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Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae

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Abstract

Ultraviolet (UV) radiation affects the chemical composition of a plant. Since young leaves are of higher value due to their increased photosynthetic activity, for these a more efficient protection and thus stronger responses to a short-term exposure to natural radiation including or excluding UV-A plus UV-B radiation (''+UV'' vs. ''−UV'') were expected than for old leaves. Nutrients and characteristic secondary metabolites of two species of Brassicaceae were analysed after two days exposure in foil-tents with different UV filtering qualities. Contents of water, carbon, nitrogen and soluble protein were found to be affected by both UV and leaf-age in *Sinapis alba* L. but mainly by leaf-age in *Nasturtium officinale* L. Glucosinolates and myrosinases, both partners of the defence system of Brassicaceae, responded highly species-specific to UV exposure. Moreover, leaf-age mainly affected total glucosinolate concentrations in *S. alba*, but myrosinase activities in *N. officinale*. The most pronounced response to UV was found in the accumulation of flavonoids which are needed to shield the leaf interior against UV. In *S. alba*, relative contents of quercetin flavonols increased at the expense of kaempferols in +UV exposed leaves. In *N. officinale*, total flavonoid quantities were 10-fold lower in −UV exposed young leaves compared to *S. alba*, and flavonoid accumulation was induced by UV specifically in old leaves. Hydroxycinnamic acid concentrations were not affected in both species. In total, these herbaceous species showed a highly species-specific and age-dependent plasticity in response to short-term exposure to UV which is discussed with respect to their defence strategies.
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Keywords: *Sinapis alba*; *Nasturtium officinale*; Brassicaceae; UV radiation; Leaf-age; Nutrients; Flavonoids; Glucosinolates; Myrosinases; Plasticity

1. Introduction

Plants have to regulate the protection of their photosynthetic active tissue when exogenous factors or other conditions change in the environment or in new habitats. Moreover, the endogenous constitution affects the chemical composition of a plant or plant organs. Even within an individual plant, the quality and quantity of metabolites may differ between young and old leaves, sun exposed parts and organs that remain in shade (Raupp and Denno, 1983; Collinge and Louda, 1988; Kursar and Coley, 2003). Since tissue damage should be minimised especially in photosynthetic active tissues, young leaves can be regarded as more valuable than old leaves and should be better protected against biotic and abiotic harms (Iwasa et al., 1996; Lambdon et al., 2003). Shifts in quality and quantity of metabolites may affect a plant's susceptibility to abiotic stresses, and thus in turn also the attractiveness to herbivorous insects (Feeny, 1976; Cornell and Hawkins, 2003; Kursar and Coley, 2003).

In order to investigate the adaptation of plants to their environment, the influence of ultraviolet-A and -B radiation (UV-B 280–315 nm, UV-A 315–400 nm) became the subject of many studies. Enhanced UV-B radiation reaches the earth's surface due to a depletion of the ozone layer, severely affecting plant life, since this radiation is strongly absorbed by proteins, nucleic acids, and macromolecules (Bassman, 2004). The number of studies on the effects of UV-B radiation, especially on plants and insects, increased

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in the last twenty years, with most studies artificially enhancing UV-B radiation using UV-B lamps under controlled conditions, e.g. in greenhouses or climate chambers (e.g. Liu et al., 1995; Feng et al., 2003; Warren et al., 2003), or in field experiments (e.g. Rinnan et al., 2003; Bjerke et al., 2005) in order to test the effects on plants and their potential for adaptation and/or self-protection. Only few studies performed outdoor experiments using UV filtering foils to expose plants to natural irradiation including or lacking the UV portion of the spectrum (e.g. Turunen et al., 1999; Kolb et al., 2001; Caputo et al., 2006).

Since UV-B induces general stress responses in plants, the syntheses of a broad variety of metabolites regarding growth, development and defence may be affected (Doughty et al., 1995; Paul et al., 1997; Mackerness et al., 1999; Mackerness, 2000). The accumulation of flavonoids and hydroxycinnamic acids in epidermal cells is the main mechanism of plants to build up protection against UV (Caldwell et al., 1983; Olsson et al., 1998; Harborne and Williams, 2000; Kolb et al., 2001).

Brassicaceae are characterised by a binary chemical defence system against herbivores and pathogens, the glucosinolate-myrosinase system (Halkier and Gershenzon, 2006). The non-volatile glucosinolates are composed of a thioglucoside moiety and an amino acid derived side chain of varying character (Andréasson et al., 2001). In case of tissue damage, the enzyme myrosinase (β-thioglucoside glucohydrolase) which is stored in separate compartments, can hydrolyse the substrate to different potentially toxic volatile break-down products (Rask et al., 2000). Myrosinases occur in various isoforms and can be either soluble or complexed with myrosinase-binding or -associated proteins (insoluble) (Rask et al., 2000; Eriksson et al., 2001). Glucosinolates and their volatile hydrolysis products can deter or repel several organisms but also serve as host plant recognition cues for specialists (Levin, 1976; Louda and Mole, 1991; Siemens and Mitchell-Olds, 1996). Concentration and accumulation of glucosinolates differ between plant organs and depend on the developmental stage of the plant (Agerbirk et al., 2001; Brown et al., 2003). Both, levels of glucosinolates and their hydrolysing enzymes can increase or decrease due to insect feeding (Siemens and Mitchell-Olds, 1998; Pontoppidan et al., 2003; Martin and Müller, 2007; Müller and Sieling, 2006), and JA- and SA-mediated pathways have been shown to be involved in glucosinolate induction (van Dam et al., 2004; Mewis et al., 2005). However, it remains unclear in how far shifts in both partners of the binary defence system are affected by UV irradiance.

Our goal was to study effects of short-term exposure to ambient visible and UV radiation on the chemical composition of two species of Brassicaceae taking into account the specific leaf-age. As young leaves are more valuable for the plant than old leaves, young leaves should build up protection mechanisms against harmful radiation with priority. Therefore, we expected stronger responses in young than in old leaves. Differences in quality and quantity of several primary and secondary plant compounds were studied, with special regard to the glucosinolate-myrosinase defence system. The plasticity of responses of young and old leaves to short-term +UV and −UV exposure is discussed with respect to the defence strategies of both species.

2. Results and discussion

2.1. Exposure conditions

Plants of *S. alba* and *N. officinale* were grown from seeds in the greenhouse for 19 days and then transferred to outside positioned foil-tents to investigate their adaptation to different UV conditions within 48 h exposure. Irradiance spectra in the greenhouse lacked UV-B and had reduced levels of photosynthetic active radiation (400–700 nm) compared to outside conditions. In +UV tents spectral irradiances included UV-B and UV-A radiation from 280 to 400 nm, whereas in −UV tents UV-B radiation was excluded, and the transmission of UV-A radiation was low between 320 and 360 nm, increasing slightly to full transmission at 400 nm (Fig. 1). Throughout the exposure period of 48 h, the maximum day time temperature in the tents did not exceed 33 °C and did not fall below 13 °C at night time, and the temperature was minutely higher in +UV tents compared to −UV tents (mean deviation 0.06 °C). The relative humidity in the tents was highest in the early morning (98.0%), and lowest in the late afternoon (44.8%), with almost equal relative humidity in both tent types (mean deviation of 0.8%).

The duration of plant exposure to UV was determined as the exposure period that is needed to achieve likely highest differences in chemical composition between −UV and +UV exposed plants, expressed as UV-A screening capacity of the leaf epidermis and measured with a UV-A-PAM chlorophyll fluorometer. In *S. alba*, highest differences were

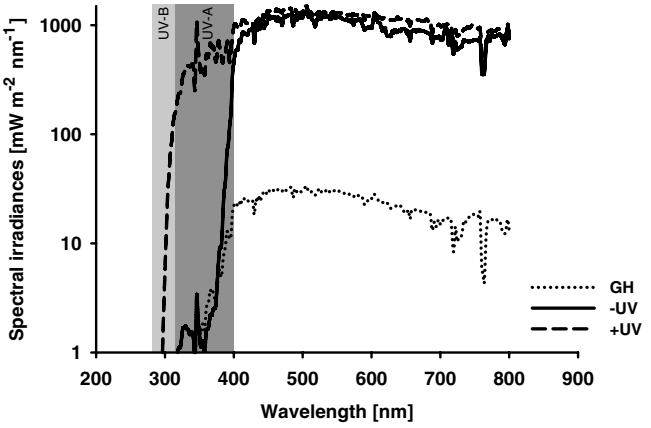

Fig. 1. Spectral irradiances in the greenhouse and in the exposure tents transmitted by both foil types, measured under cloudless sky in the early afternoon. Wavelength sections indicating UV radiation are highlighted in light grey for UV-B radiation (280–315 nm) and dark grey for UV-A radiation (315–400 nm). Note the logarithmic *y*-axis.

found after 48 hours of exposure, while for *N. officinale*, differences were higher after 3 days (Table 1). However, as in *S. alba* at that time shielding capacity already converged in leaves of −UV and +UV treatments, a 48 h exposure was used consistently for experiments. In order to study species-specific short-term exposure effects on young vs. old leaves, second youngest completely unfolded leaves and oldest non-cotyledon, primary leaves were taken for analyses.

2.2. Water and nutrients

Between young and old leaves exposed to −UV and +UV conditions, highly plant species-specific quantitative differences in water and nutrient levels (based on dry weights) were detectable. In *S. alba*, water, nitrogen and soluble protein contents and glucose concentrations were significantly affected by UV treatment, whereas leaf-age dependent effects were detected for water, carbon and soluble protein contents. Water contents were significantly higher in +UV than in −UV exposed young leaves, and higher in old than in young leaves. Soluble protein contents were higher in −UV than in +UV exposed young leaves and higher in young than in old leaves. Significant differences for glucose concentrations were only found between −UV and +UV exposed old *S. alba* leaves (Table 2, Fig. 2).

In *N. officinale*, soluble protein contents and glucose concentrations were significantly affected by UV treatment, whereas water, carbon, nitrogen and soluble protein contents were affected by leaf-age. In contrast to water contents and glucose concentrations in +UV exposed leaves, which were higher in old than in young leaves, carbon, nitrogen and soluble protein contents and glucose concentrations in −UV exposed leaves were on average higher in young than in old leaves (Table 2, Fig. 2).

Decreases in nitrogen contents with increasing leaf-age are characteristic for many plant species (McNeill and Southwood, 1978; Wait et al., 2002) and were more pronounced in *N. officinale* than in *S. alba*. Higher nitrogen contents in +UV exposed plants compared to −UV exposed plants had been also found in other plant species under supplemental UV-B (Murali and Teramura, 1985; Lindroth et al., 2000; Warren et al., 2002). Similarly, higher soluble protein levels in +UV exposed leaves were detected in *N. officinale.* In contrast, in *S. alba* levels of soluble proteins were lower after +UV exposure, as earlier detected in pea leaves under elevated UV-B conditions (He et al., 1993). Drastic shifts in water and nutrient levels due to different UV exposure conditions that are furthermore leaf-age dependent must be expected as a direct result of different photosynthetic activities in these tissues (Teramura and Caldwell, 1981; Björn et al., 1997; Kolb et al., 2001; Keiller et al., 2003). These shifts may affect the quality of the plant as a feeding source for herbivorous insects, which might adapt their consumption rates to specific nitrogen contents (Feeny, 1976; Cornell and Hawkins, 2003; Kursar and Coley, 2003; Lambdon et al., 2003). However, whereas primary compounds determine the nutritional value of plant parts, the accumulation of secondary compounds, which might differ in quality and quantity not only between young and old leaves but also due to UV exposure, might highly influence the susceptibility to harmful radiation and to herbivore attacks.

2.3. Secondary metabolites

One of the main mechanisms of protection against UV is the accumulation of flavonoids and hydroxycinnamic acids in epidermal cells (Caldwell et al., 1983; Olsson et al., 1998; Harborne and Williams, 2000; Kolb et al., 2001), driven by an increase in the activity of phenylalanin ammonia lyase (PAL) and other genes required for flavonoid biosynthesis (Mackerness, 2000). The UV dependent regulation of expression of these genes varies between tissues of different age and developmental stages (Kubasek et al., 1992).

In our study, in *S. alba* and *N. officinale* total flavonoid concentrations were significantly affected by UV treatment (higher concentrations in +UV than −UV exposed leaves, based on μmol g^{-1} DW) and leaf-age (higher concentrations in young compared to old leaves), and by the interaction between UV treatment and leaf-age in *S. alba*. Within young −UV exposed leaves and within old +UV exposed leaves total flavonoid concentrations in *S. alba* were 10-fold higher compared to *N. officinale*. In old −UV exposed *N. officinale* leaves flavonoid concentrations were below the detection limit (taken as 0 μmol g^{-1} DW).

Both plant species differed in their qualitative and quantitative composition of three groups of glycosylated flavonols, namely quercetins, kaempferols and unidentified flavonols. In *S. alba*, relative contents of all flavonols were affected by UV only, whereas in *N. officinale*, highly significant effects were evoked by UV treatment and the interaction between UV treatment × leaf-age (quercetin,

Table 1
Epidermal UV shields (%) in young leaves of *Sinapis alba* and *Nasturtium officinale* measured after three time points of exposure to ambient radiation lacking UV (−UV) and ambient radiation including UV (+UV)

		0 h	24 h	48 h	72 h
S. alba	−UV	33.7 ± 3.2	54.4 ± 1.0	62.5 ± 1.5	71.5 ± 1.7
	+UV	33.7 ± 5.1	72.8 ± 2.4	84.7 ± 1.0	84.1 ± 1.0
N. officinale	−UV	2.2 ± 1.3	4.9 ± 0.6	7.6 ± 2.7	14.7 ± 1.9
	+UV	5.5 ± 2.5	12.9 ± 4.4	26.7 ± 3.7	44.3 ± 3.0

Data were obtained with a UV-A-PAM chlorophyll fluorometer. $N = 6$ (mean ± SE).

Table 2
ANOVA *P*- and *F*-values for water, carbon and nitrogen contents (%) and soluble protein contents (μg mg^{-1} DW), glucose concentration (nmol mg^{-1} DW), total flavonoid concentration (μmol g^{-1} DW) and relative contents of individual glycosylated flavonols (% of total flavonoid concentrations, DW; not detectable concentrations were taken as zero %) and hydroxycinnamic acid concentrations (μmol g^{-1} DW), total glucosinolate concentrations (μmol g^{-1} DW) and relative contents of individual glucosinolates (% of total glucosinolate concentrations, DW); and activities of soluble and insoluble myrosinases [nmol (mg FW*min)$^{-1}$] in *Sinapis alba* and *Nasturtium officinale* leaves of different age and UV treatments

	S. alba			***N. officinale***		
	UV	**Leaf-age**	**UV × age**	**UV**	**Leaf-age**	**UV × age**
Water content	***P* = 0.005**, $F = 10.5$	***P* = 0.003***, $F = 12.2$	$P = 0.32$, $F = 1.1$	$P = 0.08$, $F = 3.9$	***P* = 0.007***, $F = 13.0$	$P = 0.45$, $F = 0.6$
Carbon content	$P = 0.20$, $F = 2.7$	***P* < 0.001***, $F = 18.6$	$P = 0.48$, $F = 0.5$	$P = 0.13$, $F = 2.9$	***P* = 0.003***, $F = 13.6$	$P = 0.14$, $F = 2.4$
Nitrogen content	***P* = 0.029**, $F = 5.6$	$P = 0.29$, $F = 1.2$	$P = 0.17$, $F = 2.0$	$P = 0.08$, $F = 3.8$	***P* = 0.002*,** $F = 14.5$	$P = 0.24$, $F = 1.5$
Soluble protein content (lo, *S. alba*, *N. officinale*)	***P* = 0.014**, $F = 7.2$	***P* = 0.023**, $F = 6.1$	***P* = 0.010**, $F = 2.9$	***P* = 0.009**, $F = 8.5$	***P* = 0.028**, $F = 5.6$	$P = 0.14$, $F = 2.4$
Glucose concentration (lo, *N. officinale*)	***P* = 0.046**, $F = 4.5$	$P = 0.46$, $F = 0.6$	***P* = 0.012**, $F = 7.7$	***P* < 0.001***, $F = 23.5$	$P = 0.89$, $F = 0.0$	$P = 0.09$, $F = 3.2$
Flavonoid concentration	***P* < 0.001***, $F = 47.4$	***P* < 0.001***, $F = 25.0$	***P* = 0.004**, $F = 10.3$	***P* < 0.001***, $F = 19.7$	***P* = 0.014**, $F = 7.3$	$P = 0.181$, $F = 1.9$
Quercetin glycosides (sqrt, *S.alba*)	***P* < 0.001***, $F = 137.5$	$P = 0.94$, $F = 0.0$	$P = 0.63$, $F = 0.3$	***P* < 0.001***, $F = 15.5$	$P = 0.34$, $F = 0.97$	***P* < 0.001***, $F = 25.1$
Kaempferol glycosides (†, *N. officinale*)	***P* < 0.001***, $F = 83.0$	$P = 0.96$, $F = 0.0$	$P = 0.90$, $F = 0.0$	***P* < 0.001***, $F = 43.8$	***P* < 0.001***, $F = 43.8$	***P* < 0.001***, $F = 43.8$
Unidentified glycosylated flavonols	***P* = 0.003**, $F = 11.1$	$P = 0.80$, $F = 0.1$	$P = 0.70$, $F = 0.2$	***P* < 0.001***, $F = 33.6$	***P* < 0.001***, $F = 83.7$	***P* < 0.001***, $F = 62.3$
Hydroxycinnamic acids (†, *N. officinale*)	$P = 0.10$, $F = 3.0$	$P = 0.20$, $F = 1.8$	$P = 0.25$, $F = 1.4$	$P = 0.63$, $F = 0.2$	$P = 0.36$, $F = 0.9$	$P = 0.09$, $F = 3.3$
Glucosinolate concentration (lo, *S. alba*)	$P = 0.26$, $F = 1.4$	***P* < 0.001***, $F = 36.2$	$P = 0.72$, $F = 0.1$	***P* < 0.001***, $F = 16.7$	$P = 0.93$, $F = 0.0$	***P* = 0.036**, $F = 5.0$
4-OH-benzyl GS (sqrt, *S. alba*)	$P = 0.57$, $F = 0.3$	***P* < 0.001***, $F = 47.4$	$P = 0.74$, $F = 0.1$	–	–	–
Arom. GS (9 min) (†, *S. alba*)	***P* = 0.009**, $F = 8.4$	$P = 0.19$, $F = 1.8$	$P = 0.19$, $F = 1.8$	–	–	–
Arom. GS (19 min)	$P = 0.43$, $F = 0.7$	$P = 1.00$, $F = 0.0$	$P = 0.17$, $F = 2.0$	–	–	–
Arom. GS (28 min)	$P = 0.85$, $F = 0.0$	***P* = 0.003***, $F = 11.2$	$P = 0.40$, $F = 0.7$	–	–	–
Benzyl GS (sqrt, *S.alba*)	$P = 0.06$, $F = 4.1$	***P* < 0.001***, $F = 20.6$	$P = 0.12$, $F = 2.6$	–	–	–
Aliphat. sulfonyl GS (24 min) (sqrt, †, *S. alba*)	$P = 0.09$, $F = 3.1$	***P* < 0.001***, $F = 49.5$	$P = 0.73$, $F = 0.1$	–	–	–
2-PE (lo,†, *N. officinale*)	–	–	–	***P* = 0.020**, $F = 6.5$	$P = 0.80$, $F = 0.1$	$P = 0.35$, $F = 0.9$
4-Pentenyl GS	–	–	–	$P = 0.51$, $F = 0.5$	***P* = 0.004***, $F = 10.5$	$P = 0.60$, $F = 0.3$
8MSOO (†, *N. officinale*)	–	–	–	$P = 0.75$ $F = 0.11$	***P* = 0.001***, $F = 13.5$	$P = 0.75$, $F = 0.1$
I3M	$P = 0.57$, $F = 0.3$	$P = 0.31$, $F = 1.1$	$P = 0.93$, $F = 0.0$	$P = 0.18$ $F = 1.9$	***P* = 0.007**, $F = 8.9$	$P = 0.44$, $F = 0.6$
4MOI3 M (at, †, *N. officinale*)	***P* = 0.006**, $F = 9.5$	$P = 0.06$, $F = 4.1$	$P = 0.27$, $F = 1.3$	***P* = 0.003***, $F = 11.1$	***P* = 0.004***, $F = 10.9$	$P = 0.40$, $F = 0.8$
Activity of soluble myrosinases	$P = 0.41$, $F = 7.1$	$P = 0.34$, $F = 1.0$	***P* = 0.040**, $F = 5.0$	$P = 0.37$, $F = 0.8$	***P* < 0.001***, $F = 17.4$	$P = 0.15$, $F = 2.3$
Activity of insoluble myrosinases	***P* < 0.001***, $F = 18.0$	$P = 0.12$, $F = 2.8$	***P* = 0.049**, $F = 4.7$	$P = 0.86$, $F = 0.0$	n.t.	n.t.

Abbreviations for glucosinolates (GS): 2PE, 2-phenylethyl GS; 8MSOO, 8-metylsulfinyloctyl GS; I3M = indol-3-yl-methyl GS; 4MOI3M, 4-methoxy-indol-3-yl-methyl GS; arom. GS (9 min, 19 min, 28 min; minutes refer to retention times) = glucosinolates with aromatic side chains, not further identified; aliphat. sulfonyl GS (24 min) = glucosinolate with an aliphatic sulfonyl side chain, not further identified. Data were square root-transformed (sqrt), arcsin-transformed (at) or log-transformed (lo) to achieve homogeneity of variances (data are marked with (†) where homogeneity of variances could not be achieved, but data were transformed to approximate homogeneity). $N = 4$–7, n.t., not tested due to sample sizes <4. Boldface indicates $P < 0.05$. Asterisks denote significant *P* values after a sequential Bonferroni correction for each effect (UV treatment, leaf-age) per plant, respectively.

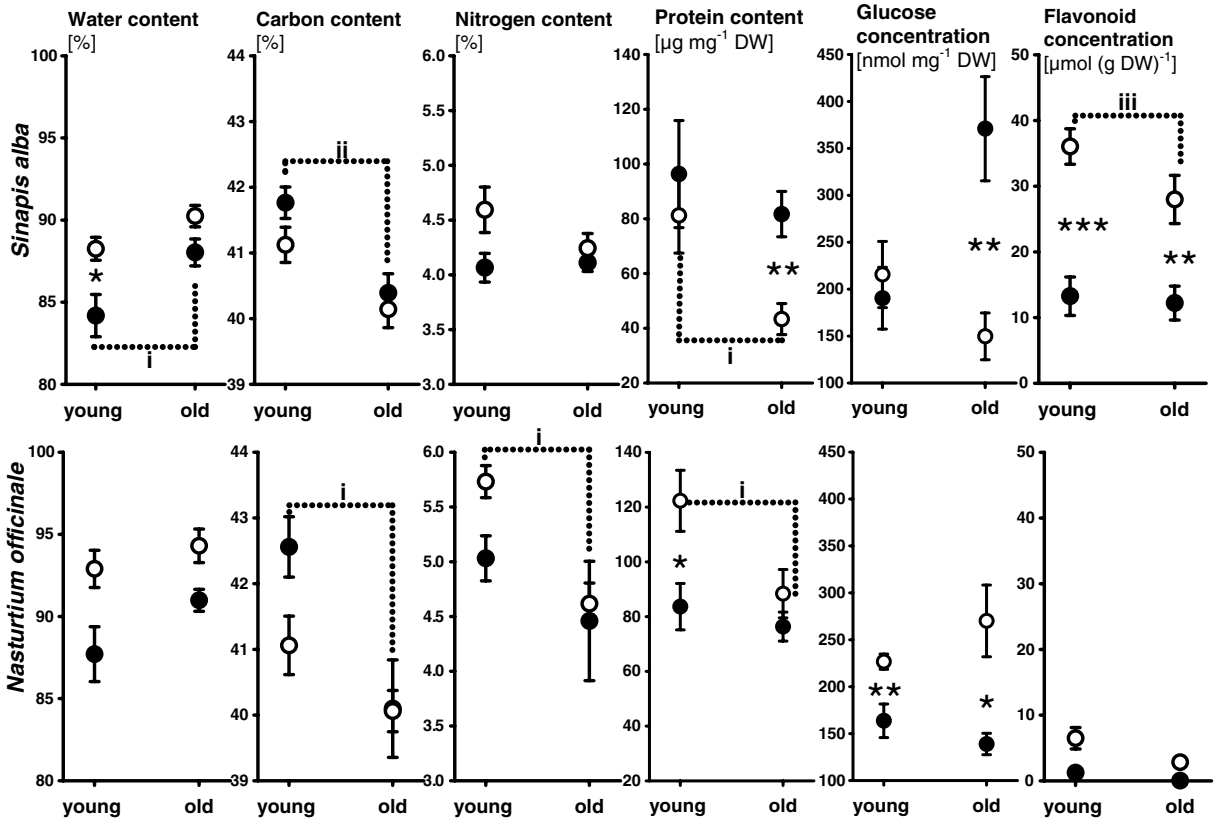

Fig. 2. Metabolite quantities in young and old leaves of *Sinapis alba* and *Nasturtium officinale* after 48 h short-term exposure to natural radiation ±UV ($N = 4$–7, mean ± SE). Open circles = +UV exposed leaves, filled circles = −UV exposed leaves. Statistical comparisons for differences in chemical compositions of leaves within each leaf-age class due to exposure to −UV and +UV, and within each UV condition due to leaf-age, were performed with Student's *t*-tests (since flavonoid contents of −UV exposed old leaves of *N. officinale* were below detection limit, this data set could not be statistically tested). For UV affected differences asterisks indicate $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, for leaf-age differences (i) indicate $P < 0.05$, (ii) $P < 0.01$, (iii) $P < 0.001$. After a sequential Bonferroni-correction, only significances $P < 0.001$ remain.

kaempferol and unidentified flavonol glycosides) and by leaf-age (kaempferol and unidentified flavonol glycosides) (Table 2, Fig. 2). In *S. alba*, +UV exposed leaves showed an approximately sevenfold higher relative quercetin glycoside content than −UV exposed leaves (young leaves: −UV 6.7%, +UV 47.8%; old leaves: −UV 7.6%, +UV 46.6% of total flavonoid content), whereas relative contents of kaempferol glycosides were approximately twofold higher in −UV exposed than in +UV treated leaves (young leaves: −UV 84.3%, +UV 48.3%; old leaves: −UV 83.6%, +UV 48.6% of total flavonoid content). Consequently, relative quercetin glycoside contents highly increased at the expense of relative kaempferol glycoside contents in +UV compared to −UV exposed leaves. This flavonol shift in ratios was based on approximately 22-fold higher concentrations of glycosylated quercetins in young +UV exposed leaves and about 14-fold higher concentrations in old +UV exposed leaves compared to those exposed to −UV. Concentrations of kaempferol flavonols were hardly affected by UV treatment in leaves of both age classes.

In *N. officinale* equally low relative contents of quercetin glycosides were found for both −UV and +UV exposed young leaves (−UV 28.5%, +UV 23.4%), whereas the absolute quercetin glycoside concentration showed an approximate fivefold increase between −UV and +UV exposed young leaves. A high relative quercetin glycoside content was found for old +UV exposed leaves (42.6% of total flavonoid content), whereas kaempferol glycosides were only detectable in young leaves exposed to +UV (13.9% of total flavonoid content).

Olsson et al. (1998) detected relatively lower kaempferol but higher quercetin glycoside levels in *Brassica napus* L. leaves exposed to supplemental UV-B radiation than in control plants under natural radiation conditions, similar to the flavonol shift we found in *S. alba*. An accumulation of 3′4′-dihydroxyflavonoids at the expense of 4′-hydroxyflavonoid synthesis was also observed for *Oryza sativa* (Poaceae) (Markham et al., 1998). This might result in an increased antioxidant activity of 3′4′-dihydroxyflavonoids due to an additional ortho-dihydroxyl group in the B-ring of the flavonoid skeleton. Therefore, quercetin flavonols, prominent flavonols in *S. alba* and *N. officinale*, might play a more important role in free radical scavenging than kaempferol flavonols (Markham et al., 1998; Olsson et al., 1998; Harborne and Williams, 2000).

Hydroxycinnamic acids support the protection of the plant tissue against harmful radiation (Caldwell et al.,

1983; Olsson et al., 1998; Kolb et al., 2001). In both plant species investigated, their concentrations were neither affected by UV treatment, nor by leaf-age or the interaction between UV treatment × leaf-age. Concentrations of hydroxycinnamic acids in *N. officinale* (young leaves: −UV 14.2 ± 3.0 μmol g^{-1} DW, +UV 11.3 ± 1.3 μmol g^{-1} DW; old leaves: −UV 9.6 ± 0.5 μmol g^{-1} DW, +UV 12.7 ± 1.3 μmol g^{-1} DW; mean ± SE, $N = 6$; Table 2) were about eightfold higher compared to those in *S. alba* (young leaves: −UV 1.2 ± 0.3 μmol g^{-1} DW, +UV 1.5 ± 0.3 μmol g^{-1} DW; old leaves: −UV 1.3 ± 0.1 μmol g^{-1} DW, +UV 3.0 ± 0.9 μmol g^{-1} DW; mean ± SE, $N = 6$; Table 2). Kolb et al. (2001) observed a high increase in the accumulation of hydroxycinnamic acids in leaves of greenhouse grown *Vitis vinifera* L. cv. Silvaner (Vitaceae) after placing them outside under different radiation regimes. These shifts started at the second day of outdoor exposure and were highest after seven days in conditions lacking UV irradiance. Increased levels of hydroxycinnamic acids might be also found in *S. alba* and *N. officinale* after longer exposure periods.

The different patterns of accumulation and distribution of phenolic compounds within both Brassicaeae due to variable UV conditions might represent responses to ecological adaptations to the original habitats of each species. Naturally, *S. alba* grows in sunny and open habitats, and thus individuals might be potentially well adapted to high UV radiation, reflected by accumulation of large quantities of flavonols with improved scavenging activities. In contrast, *N. officinale* with comparably low flavonoid concentrations naturally grows in shady and moist places with only moderate UV radiation. However, variable flavonoid contents and compositions between different plant species and different plant organs might also determine their acceptability by herbivorous insects, since these compounds can be involved in stimulation or deterrence of feeding and oviposition on a host plant (e.g. Harborne and Williams, 2000; Warren et al., 2002; Caputo et al., 2006).

Apart from an induction and accumulation of flavonoids, UV induces further defence responses. A highly species-specific activation of sets of antioxidant enzymes and of hormones, e.g. jasmonic acid, salicylic acid and ethylene due to UV exposure has been described (Mackerness et al., 1999; Mackerness, 2000; and references therein). Jasmonic acid and its methyl ester are known to regulate gene expression affecting plant growth and development (Mackerness et al., 1999; Mackerness, 2000), but also mediate defence responses against antagonists, such as an accumulation of glucosinolates (Doughty et al., 1995). In Brassicaceae, the glucosinolate-myrosinase system plays an important role in defence against herbivores and pathogens (Rask et al., 2000; Halkier and Gershenzon, 2006). Its effectiveness might depend on the quality and quantity of glucosinolates as well as on the activity of myrosinases.

In *S. alba*, total glucosinolate concentrations (based on μmol g^{-1} DW) were significantly affected by leaf-age, with higher concentrations in young compared to old leaves (−UV exposed young vs. old leaves: $P = 0.006$, $t = 4.0$, +UV exposed young vs. old leaves: $P < 0.001$, $t = 6.5$; Welch-*t*-test). Eight main glucosinolates (GS) were identified in *S. alba*. The relative contents of some minor GS were significantly affected by UV treatment [unidentified aromatic GS (at 9 min retention time), 4-methoxy-indol-3-yl-methyl GS], whereas others were affected by leaf-age [main GS 4-hydroxybenzyl GS, an unidentified aromatic GS (28 min), benzyl GS, an aliphatic sulfonyl GS (24 min)] (Table 2). In combination, the activity of soluble myrosinases [nmol (mg FW^* $min)^{-1}$] in young and old *S. alba* leaves were rather low. The activity of insoluble myrosinases was strongly affected by UV treatment with highest activity in −UV leaves, but differences were statistically significant only for old leaves ($P = 0.010$, $t = 4.5$; Welch *t*-test; Table 2, Fig. 3). The interaction between UV treat-

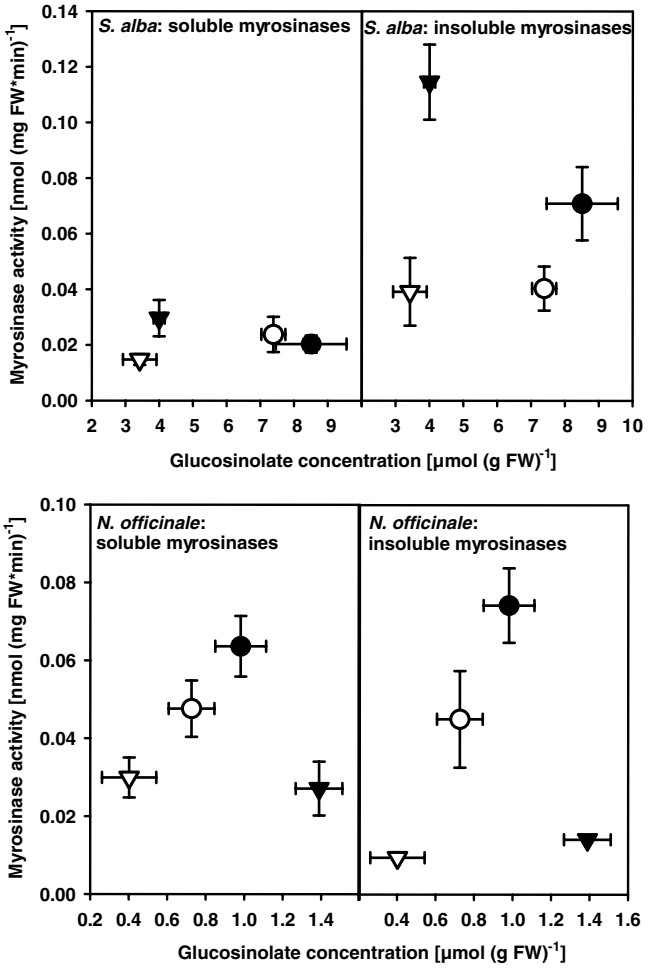

Fig. 3. Glucosinolate concentrations (μmol g^{-1} FW) and activities of soluble and insoluble myrosinases [nmol (mg $FW^*min)^{-1}$] in young and old leaves of *Sinapis alba* and *Nasturtium officinale* after 48 h exposure to natural radiation ±UV ($N = 5$–7, mean ± SE). Myrosinase activities were quantified as nmol glucose released per minute and mg fresh weight. Open circles = +UV exposed young leaves, filled circles = −UV exposed young leaves, open triangles = +UV exposed old leaves, filled triangles = −UV exposed old leaves.

ment × leaf-age significantly affected the activity of both, soluble and insoluble myrosinases.

In *N. officinale* the pattern was rather different: glucosinolate concentrations were significantly affected by UV treatment and the interaction between UV treatment × leaf-age. Compared to *S. alba*, glucosinolate quantities in *N. officinale* were three- to ninefold lower in young leaves and 10- to 12-fold lower in old leaves. Whereas in young leaves differences between differently UV treated leaves were only weak, in old leaves significantly lower glucosinolate concentrations were measured in +UV than in −UV exposed leaves ($P = 0.001$, $t = 4.2$; Welch t-test). Five glucosinolates were identified; their relative content was affected by UV treatment (main GS 2-phenylethyl GS, 4-methoxy-indol-3-yl-methyl GS) or leaf-age (4-pentenyl GS, 8-metylsulfinyloctyl GS, indol-3-yl-methyl GS, 4-methoxy-indol-3-yl-methyl GS). 4-methoxy-indol-3-yl-methyl GS could neither be detected in old leaves nor in −UV treated young leaves, but was found in +UV exposed young leaves (Table 2). The activities of soluble and insoluble myrosinases were not affected by different UV treatments, but soluble myrosinase activities were significantly higher in young than in old leaves of −UV exposed plants ($P = 0.004$, $t = 4.0$; Welch t-test; Table 2, Fig. 3). In a few leaf samples, enzyme activities were under the detection limit due to small tissue sizes (<15 mg FW).

Plant species-specific and leaf-age dependent differences in the quantitative and qualitative composition of glucosinolates might variably affect the host plant recognition of specialist herbivores, whereas the combination with hydrolysing myrosinases determines the defence against generalists. Thereby the quantity of glucosinolate hydrolysis products was shown to depend on the reaction duration of active myrosinases (Lazzeri et al., 2004). However, an optimal defence against plant enemies should be obtained by a constitutive optimal ratio of myrosinase activity to glucosinolate concentration. In any plant part that needs to be protected with priority the concentrations of both partners should correspond to this ratio or be higher. This optimal ratio is unknown so far, but probably depends among others on the kind of myrosinase (soluble vs. insoluble) and on the substrate, namely specific glucosinolates. Nevertheless, high substrate levels can act already deterrent in itself.

In leaves of *S. alba* and *N. officinale* UV treatments affected both partners of the defence system. In *S. alba*, the activities of myrosinases were similar in leaves of both age, but glucosinolate concentrations were quite different. Rather high levels of the substrate in young leaves might provide an efficient protection. In contrast, in *N. officinale* differences between young and old leaves were more pronounced in myrosinase activities than glucosinolate concentrations (Fig. 3). High myrosinase activities in young leaves might be the most important factor in the defence system of *N. officinale*. However, extraordinary low substrate levels might also weaken the defence independent of myrosinase activities, since a threshold value of breakdown products might be necessary to efficiently repel enemies.

The different responses of glucosinolates and myrosinases to UV treatments between both plant species might be again related to contrasting ecological requirements of the species' original habitats. To our knowledge, our study is the first that investigated effects of UV on both, glucosinolates as well as myrosinase activities in leaves, taking also the leaf-age into account.

3. Conclusions

Responses to an environmental stress such as short-term exposure to different light regimes with increased photosynthetic active radiation levels and including or excluding UV-A plus UV-B radiation were shown to be highly species-specific and leaf-age dependent in the two investigated Brassicaceae. UV induces probably a plethora of key enzymes transmitting a general stress response. Thereby not only UV absorbing metabolites are affected but also other primary and secondary metabolites including several compounds that might influence members of the next trophic level by affecting host plant recognition, feeding and oviposition behaviour of herbivores and their performance to various degrees (e.g. McCloud and Berenbaum, 1999; Lindroth et al., 2000; Warren et al., 2002). The level of plasticity in response to UV might be closely related to the species' needs for adaptation to their specific environment, where plants might have to adjust their defence not only against harmful radiation, but also against feeding insects. Increasing costs for synthesis or reallocation of defence chemicals might accompany this process of adaptation, which might be furthermore influenced by the abundance and sensitivity of generalist insects (Iwasa et al., 1996). With regard to tissue-age, old leaves are often less nutritious than young leaves (e.g. Raupp and Denno, 1983; Lambdon et al., 2003), and optimal levels of chemical defence were also found to decrease with leaf-age (Iwasa et al., 1996). Similarly, young +UV exposed *S. alba* leaves with high levels of nitrogen and soluble proteins should provide a nutritive feeding source, but due to corresponding high flavonoid and glucosinolate levels and comparably low myrosinase activities these leaves might be efficiently defended against both harmful radiation and herbivore attacks. In contrast, old +UV and −UV exposed leaves of *S. alba* with low nutritional values, low flavonoid and glucosinolate contents and at least variable activities of insoluble myrosinases could be regarded as less protected. Old *N. officinale* leaves also contained low nutrient levels, low myrosinase activities and particularly low flavonoid levels which were even not measurable in −UV exposed leaves, and therefore are probably poorly protected against UV and herbivory. In young *N. officinale* leaves flavonoid levels were still rather low compared to *S. alba*, probably reflecting the habitat of the species in shady areas of the former and in open, sunny habitats of the latter. Against

herbivores, myrosinase activities might protect the young tissue of *N. officinale* which was rich in nitrogen and soluble proteins.

The interaction between qualitative and quantitative shifts of primary and secondary metabolites of plants and their role for host plant perception of insects should be analysed in order to estimate the variable susceptibility to herbivores of different plant parts and plant species exposed to different radiation regimes. Studies of herbivore performance and preferences using *S. alba* and *N. officinale* after different UV treatments as food will provide a further base for the interpretation of the high phenotypic plasticity of leaves due to UV treatment in the two investigated Brassicaceae.

4. Experimental

4.1. Plant species

White mustard (*Sinapis alba* L. cv. Silenda, Kiepenkerl, Norken, Germany) and water cress (*Nasturtium officinale* L., Rühlemann's, Horstedt, Germany) were grown from seeds in unfertilised soil (peat, pH 6) in the greenhouse (20: 15 °C, 16: 8 h L: D, 70% r.h.). In the greenhouse irradiance spectra lacked UV-B, whereas low levels of UV-A were detected starting at 320 nm, but not exceeding 21 mW m^{-2} nm^{-1}. Photosynthetic active radiation (400–700 nm) was approximately 10.3×10^{-6} mW m^{-2} nm^{-1}. Irradiance spectra were measured in the early afternoon under cloudless sky with a portable high accuracy UV–visible spectroradiometer configured with 0.25 mm entrance and exit slits that result in a half bandwidth of $\leqslant$2 nm, detected at 400 nm (OL 754, Optronic Laboratories, Orlando, USA; Fig. 1). At an age of 19 days (4–5 leaf stage for *S. alba*, multiple leaf stage for *N. officinale*) individual plants were transferred outdoors in two types of foil-tents to allow adaptations to two different radiation regimes including and excluding ultraviolet radiation. A minimum of 12 plant individuals of *S. alba* and six pots with *N. officinale* were exposed in each tent, resulting in 24 *S. alba* individuals and 20 *N. officinale* pots per exposure condition. Samples of the second youngest completely unfolded leaves and the primary and thus oldest non-cotyledon leaves of each plant individual were collected after short-term UV exposure; harvest time was at about 10 o'clock in the morning. Exposure was performed in July 2005.

4.2. UV exposure and climate conditions

Four exposure tents provided with two types of foils were situated outdoors in the Botanical Garden of Würzburg. Tents (3×1 m ground area) were constructed of wooden frames (beam width: 5 cm) with roofs and walls of foils, and aligned with the longer axis in an east–west direction. The roof sloped from 1.8 m (north) to 1.2 m (south), and the northern wall remained open for ventilation; diffuse radiation from the north was minimised by shielding the open end with the appropriate foil (3.5×1.5 m) mounted at 45° and 20 cm from the top of the tent (Kolb et al., 2001). Two tents were provided with a teflon foil (Nowofol, Siegsdorf, Germany) transmitting the complete visible light spectrum plus UV-A (315–400 nm) and UV-B (280–315 nm) radiation. Spectral irradiances in +UV tents ranged from 280 to 900 nm. The two other tents were provided with a "Lee 226 UV" foil (FFL-Rieger, Munich, Germany) cutting off UV-B, transmitting visible light, and transmitting low levels of UV-A between 320 and 360 nm, increasing slightly to full transmission at 400 nm. Spectral irradiances in −UV tents ranged approximately from 400 to 900 nm (Fig. 1). Transmitted radiation was measured in the early afternoon under cloudless sky with a spectroradiometer. Tents of both foil types were situated alternating in a distance of 3 m to each other, in order to avoid shading. Outside the tents radiation parameters (UV-A and UV-B-radiation, global radiation) were recorded by a meteorological station (Thies Clima, Göttingen, Germany) in 25 m distance from the tents. A maximum UV-A radiation of 61 W m^{-2}, UV-B radiation of 1.12 W m^{-2} and global radiation of 815 W m^{-2} were recorded. Furthermore, in both types of foil-tents, humidity and temperature were measured (Tiny Tag Ultra, Gemini Data Loggers, UK) over the whole exposure period.

4.3. Physical analysis of plant material

The UV-A screening of the leaf epidermis was measured with a UV-A-PAM chlorophyll fluorometer (Gademann Meßinstrumente, Würzburg, Germany). By use of light-emitting diodes, a quasi-simultaneous excitation of chlorophyll fluorescence at 375 nm, F(UV-A), and 470 nm (blue-green light), F(BL), is achieved. The resulting UV shield (%) is calculated from $100 \times [1 - F(\text{UV-A})/F(\text{BL})]$ and gives a measure for the content of screening pigments, mainly phenolic compounds like flavonoids, and hydroxycinnamic acids (Bilger et al., 1997). Maximum differences in epidermal UV shields between individual plants reflect highest differences in pigment accumulation and were taken as indicators for additional differences in the chemical composition of differently treated plants. UV shields of adaxial leaf sides of youngest leaves of six individual plant species were measured per species daily over four days of exposure at 10 o'clock in the morning in a preceding experiment. Per type of foil-tent three plant species were investigated in each of both tents, but no differences in UV shields were detectable between these tents. Therefore, data were pooled per type of foil-tent. The optimum exposure time was determined and used for UV treatment of plants for the main experiment.

4.4. Chemical analyses

For chemical analyses, leaf samples were taken from the second youngest and the oldest leaf of each plant individual exposed in the tents for 48 h, and frozen in liquid nitrogen

right after harvest. Plants exposed in both tents provided with the same type of foil were haphazardly taken for leaf samples; per plant individual only one leaf sample was used for each analysis. Number of replicates varied between 4 and 7. All samples were stored at −80 °C until analysis. Leaf samples for analyses of glucosinolate concentration and myrosinase activity were collected from corresponding leaf halves of longitudinally sectioned leaves, respectively, in *S. alba*. As far as leaf size allowed, further samples for analysis of flavonoid concentration and C/N content were also taken from the same leaf. Otherwise, and in *N. officinale*, these were taken from additional plant individuals of the same treatment. Since *N. officinale* leaves are of small size, three or four leaves of identical age were pooled and longitudinally sectioned, in order to ensure two chemically identical samples for analyses of glucosinolates and myrosinases, respectively. Glucose concentration and soluble protein contents were analysed from samples taken for myrosinase analysis.

Water content. Leaf samples were taken from freshly collected leaf material, weighed and lyophilised. Dry weights were measured, and weight differences of fresh and dry samples were taken as water content (in percent).

Carbon and nitrogen content. Carbon and nitrogen content of lyophilised leaf samples were determined by quantitative decomposition of substances via oxidative combustion (CHN-O-Rapid, Elementar, Hanau, Germany).

Soluble protein content. Frozen leaf discs were ground and extracted three times with buffer (200 mM Tris, 10 mM EDTA, pH 5.5). The protein contents of the resulting extracts were determined with Bradford reagent (Sigma, Taufkirchen, Germany) by using bovine serum albumin (1.4 mg ml^{-1} in extraction buffer) as a standard. The absorbance was measured with a photometer (Multiskan EX, Thermo Labsystems, Vantaa, Finland) at 595 nm. Obtained absorption values were used for calculation of soluble protein contents using a cubic polynomial standard curve.

Flavonoid classification and quantification. The frozen leaf material was lyophilised, weighed, ground, and extracted in aqueous 80% MeOH with catechin (Phytoplan, Heidelberg, Germany) as internal standard. In order to remove chlorophyll, petrolether (Fluka, Taufkirchen, Germany) was added to the extracts and the resulting upper phases were discarded. These purified extracts were analysed by HPLC (1100 Series, Hewlett-Packard, Waldbronn, Germany) with a quaternary pump and a 1040M diode array detector. Gradient separation of flavonoids was achieved on a Supelco C-18 column (Supelcosil LC-18, 250 × 4.6 mm, 5 μm, Supelco) with an eluent gradient (solvent A: 0.1% formic acid in aqua bidest, solvent B: acetonitril, Rotisol >99.9%, Roth, Karlsruhe, Germany) of 8–15% B (5 min), 15–22% B (15 min), 22% B (5 min hold), 22–27% B (5 min), 27–40% B (5 min), followed by a cleaning cycle (40–100% B in 5 min, 5 min hold, 100–8% B in 1 min, 10 min hold). Glycosylated flavonols and hydroxycinnamic acids were classified by comparison of retention times and UV spectra to those of purified standards. Compounds were quantified by calculating the peak area at 254 nm (bandwidth 4 nm) relative to the area of the internal standard peak. In order to determine response factors of flavonol compounds and hydroxycinnamic acids to the internal standard catechin, known concentrations of the internal standard and quercetin-3-*O*-glucoside (Phytoplan, Heidelberg, Germany), kaempferol-7-*O*-neohesperidoside (Extrasynthèse, Genay, France), and ferulic acid (Fluka, Taufkirchen, Germany), respectively, were repeatedly injected. Relative ratios of integrated peak areas were calculated and used as response factors for quantification of quercetin glycosides (response factor 43), kaempferol glycosides (36.9), other unidentified glycosylated flavonols (40, estimated) and hydroxycinnamic acids (13).

Glucosinolate identification and quantification. The frozen leaf material was lyophilised, weighed, ground, and extracted in aqueous 80% MeOH with 2-propenyl glucosinolate (⩾99%, Fluka, Taufkirchen, Germany) as internal standard. Glucosinolates were converted to desulfoglucosinolates using purified sulfatase [E.C. 3.1.6.1, 'type H-1, from *Helix pomatia*, 15,100 units (gram solid)$^{-1}$; Sigma, Taufkirchen, Germany] (Graser et al., 2001). The resulting desulfoglucosinolates were analysed by HPLC and identified by comparison of retention times and UV spectra to those of purified standards, which were further confirmed by LC-ESI-MS as described earlier (Reifenrath et al., 2005). Desulfoglucosinolates were quantified by calculating the peak area at 229 nm (bandwidth 4 nm) relative to the area of the internal standard peak, corrected by the response factors as in Brown et al. (2003). A few aromatic glucosinolates of *S. alba* could not be further identified. For these, a response factor of 0.5 was assumed. For another glucosinolate of *S. alba* with an aliphatic sulfonyl side chain at 24 min retention time, a response factor of 1 was taken.

Myrosinase activity and glucose concentration. The activity of soluble and insoluble myrosinases was determined by photometric quantification of released glucose from externally added substrate. For *S. alba*, 4-hydroxybenzyl glucosinolate (obtained from glucosinolates.com, Denmark) and for *N. officinale*, 2-phenylethyl glucosinolate (Phytoplan, Germany) were used as substrates, which are the most dominant glucosinolates in both species, respectively. The extraction and determination of myrosinase activity followed slightly modified protocols of Müller and Martens (2005) and Martin and Müller (2007). Frozen leaf samples were extracted three times in 500 μl extraction buffer (200 mM Tris, 10 mM EDTA, pH 5.5) on ice and supernatants applied to Sephadex A25-columns (Sigma, Taufkirchen, Germany) to remove internal glucosinolates. Extracts were eluted with further 500 μl buffer. One hundred and fifty microliters of each extract were added to four individual cells on a 96-cell microplate with 25 μl of 1.9 M glucosinolate/phosphate buffer in two cells as substrate. The other two cells served as background controls and 25 μl phosphate buffer were added instead of glucosinolate. Released glucose was measured by addition of 50 μl freshly mixed colour reagent, including glucose oxidase,

peroxidase, 4-aminoantipyrine and phenol (Siemens and Mitchell-Olds, 1998) and determined by measuring the absorbance at 490 nm on a photometer for 90 min at room temperature. Means of the two replicate measurements were calculated after subtraction of the means of the background controls. A glucose standard curve was included for each assay. Maximum rates of colour production were observed between 5 and 15 min. Glucose concentrations were calculated by relating sample maximum rates to those of the standard curve (Martin and Müller, 2007). The resulting enzyme kinetics of myrosinases were analysed for a linear range of enzyme activity (usually between 30 and 70 min). Myrosinase activities were quantified as nmol glucose released per minute and mg fresh weight. To detect activity of insoluble myrosinases in the remaining pellets, these were dissolved in 500 µl buffer, and measured in the same way as the supernatants, with two background controls per sample. Under our assay conditions, rates of colouration were not affected by ascorbate (data not shown).

4.5. Statistical analyses

Multifactorial ANOVA were used to determine the effects of UV exposure, leaf-age and the interaction term UV treatment × leaf-age (Table 2). Data were square root-, arcsin- or log-transformed to achieve homogeneity of variances. For further analyses Student's *t*-tests for independent variables were used instead of post hoc tests following the ANOVA, since only comparisons of chemical composition within leaf-age classes due to UV exposure and within UV treatments due to leaf-age classes, respectively, were of interest (Fig. 2). In case of heterogeneity of variances, Welch correction was used, and two-tailed *P*-values are given. Data were analysed with Statistica 7 (StatSoft, Tulsa, USA).

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References

Agerbirk, N., Olsen, C.E., Nielsen, J.K., 2001. Seasonal variation in leaf glucosinolates and insect resistance in two types of *Barbarea vulgaris* ssp. *arcuata*. Phytochemistry 58, 91–100.

Andréasson, E., Wretblad, S., Granér, G., Wu, X., Zhang, J., Dixelius, C., Meijer, J., 2001. The myrosinase-glucosinolate system in the interaction between *Leptosphaeria maculans* and *Brassica napus*. Mol. Plant Pathol. 2, 281–286.

Bassman, J.H., 2004. Ecosystem consequences of enhanced solar ultraviolet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. Photochem. Photobiol. 79, 382–398.

Bilger, W., Veit, M., Schreiber, L., Schreiber, U., 1997. Measurements of leaf epidermal transmittance of UV radiation by chlorophyll fluorescence. Physiol. Plant. 101, 754–763.

Bjerke, J.W., Gwynn-Jones, D., Callaghan, T.V., 2005. Effects of enhanced UV-B radiation in the field on the concentration of phenolics and chlorophyll fluorescence in two boreal and arctic-alpine lichens. Environ. Exp. Bot. 53, 139–149.

Björn, L.O., Callaghan, T.V., Johnson, I., Lee, J.A., Manetas, Y., Paul, N.D., Sonesson, M., Wellburn, A.R., Coop, D., Heide-Jørgensen, H.S., Gehrke, C., Gwynn-Jones, D., Johanson, U., Kyparissis, A., Levizou, E., Nikolopoulos, D., Petropoulou, Y., Stephanou, M., 1997. The effects of UV-B radiation on European heathland species. Plant Ecol. 128, 253–264.

Brown, P.D., Tokuhisa, J.G., Reichelt, M., Gershenzon, J., 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. Phytochemistry 62, 471–481.

Caldwell, M.M., Robberecht, R., Flint, S.D., 1983. Internal filters: prospects for UV-acclimation in higher plants. Physiol. Plant. 58, 445–450.

Caputo, C., Rutitzky, M., Ballaré, C.L., 2006. Solar ultraviolet-B radiation alters the attractiveness of *Arabidopsis* plants to diamondback moths (*Plutella xylostella* L.): impacts on oviposition and involvement of the jasmonic acid pathway. Oecologia 149, 81–90.

Collinge, S.K., Louda, S.M., 1988. Herbivory by leaf miners in response to experimental shading of a native crucifer. Oecologia 75, 559–566.

Cornell, H.V., Hawkins, B.A., 2003. Herbivore responses to plant secondary compounds: a test of phytochemical coevolution theory. Amer. Nat. 161, 507–522.

Doughty, K.J., Kiddle, G.A., Pye, B.J., Wallsgrove, R.M., Pickett, J.A., 1995. Selective induction of glucosinolates in oilseed rape leaves by methyl jasmonate. Phytochemistry 38, 347–350.

Eriksson, S., Ek, B., Xue, J., Rask, L., Meijer, J., 2001. Identification and characterization of soluble and insoluble myrosinase isoenzymes in different organs of *Sinapis alba*. Physiol. Plant. 111, 353–364.

Feeny, P., 1976. Plant apparency and chemical defence. In: Wallace, J.W., Mansell, R.L. (Eds.), Biochemical Interaction between Plants and Insects. Plenum Press, New York, pp. 1–41.

Feng, H., An, L., Chen, T., Qiang, W., Xu, S., Zhang, M., Wang, X., Cheng, G., 2003. The effect of enhanced ultraviolet-B radiation on growth, photosynthesis and stable isotope composition (δ ^{13}C) of two soybean cultivars (*Glycine max*) under field conditions. Environ. Exp. Bot. 49, 1–8.

Graser, G., Oldham, N.J., Brown, P.D., Temp, U., Gershenzon, J., 2001. The biosynthesis of benzoic acid glucosinolate esters in *Arabidopsis thaliana*. Phytochemistry 57, 23–32.

Halkier, B.A., Gershenzon, J., 2006. Biology and biochemistry of glucosinolates. Ann. Rev. Plant Biol. 57, 303–333.

Harborne, J.B., Williams, C.A., 2000. Advances in flavonoid research since 1992. Phytochemistry 55, 481–504.

He, J., Huang, L.-K., Chow, W.S., Whitecross, M.I., Anderson, J.M., 1993. Effects of supplementary ultraviolet-B radiation on rice and pea plants. Aust. J. Plant Physiol. 20, 129–142.

Iwasa, Y., Kubo, T., van Dam, N., de Jong, T., 1996. Optimal level of chemical defence decreasing with leaf age. Theor. Popul. Biol. 50, 124–148.

Keiller, D.R., Mackerness, S.A.-H., Holmes, M.G., 2003. The action of a range of supplementary ultraviolet (UV) wavelengths on photosynthesis in *Brassica napus* L. in the natural environment: effects on PS II,

CO_2 assimilation and level of chloroplast proteins. Photosynth. Res. 75, 139–150.

Kolb, C.A., Käser, M.A., Kopecký, J., Zotz, G., Riederer, M., Pfündel, E., 2001. Effects of natural intensities of visible and ultraviolet radiation on epidermal ultraviolet screening and photosynthesis in grape leaves. Plant Physiol. 127, 863–875.

Kubasek, W.I., Shirley, B.W., McKilop, A., Goodman, H.M., Briggs, W., Ausubel, F.M., 1992. Regulation of flavonoid biosynthetic genes in germinating *Arabidopsis* seedlings. Plant Cell 4, 1229–1236.

Kursar, T.A., Coley, P.D., 2003. Convergence in defense syndromes of young leaves in tropical rainforests. Biochem. Syst. Ecol. 31, 929–949.

Lambdon, P.W., Hassall, M., Boar, R.R., Mithen, R., 2003. Asynchrony in the nitrogen and glucosinolate leaf-age profiles of *Brassica*: is this a defensive strategy against generalist herbivores? Agric. Ecosyst. Environ. 97, 205–214.

Lazzeri, L., Curto, G., Leoni, O., Dallavalle, E., 2004. Effects of glucosinolates and their enzymatic hydrolysis products via myrosinase on the root-knot nematode *Meloidogyne incognita* (Kofoid et White) Chitw. J. Agric. Food Chem. 52, 6703–6707.

Levin, D.A., 1976. Chemical defenses of plants to pathogens and herbivores. Ann. Rev. Ecol. Syst. 7, 121–159.

Lindroth, R.L., Hofmann, R.W., Campbell, B.D., McNabb, W.C., Hunt, D.Y., 2000. Population differences in *Trifolium repens* L. response to ultraviolet-B radiation: foliar chemistry. Oecologia 122, 20–28.

Liu, L., Gitz, D.C., McClure, J.W., 1995. Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. Physiol. Plant 93, 725–733.

Louda, S., Mole, S., 1991. Glucosinolates: chemistry and ecology. In: Rosenthal, G.A., Berenbaum, M. (Eds.), Herbivores: their Interactions with Secondary Plant Metabolites. Academic Press, San Diego, pp. 123–164.

Mackerness, S.A.-H., 2000. Plant responses to ultraviolet-B (UV-B: 280–320 nm) stress: what are the key regulators? Plant Growth Regul. 32, 27–39.

Mackerness, S.A.-H., Surplus, S.L., Blake, P., John, C., Buchanan-Wollaston, V., Jordan, B.R., Thomas, B., 1999. Ultraviolet-B-induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signalling pathways controlled by jasmonic acid, ethylene and reactive oxygene species. Plant Cell Environ. 22, 1413–1423.

Markham, K.R., Tanner, G.J., Caasi-Lit, M., Whitecross, M.I., Nayudu, M., Mitchell, K.A., 1998. Possible protective role for 3′,4′-dihydroxyflavones induced by enhanced UV-B in a UV-tolerant rice cultivar. Phytochemistry 49, 1913–1919.

Martin, N., Müller, C., 2007. Induction of plant responses by a sequestering insect: relationship of glucosinolate concentration and myrosinase activity. Basic Appl. Ecol. 8, 13–25.

McCloud, E.S., Berenbaum, M.R., 1999. Effects of enhanced UV-B radiation on a weedy forb (*Plantago lanceolata*) and its interactions with a generalist and specialist herbivore. Entomol. Exp. Appl. 93, 233–247.

McNeill, S., Southwood, T.R.E., 1978. The role of nitrogen in the development of insect/plant relationships. In: Harborne, J.B. (Ed.), Biochemical Aspects of Plant and Animal Coevolution. Academic Press, London, pp. 77–98.

Mewis, I., Appel, H.M., Hom, A., Raina, R., Schultz, J.C., 2005. Major signaling pathways modulate *Arabidopsis* glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol. 138, 1149–1162.

Müller, C., Martens, N., 2005. Testing predictions of the 'evolution of increased competitive ability hypothesis' for an invasive crucifer. Evol. Ecol. 19, 533–550.

Müller, C., Sieling, N., 2006. Effects of glucosinolate and myrosinase levels in *Brassica juncea* on a glucosinolate-sequestering herbivore – and vice versa. Chemoecology 16, 191–201.

Murali, N.S., Teramura, A.H., 1985. Effects of ultraviolet-B irradiance on soybean VII. Biomass and concentration and uptake of nutrients at varying P supply. J. Plant Nutr. 8, 177–192.

Olsson, L.C., Veit, M., Weissenböck, G., Bornman, J.F., 1998. Differential flavonoid response to enhanced UV-B radiation in *Brassica napus*. Phytochemistry 49, 1021–1028.

Paul, N.D., Rasanayagam, S., Moody, S.A., Hatcher, P.E., Ayres, P.G., 1997. The role of interactions between trophic levels in determining the effect of UV-B on terrestrial ecosystems. Plant Ecol. 128, 297–308.

Pontoppidan, B., Hopkins, R., Rask, L., Meijer, J., 2003. Infestion by cabbage aphid (*Brevicoryne brassicae*) on oilseed rape (*Brassica napus*) causes a long lasting induction of the myrosinase system. Entomol. Exp. Appl. 109, 55–62.

Rask, L., Andréasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B., Meijer, J., 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol. Biol. 42, 93–113.

Raupp, M.J., Denno, R.F., 1983. Leaf age as predictor of herbivore distribution and abundance. In: Denno, R.F., McClure, M.S. (Eds.), Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York, pp. 91–124.

Reifenrath, K., Müller, C., Riederer, M., 2005. Leaf surface wax layers of Brassicaceae lack feeding stimulants for *Phaedon cochleariae*. Entomol. Exp. Appl. 115, 41–50.

Rinnan, R., Impiö, M., Silvola, J., Holopainen, T., Martikainen, P.J., 2003. Carbon dioxide and methane fluxes in boreal peatland microcosms with different vegetation cover – effects of ozone or ultraviolet-B exposure. Oecologia 137, 475–483.

Siemens, D.H., Mitchell-Olds, T., 1996. Glucosinolates and herbivory by specialists (Coleopterea: Chrysomelidae, Lepidoptera: Plutellidae): consequences of concentration and induced resistance. Environ. Entomol. 25, 1344–1353.

Siemens, D.H., Mitchell-Olds, T., 1998. Evolution of pest-induced defences in *Brassica* plants: tests of theory. Ecology 79, 632–646.

Teramura, A.H., Caldwell, M.M., 1981. Effects of ultraviolet-B irradiances on soybean. IV. Leaf ontogeny as a factor in evaluating ultraviolet-B irradiances effects on net photosynthesis. Am. J. Bot. 68, 934–941.

Turunen, M., Heller, W., Stich, S., Sandermann, H., Sutinen, M.-L., Norokorpi, Y., 1999. The effects of UV exclusion on the soluble phenolics of young Scots pine seedlings in the subarctic. Environ. Pollut. 106, 219–228.

van Dam, N.M., Witjes, L., Svatoš, A., 2004. Interaction between aboveground and belowground induction of glucosinolates in two wild *Brassica* species. New Phytol. 161, 801–810.

Wait, D.A., Coleman, J.S., Jones, C.G., 2002. *Chrysomela scripta*, *Plagiodera versicolora* (Coleoptera: Chrysomelidae), and *Trichoplusia ni* (Lepidoptera: Noctuidae) track specific leaf developmental stages. Popul. Ecol. 31, 836–843.

Warren, J.M., Bassman, J.H., Eigenbrode, S., 2002. Leaf chemical changes induced in *Populus trichocarpa* by enhanced UV-B radiation and concomitant effects on herbivory by *Chrysomela scripta* (Coleoptera: Chrysomelidae). Tree Physiol. 22, 1137–1146.

Warren, J.M., Bassman, J.H., Fellman, J.K., Mattinson, D.S., Eigenbrode, S., 2003. Ultraviolet-B radiation alters phenolic salicylate and flavonoid composition of *Populus trichocarpa* leaves. Tree Physiol. 23, 527–535.