was carried out during the summer in a field site in the Sierras Chicas (31°S, Córdoba, central Argentina). *Nicotiana longiflora* seedlings were grown for 1 week in a glasshouse, transferred to the field plots (1 × 1.4 m), and assigned to the experimental UV-B treatments. There were two treatments: attenuated UV-B and near-ambient UV-B, with three replicates per treatment. The contrasting UV-B levels were obtained by covering the plots with clear plastic filters: clear polyester for the attenuated UV-B treatment (filtered out >90% of the UV-B component of solar radiation), and ‘Stretch’ film for the near-ambient UV-B treatment (>80% transmittance in the solar UV-B region). Both films had very high, similar transmittance in the UV-A (315–400 nm) and photosynthetically active radiation (PAR; 400–700 nm) regions of the spectrum (Fig. 1). The plants remained under the treatments until they were harvested to determine leaf phenolics.

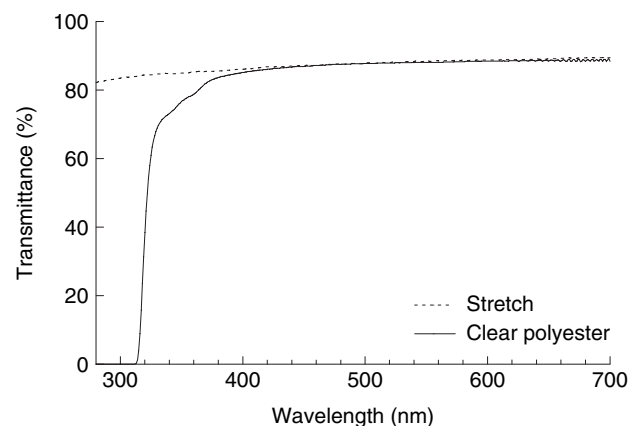


FIG. 1. Transmittance spectra of the films used for UV-B attenuation in the field study. Clear polyester (Oeste Aislante, Buenos Aires, 0.1 mm thick) and ‘Stretch’ polyethylene film (URFLEX S.A. Argentina, Buenos Aires, 0.025 mm thick) were used to create the ‘attenuated UV-B’ and ‘near-ambient UV-B’ treatments, respectively.

Simulated herbivory

Three weeks after the beginning of the UV-B manipulations, the plants of the no-UV-B and attenuated UV-B treatments were divided into two groups. In one of the groups, the plants were subjected to a simulated herbivory treatment; in the other group, the plants were left undisturbed and served as a control. The simulated herbivory treatment was applied to the two youngest fully expanded leaves of each plant. Three rows of puncture wounds were made with a fabric pattern wheel on each side of the mid-vein and 20 μL per plant of a 1:6 (v:v) diluted *Manduca sexta* regurgitate collected from fourth instar caterpillars were applied to the fresh wounds. This treatment induces defence-related gene expression responses that are equivalent to those elicited by actual insect herbivory (for details, see Halitschke *et al.*, 2001 and references therein). Plants remained under the light treatments for an additional 72 h, until they were harvested for determinations of leaf phenolics.

Determination of leaf phenolics

Tissue samples were collected from wounded leaves and from leaves of the equivalent nodal position in the control plants of the attenuated UV-B and no UV-B treatments, 72 h after the simulated herbivory treatment. Undamaged plants from the near-ambient and simulated ambient UV-B treatments were also harvested at that time, collecting the same cohorts of leaves that were harvested in plants of the attenuated-UV-B and no-UV-B treatments. Leaf disks were taken at the time of harvest to determine the content of methanol-soluble, UV-absorbing phenolic compounds by measuring the absorbance of the extracts at 305 nm (A_{305}), essentially as described in Izaguirre *et al.* (2006). The remaining leaf tissue was immediately frozen in liquid nitrogen.

Extraction of phenolic compounds for high-performance liquid chromatography (HPLC) analysis was performed following Keinänen *et al.* (2001). Briefly, green tissue (approx. 100–200 mg) without the mid-vein was ground frozen in liquid nitrogen and transferred to a centrifuge tube with 1.5 mL of 40 % aqueous methanol, containing 0.5 % acetic acid. After shaking for 2 h, samples were centrifuged (12 min, 13 000 rpm) and the supernatant was used for HPLC and mass spectrometry (HPLC-MS) analysis.

Details of the HPLC analysis have been described in Tan *et al.* (2004). In brief, samples (20 μL) were subjected to HPLC on a Nucleosil C-18 column (EC 250/4,120-5; Macherey and Nagel), with 0.1 % trifluoroacetic acid (TFA) as solvent A and 98 % acetonitrile/0.1 % TFA as solvent B, at a flow rate of 1 ml min^{-1} at 24 °C (gradient of solvent A: 100 % at 0, 94 % at 3, 80 % at 13, 76 % at 20, 37 % at 44, 0 % at 46 min), using a photodiode array detector 540 at 254 nm as part of the Biotech System (Solvent Delivery System 522, Autosampler 565, Jet-Stream plus, Degasy DG 1210, software CHROMA 2000; Biotech, Neufahrn, Germany).

Chemical structures were determined according to Tan *et al.* (2004) by electrospray ionization (ESI)-MS

using a Hewlett-Packard (Avondale, PA, USA) HP 1100 HPLC coupled to a Micromass Quattro II (Waters, Micromass, Manchester, UK) tandem quadrupole mass spectrometer (geometry quadrupole–hexapole–quadrupole) equipped with an ESI source. The capillary and cone voltages in ESI mode were 3.3 kV and 18 V, respectively. Nitrogen for nebulization was applied at 15 L h^{-1} , and drying gas at 250 L h^{-1} and 250 °C. The source and capillary were heated at 80 and 250 °C, respectively. The mass spectrometer was operated in conventional scanning mode using the first quadrupole. Positive-ion full-scan mass spectra were recorded from mass-to-charge ratio 90 to 650 (scanning time 1.5 s). Separation of compounds was achieved on a reverse phase column (5 μm Supelcosil C18 DB phase, 250 \times 2.1 mm id, Supelco, Bellefonte, PA, USA) equipped with a Supelguard pre-column (C18 DB phase, 20 \times 2.1 id, Supelco). The solvent system was H_2O with 0.1 % HCO_2H (A) or acetonitrile (B). The following gradient was used: 0–6 min, 2–4 % of B; 6–13 min 4–18 % of B; 13–17 min, 18–28 % of B; 17–22 min, 28–53 % of B; 22–24 min, 53–93 % of B and 24–29 min, hold 93 % of B. The flow was maintained at 0.4 mL min^{-1} and the column temperature was set at 30 °C. Effluent passed through a serially connected UV detector set at 254 or 320 nm before splitting to a waste and to the API source (flow approx. 30 μL min^{-1}).

Statistical analysis

Analysis of variance (ANOVA) was used, performing multiple comparisons on least-squares means ($\alpha = 0.05$) to detect significant treatment effects on phenolic compounds in each species (total soluble phenolics and individual phenolic peaks). When necessary, data were transformed to meet the assumptions of ANOVA. To assess the similarities in the phenolic response elicited by UV-B and

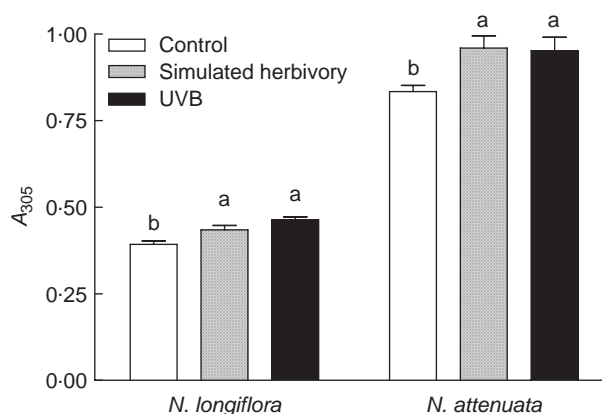


FIG. 2. Comparison of the effects of UV-B and simulated herbivory on the abundance of methanol-extractable, UV-absorbing phenolic compounds ($n = 3$). *Nicotiana attenuata* was grown in the glasshouse. The 'Control' and 'UVB' conditions correspond to the 'no-UV-B' and 'simulated ambient UV-B' treatments, respectively. *Nicotiana longiflora* was grown in the field. In this case, the 'Control' and 'UVB' conditions correspond to the 'attenuated UV-B' and 'near-ambient UV-B' treatments, respectively. For a full description of the UV-B supplementation and attenuation treatments, see Materials and methods.

simulated *M. sexta* herbivory, correlation analysis was used. This analysis was applied to the induction ratios calculated for each of the individual compounds identified by HPLC-MS [induction ratio = abundance of a compound in plants of the stress condition (UV-B or simulated herbivory) divided by its abundance in the control condition].

RESULTS

Plants of *N. attenuata* and *N. longiflora* responded to simulated herbivory and UV-B treatment with a significant increase in the pool of methanol-soluble UV-absorbing leaf phenolics (Fig. 2). Under the experimental conditions, the abundance of methanol-extractable leaf phenolics was lower in *N. longiflora* than in *N. attenuata*. However, the effects of simulated herbivory and UV-B were similarly expressed in both species (Fig. 2).

HPLC elution chromatograms indicated the presence of a variety of UV-absorbing compounds in the extracts of *N. attenuata* leaves. Nine of the peaks, which accounted for a large percentage (>90 %) of the total UV absorbance, displayed a significant response to UV-B and/or simulated herbivory (Table 1). MS analysis allowed these

stress-responsive peaks to be identified as phenolic compounds belonging to the following categories: phenolic acids (three isomers of CHA); polyamines (three isomers of dicaffeoylspermidine and two isomers of an unknown compound closely related to dicaffeoylspermidine); and flavonoids (rutin) (Table 1). CHA and rutin were, by far, the most abundant compounds, accounting for >80 % of the total UV absorbance of the extracts.

Both the basal levels of leaf phenolics and the magnitude of the phenolic response induced by UV-B and simulated herbivory were higher in *N. attenuata* than in *N. longiflora*. Comparisons between species are difficult to interpret, however, since irradiation treatments and growing conditions differed between experiments. Fewer peaks in *N. longiflora* showed statistically significant changes compared with *N. attenuata*; however, the pattern of response of the two species was similar for the flavonoid rutin and the most abundant isomers of CHA and dicaffeoylspermidine (Table 1).

The effects of simulated herbivory and UV-B on the abundance of UV-absorbing phenolic compounds were compared using correlation analysis. The induction ratios for many of the compounds showed similar responses to

TABLE 1. Abundance of phenolic compounds in the control and the two stress conditions (TIC, total ion counts, per g of dry mass)

	Isomer	Condition*	Abundance ($\times 10^4$ cpm g ⁻¹)		Relative abundance (%)	
			<i>N. attenuata</i>	<i>N. longiflora</i>	<i>N. attenuata</i>	<i>N. longiflora</i>
Chlorogenic acid	A	Control	15.4 ^a	14.0	12.9	16.6
		Simulated herbivory	21.0 ^a	19.6		
		UVB	28.4 ^b	16.6		
	B	Control	34.0 ^a	42.8 ^a	28.5	50.7
		Simulated herbivory	58.0 ^b	64.4 ^{ab}		
		UVB	71.2 ^b	65.3 ^b		
	C	Control	3.0 ^a	4.4	2.5	5.2
		Simulated herbivory	4.9 ^b	7.0		
		UVB	6.0 ^b	6.0		
Dicaffeoylspermidine	A	Control	1.0 ^a	0.5	0.8	0.6
		Simulated herbivory	7.5 ^b	0.3		
		UVB	5.1 ^b	0.8		
	B	Control	4.1 ^a	0.8 ^a	3.4	1.0
		Simulated herbivory	25.0 ^b	1.2 ^a		
		UVB	24.0 ^b	2.3 ^b		
	C	Control	5.2 ^a	1.3 ^a	4.4	1.5
		Simulated herbivory	37.0 ^b	4.5 ^b		
		UVB	25.9 ^b	2.9 ^b		
Unknown	A	Control	4.8 ^a	1.2 ^a	4.0	1.4
		Simulated herbivory	25.0 ^b	2.7 ^a		
		UVB	11.8 ^b	3.3 ^b		
	B	Control	10.1 ^a	2.5	8.5	3.0
		Simulated herbivory	41.4 ^b	6.1		
		UVB	29.0 ^b	4.5		
Rutin	Control	41.8 ^a	16.9 ^a	35.0	20.0	
	Simulated herbivory	51.7 ^a	21.5 ^a			
	UVB	106.0 ^b	37.4 ^b			
			Total		100.0	100.0

The relative abundances of the various peaks were calculated based on ion count data for the control condition. Within each species and for each of the compounds, different letters indicate significant differences ($P < 0.05$; $n = 3$) between treatments.

* *Nicotiana attenuata* was grown in the glasshouse. The 'Control' and 'UVB' conditions correspond to the 'no-UV-B' and 'simulated ambient UV-B' treatments, respectively. *Nicotiana longiflora* was grown in the field. In this case, the 'Control' and 'UVB' conditions correspond to the 'attenuated UV-B' and 'near-ambient UV-B treatments', respectively. For a full description of the UV-B supplementation and attenuation treatments, see Materials and Methods.

UV-B and simulated herbivory; all but one of the data points were found in the North-east (positive) quadrant of the simulated herbivory vs. UV-B plot, and the correlation coefficient was highly significant (Fig. 3). This convergent pattern of induction was particularly clear for phenylpropanoid derivatives, such as the most abundant isomers of CHA and the polyamines (dicaffeoylspermidine-related compounds). In contrast, for rutin, the only flavonoid that accumulated in significant quantities in the experiments, there was a clear response to UV-B radiation, but little or no response to simulated herbivory (Fig. 3, circled data points).

DISCUSSION

The effects of UV-B on flavonoid levels in *N. attenuata* were similar to the effects described in previous work with woody and herbaceous species. The increase in the flavonoid rutin was consistent with previous observations showing increased levels of quercetin glucosides (Lavola *et al.*, 1998; Ryan *et al.*, 1998; Wilson *et al.*, 1998; Olsson *et al.*, 1999; de la Rosa *et al.*, 2001; Hofmann *et al.*, 2003; Tegelberg *et al.*, 2004). Rutin is not typically induced by insect herbivory in *N. attenuata* (Kessler and Baldwin, 2004), which is also consistent with the lack of effect of the simulated-herbivory treatment in the present experiments.

Interestingly, UV-B caused significant increases in the levels of the most abundant isomer of CHA (Table 1). CHA is a potent cellular antioxidant (Grace and Logan,

2000; Niggeweg *et al.*, 2004). Previous work has shown that natural doses of UV-B increase the foliar content of other non-enzymatic (Giordano *et al.*, 2004) and enzymatic antioxidants (Mazza *et al.*, 1999a), and this antioxidant response is thought to be an important component of the acclimation mechanisms that prevent (or minimize) UV-B-induced oxidative damage under natural conditions (Giordano *et al.*, 2004).

In addition to its antioxidant function, CHA appears to play a significant role as a defence molecule in plants that are under attack from chewing insects (Stamp and Osier, 1998). In *Nicotiana* spp, CHA is strongly induced in response to natural herbivory (Kranthi *et al.*, 2003; Kessler and Baldwin, 2004), simulated herbivory (Izaguirre *et al.*, 2006; Table 1 and Fig. 3) and treatment with the defence-related hormone jasmonic acid (JA) (Keinänen *et al.*, 2001). Both in *N. attenuata* (Kessler and Baldwin, 2004) and in *N. longiflora* (Izaguirre *et al.*, 2006), increased levels of CHA correlate with a decrease in the performance of *M. sexta* caterpillars. Therefore, the present results (Table 1, Fig. 3) suggest that elevated levels of CHA and related compounds may explain, at least in part, the negative effect of plant exposure to solar UV-B on the performance of chewing insects that have been documented in *Nicotiana* (Izaguirre *et al.*, 2003) and other species of the Solanaceae (Ballaré *et al.*, 1996). The available evidence from manipulative field experiments suggests a negative, continuous relationship between UV-B irradiance and intensity of insect herbivory (Ballaré *et al.*, 1996; Mazza *et al.*, 1999b). Under natural conditions, exposure to solar UV-B varies with a number of factors, including shading by neighbouring plants. In this context, it is interesting to note that reduced levels of defences (including CHA) have been reported in response to shading and competition (Stamp *et al.*, 2004; see also Roberts and Paul, 2006).

Previous work in the genus *Nicotiana* has indicated that solar UV-B and herbivory induce convergent responses at the gene expression level. In particular, solar UV-B and herbivory appear to have similar effects in downregulating a number of genes encoding proteins of the photosynthetic apparatus, and upregulating genes encoding enzymes of the octadecanoid pathway, which leads to the formation of JA and other oxylipins (Izaguirre *et al.*, 2003). JA is known to regulate many of the defence responses that plants mount in response to insect attack (Schilmiller and Howe, 2005). The role of JA in UV-B-induced responses is not clear, however. In *Arabidopsis*, UV-B induces increased levels of JA (at least in plants grown under low levels of PAR) (A.-H.-Mackerness *et al.*, 1999). In tomato, Stratmann *et al.* (2000) failed to detect any effects of UV-B on JA levels, but they found that UV-B treatments enhanced certain JA-dependent anti-herbivore responses induced by wounding. These observations have lent support to the idea that UV-B and JA activate signalling cascades that may share one or more functional elements.

The function of JA in the regulation of the biosynthesis of phenolic compounds has not been investigated in detail. It is known that JA can regulate the expression of some of the enzymes of this biosynthetic pathway. For example, in several plant species, treatment with JA or methyl-JA

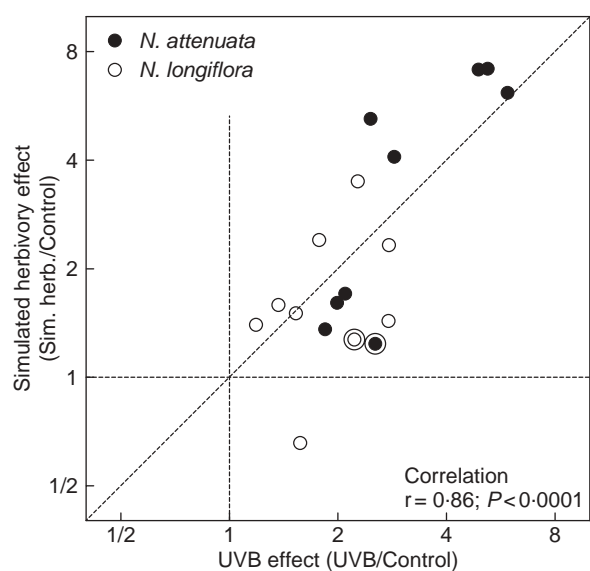


FIG. 3. Comparison of the effects of UV-B and simulated herbivory on the accumulation of UV-absorbing phenolic compounds. Each data point indicates the induction ratio for each of the nine individual compounds identified in Table 1 (i.e. the abundance of the compound in plants of the stress treatment divided by its abundance in the control condition). Circled data points correspond to rutin (a flavonoid); all others correspond to phenylpropanoid derivatives. All data ($n = 2$ species \times 9 peaks = 18) were used in the correlation analysis ($r = 0.86$ for *N. attenuata*, and $r = 0.36$ for *N. longiflora*). The diagonal line represents a 1:1 relationship.

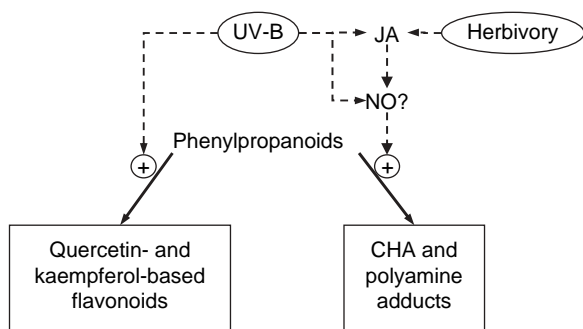


FIG. 4. Schematic diagram showing possible interactions between herbivory and UV-B in the induction of UV-absorbing phenolic compounds in leaves of *Nicotiana* spp. Both stimuli result in increased accumulation of phenylpropanoids, CHA and polyamine adducts, presumably using JA and/or NO as intermediate signalling molecules. UV-absorbing flavonoids (such as rutin) are clearly induced by UV-B, but not by insect herbivory.

(MeJA) increases the expression of phenylalanine ammonia-lyase (PAL; Gundlach *et al.*, 1992; McConn *et al.*, 1997; Thoma *et al.*, 2003; Wang and Wu, 2005) and chalcone synthase (CHS; Creelman *et al.*, 1992; Rojo *et al.*, 1998; Richard *et al.*, 2000; Ali *et al.*, 2003). It has also been shown that *jar-1*, an *Arabidopsis* mutant impaired in JA signalling, has reduced levels of UV-B-absorbing leaf phenolics (Caputo *et al.*, 2006).

A possible explanation for the partially convergent phenolic responses elicited by UV-B and herbivory in the present experiments is that UV-B upregulates genes of the JA pathway, which in turn controls the expression of genes involved in the regulation of phenylpropanoid and CHA (but not flavonoid) biosynthesis (Fig. 4). JA has been reported to cause increased levels of CHA in *N. attenuata*, but without affecting rutin accumulation (Keinänen *et al.*, 2001; Lou and Baldwin, 2003). Alternatively, UV-B could activate signalling components downstream of JA, such as nitric oxide (NO). NO accumulation is induced by MeJA in *Taxus* cell cultures (Wang and Wu, 2005) and by MeJA and wounding in *Arabidopsis* (Huang *et al.*, 2004). UV-B has also been shown to increase NO levels in broad bean (Zhang *et al.*, 2003; He *et al.*, 2005). The role of NO in the control of phenolic responses is not well understood, but it has been shown that NO can increase PAL activity (Durner *et al.*, 1998). Further testing of the model outlined in Fig. 4 will be required. The use of transgenic lines of *N. attenuata* with reduced expression of key enzymes of the JA pathway (Halitschke *et al.*, 2004) should allow a direct evaluation of the involvement of JA in the UV-induced regulation of leaf phenolics.

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