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Leaf waxes of slow-growing alpine and fast-growing lowland *Poa* species: inherent differences and responses to UV-B radiation

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Abstract

We investigated whether alpine and lowland *Poa* species exhibit inherent differences in leaf cuticular waxes, leaf UV absorbing compounds and/or growth responses to UV-B treatment. All plants were grown hydroponically in a growth cabinet (constant 20°; 14 hr photoperiod; 520 μmol photons $m^{-2} s^{-1}$ PAR). Two alpine (*P. fawcettiae* and *P. costiniana*), one sub-alpine (*P. alpina*) and three temperate lowland species (*P. pratensis*, *P. compressa* and *P. trivialis*) were grown under conditions without UV radiation for 36 days. In a subsequent experiment, four *Poa* species (*P. costiniana, P. alpina, P. compressa* and *P. trivialis)* were also exposed for 21 days to UV-B/(UV-A) radiation ('UV-B treatment') that resulted in daily UV-B radiation of 7.5 kJ m^{-2} day^{-1}, with control plants being grown without UV-B ('UV-A control treatment'). All treatments were carried out in the same growth cabinet. There was no altitudinal trend regarding wax concentrations per unit leaf area, when the six species grown under UV-less conditions, were compared at similar developmental stage (20–30 g shoot fresh mass). However, large differences in cuticular wax chemical composition were observed between the alpine and lowland species grown under UV-less conditions. For example, a single primary alcohol was present in the waxes of the lowland and sub-alpine species ($C_{26}H_{53}OH$), but was virtually absent in the alpine species. Although alkanes were present in all six species (primarily $C_{29}H_{60}$ and $C_{31}H_{64}$), the proportion of total wax present as alkanes was highest in the alpine species. Aldehydes were only present in the waxes of the alpine species. Conversely, substantial amounts of triterpenoids were mainly present in the three lowland species (squalene and lupeol were the dominant forms). The proportion of total wax present as long-chain esters (LCE-s) was similar in all six species grown in the absence of UV radiation. Acetates were observed only in the wax of *P. trivialis*. Of the four species exposed to UV-B, only *P. costiniana* and *P. compressa* showed any differences in wax chemical composition (compared to UV-A control plants). In *P. costiniana*, the proportion of total waxes present as alkanes was substantially lower in the UV-B grown plants (replaced by an unknown compound with a high retention time value). UV-B grown *P. costiniana* and *P. compressa* both exhibited less wax per unit leaf area. In *P. compressa*, this resulted from a lower absolute amount of alcohol per unit leaf mass in the UV-B treatment. The concentrations of UV absorbing compounds in whole leaf extracts differed between the four investigated species. However, no systematic differences were apparent between the alpine, sub-alpine and lowland species. Exposure to UV-B radiation had no effect on the specific leaf area (ratio of leaf area to leaf dry mass). We conclude that the alpine and lowland *Poa* species have a cuticular wax composition that is inherently different.

Keywords: Poa; Gramineae; Absorption; Alpine; Cuticular wax; Growth analysis; Leaf wax; Lowland; Reflectance; Relative growth rate; Specific leaf area; UV-B absorbing compounds; UV-B radiation

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1. Introduction

Alpine plants exhibit inherently low relative growth rates (RGR) compared to lowland species (Körner & Pelaez Menendez-Riedl, 1989; Atkin & Day, 1990; Atkin, Botman & Lambers, 1996a,b). The low RGR of alpine *Poa* species is associated with leaf characteristics (e.g. increased thickness and leaf density) that reduce the specific leaf area (SLA, the ratio of leaf area to leaf mass) (Atkin et al., 1996b). Other alpine species also exhibit low SLA values, both in the field and under controlled-environment conditions (Atkin et al., 1996b; Woodward, 1979a,b, 1983; Körner, Neumayer, Menendez-Riedl & Smeets-Scheel, 1989; Vitousek, Field & Matson, 1990).

In addition to differences in SLA, alpine and lowland species may also exhibit inherent differences in the amount and/or type of leaf cuticular wax. Cuticular waxes form a barrier between the leaf and the surrounding environment. For example, cuticular waxes appear to protect some leaves from water loss due to high visible irradiances (Robinson, Lovelock & Osmond, 1993) or high UV-B radiation, via increased reflection and/or absorptive screening of the incoming radiation (Caldwell, 1981). The fact that alpine plants are typically exposed to higher irradiances including UV-B radiation than lowland plants, raises the possibility that alpine plants will exhibit inherently different leaf wax properties than lowland plants. There is evidence that some high-altitude plants have highly reflective waxy layers (Thomas & Barber, 1974). However, it is not known whether such differences are due to different amounts or the three dimensional structure of the cuticular waxes. It is also not known if alpine plants *per se* exhibit inherently different wax properties from their lowland counterparts.

The role of leaf waxes in ameliorating the effects of enhanced UV-B radiation has yet to be fully elucidated. Barnes, Paul, Percy, Broadbent, McLaughlin et al. (1994) hypothesised that leaf waxes may reduce UV-B sensitivity, as the susceptibility of pea plants to enhanced UV-B radiation is greatest in plants with low glaucousness. Glaucousness and the amount of leaf wax are positively correlated in genotypes of several species (Gonzalez, Paul, Percy, Ambrose, McLaughlin et al., 1996, and references therein). However, other reports have suggested that the effect of leaf waxes on UV-B tolerance (via reflectance and/or absorbance) is minor (e.g. Gonzalez et al., 1996; Day, Martin & Vogelmann, 1993). Nevertheless, exposure to UV-B radiation is known to alter the chemical composition of leaf cuticular waxes in several species (Gonzalez et al., 1996; Barnes, Percy, Paul, Jones, McLaughlin et al., 1996; Cen & Bornman, 1993): such alterations could, conceivably, alter the degree of UV-B reflectivity/absorption. The degree of tolerance to UV-B radiation in alpine plants is also likely to depend on other leaf characteristics, such as the levels of UV-B absorbing compounds (e.g. flavonoids).

Alpine plants are reported to possess constitutively higher concentrations of UV-B absorbing compounds than lowland species (Ziska, Teramura & Sullivan, 1992). Several alpine species that typically experience high UV-B levels (Caldwell, 1968; Caldwell, Teramura & Tevini, 1989; Tevini & Teramura, 1989) also appear to exhibit greater tolerance of UV-B radiation than lowland species (Ziska et al., 1992; Sullivan, Teramura & Ziska, 1992; Larson, Garrison & Carlson, 1990). However, the recent work of Van de Staaij (1994) suggests that not all alpine and lowland plant species differ in their sensitivity to UV-B radiation. Further work is therefore needed to assess whether alpine plants are indeed more tolerant of UV-B radiation, and if so, what the mechanism(s) of such tolerance is.

This study investigates whether slow-growing alpine and fast-growing lowland species differ in amount and composition of their constitutive cuticular waxes in the absence of UV radiation. To determine the effect of altitudinal origin on these parameters in congeneric plant material, we compared cuticular wax characteristics of two alpine, one sub-alpine and three lowland *Poa* species. Furthermore, we also assess whether four of the six *Poa* species differ in their responses to UV-B radiation with respect to wax or UV-absorbing compound concentration and leaf morphology. Our hypothesis was that the alpine species would exhibit inherently greater concentrations of wax per unit leaf area, and that the chemical composition of the alpine waxes would be inherently different from that of the lowland species. We also expected that UV-B radiation would result in an increase in the cuticular wax production in the alpine *Poa* species as compared with the lowland species.

2. Results

2.1. Cuticular wax characteristics under conditions without UV radiation

2.1.1. Developmental changes in leaf wax composition

To assess the appropriate developmental stage in which to compare the six *Poa* species grown under conditions without UV radiation (Table 1) we measured the developmental changes in leaf wax composition in two of the six *Poa* species: *P. alpina* and *P. compressa* 10, 21 and 36 days after commencement of growth (Fig. 1). The percentage of total waxes present as primary alcohols clearly decreased with age in both species (Fig. 1). This decrease was not due to a decrease in alcohol content *per se*, but rather to an increase in the absolute concentration of other com-

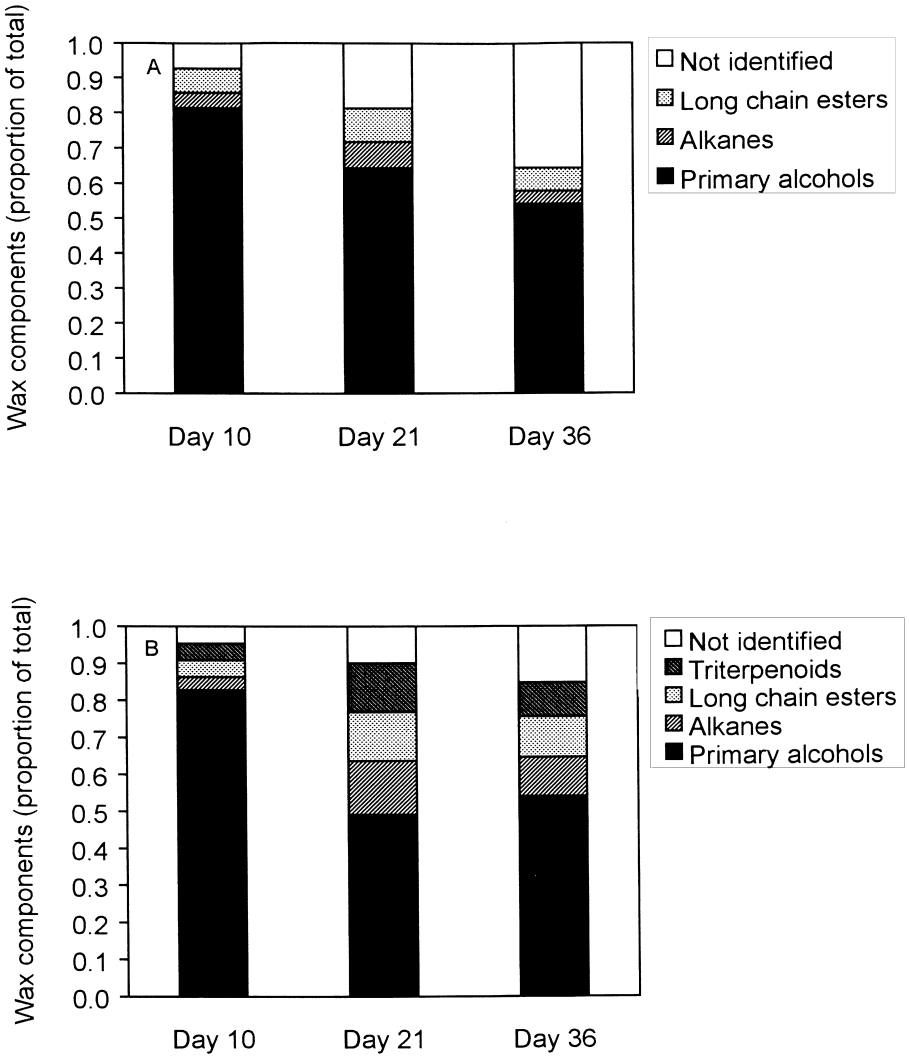

Fig. 1. Ontogenetic changes in wax chemical composition (expressed as a percentage of total wax content) in two *Poa* species (A, *Poa alpina* and B, *Poa compressa)* grown under UV-less conditions. Chemical composition on days 10, 21 and 36 after commencement of the growth period are shown.

pounds, such as alkanes, long-chain esters (LCE-s) and unidentified components (Fig. 1). Given that there were few differences in the composition of the cuticular waxes of plants sampled at 20 and 100 g shoot fresh mass, we decided to compare the cuticular wax composition of all six *Poa* species under conditions with and without UV, at the 20–30 g shoot fresh mass stage.

Table 1
Experimental treatments for the different *Poa* species (irradiance: 520 ± 24 μmol $m^{-2} s^{-1}$; UV-B_{BE}: 7.5 kJ m^{-2} day^{-1})

Poa species	Treatment	Lamps and filters	Type of radiation
P. costiniana, P. fawcettiae, P. alpina, P. pratensis, P. compressa, P. trivialis	No UV radiation	Philips HPI/T no UV-B tubes (no filter)	+ PAR no UV-B no UV-A
P. costiniana, P. alpina, P. compressa, P. trivialis	UV-A control	Philips HPI/T UV-B tubes (Philips TL 40W/12) (Mylar filter)	+ PAR no UV-B + UV-A
P. costiniana, P. alpina, P. compressa, P. trivialis	UV-B treatment	Philips HPI/T UV-B tubes (Philips TL 40W/12) (Cellulose diacetate filter)	+ PAR + UV-B + UV-A

Table 2
Comparison of wax properties (total amount and chemical composition) of leaf waxes in six altitudinally contrasting *Poa* species grown under UV-less conditions. Total wax concentration is expressed as concentration per unit leaf dry mass (DM) and leaf area basis. The wax chemical components are expressed as a percentage of the total wax concentration. RGR values as reported by Atkin et al. (1996b).

Species	Origin	RGR	Wax concentration		Alkanes	Primary alcohols	Acetates	Aldehydes	Triterpenoids	Long chain esters	Not identified
		($mg\ g^{-1}\ d^{-1}$)	($\mu g\ g^{-1}$ DM)	($mg\ m^{-2}$)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
P. costiniana	alpine	111	282.3	10.4	47.0	trace	—	27.2	0.8	10.0	15.1
P. fawcettiae	alpine	125	231.2	8.3	76.4	trace	—	1.4	—	6.5	15.7
P. alpina	sub-alpine	166	775.2	21.8	7.4	64.4	—	—	—	9.5	18.7
P. pratensis	lowland	179	425.9	13.6	55.6	18.1	—	1.3	8.1	8.3	8.5
P. compressa	lowland	188	987.6	28.5	14.3	49.3	—	0.2	13.3	13.2	9.8
P. trivialis	lowland	255	551.0	12.4	8.8	74.0	4.3	—	2.5	6.8	3.6

2.1.2. Chemical composition of leaf waxes in the six Poa species under conditions without UV radiation

Large differences in the chemical composition of the cuticular waxes were observed between the six *Poa* species grown without UV-B, when each species was sampled at the 20–30 g shoot fresh mass stage (Table 2). For example, primary alcohols were present in very low concentrations in the waxes of the two slow-growing alpine species (*P. costiniana* and *P. fawcettiae*). In contrast, a large percentage of total waxes was present as alcohols in the fastest growing lowland species, *P. trivialis*. The two other lowland species (*P. pratensis* and *P. compressa*) and the sub-alpine species (*P. alpina*) also exhibited a substantial percentage of primary alcohols in their cuticular waxes (Table 2).

The results in Fig. 2 demonstrate that there is an apparent negative correlation between the leaf cuticular primary alcohols and alkanes, when expressed as a percentage of total wax: the lowland species that contained the highest percentage of alcohols in their leaf cuticular waxes also exhibited the lowest percentage of alkanes. With the exception of *P. compressa*, all species contained similar proportions of LCE-s (percentage of total cuticular waxes; Table 2). Aldehydes were only present in the alpine species *P. costiniana*, whereas triterpenoids were mainly present in the lowland species (Table 2).

Closer inspection of the differences in chemical composition of the cuticular waxes in the six *Poa* species revealed that there was a single primary alcohol ($C_{26}H_{53}OH$; data not shown) in the four species that contained a substantial proportion of alcohols in their waxes. Although two alkanes ($C_{29}H_{60}$ and $C_{31}H_{64}$) dominate the alkanes in all species, several others were also observed. The dominant form of alkanes were the ones with an odd number of carbon atoms rather than the forms with an even number of carbons. Of the LCE-s found in all species, the dominant forms had a carbon-chain length of 42 and 44 (Fig. 3). Although the LCE-s with an even carbon-chain length were dominant in all chromatograms, trace amounts of LCE-s with uneven carbon-chain lengths were found

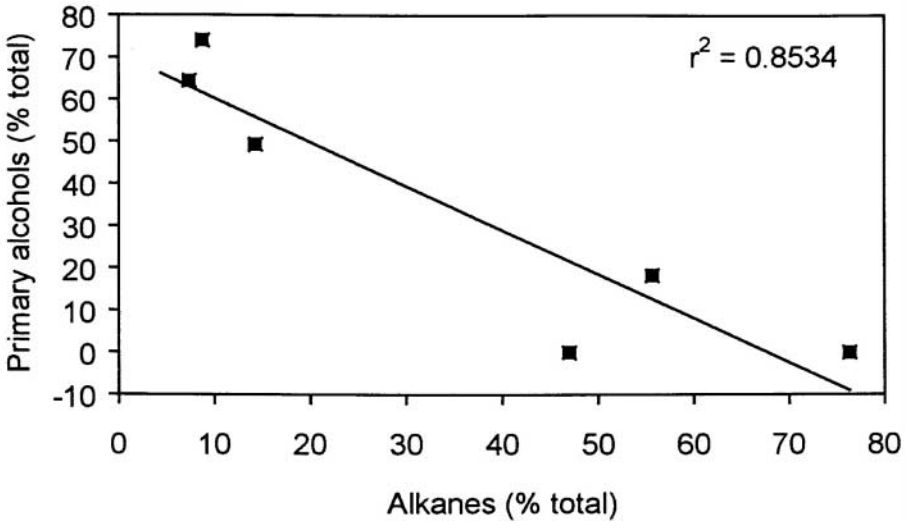

Fig. 2. Proportion of total waxes present as alkanes plotted against proportion present as primary alcohols in six *Poa* species grown under UV-less conditions. The different species can be identified from the chemical composition data shown in Table 2. The line indicates a significant regression (r^2: 0.8534, $p < 0.0085$, $n = 6$).

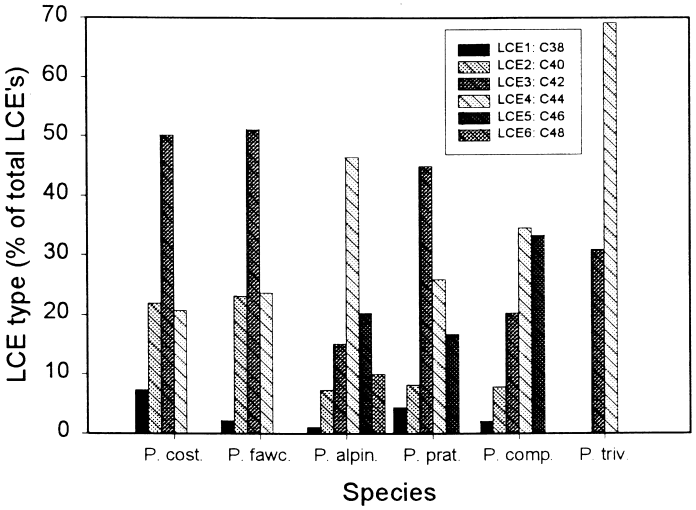

Fig. 3. Breakdown of long-chain esters (LCE-s) into the various types that differ in carbon-chain length (C_{38} to C_{48}) in six *Poa* species grown under UV-less conditions.

for nearly all the *Poa* species that were included in this study. A clear identification of the triterpenoids was only possible in the waxes of one of the three triterpenoid-containing species (*P. compressa*): squalene (38% of total triterpenoids) and lupeol (43% of total triterpenoids) being the dominant forms. Smaller amounts of β-amyrin (19% of total triterpenoids) were also present. The triterpenoids were made up of squalene and lupeol in all species, although the percentage of both compounds differed between the species (data not shown). Trace amounts of β-amyrin were identified in the waxes of *P. compressa* and *P. costiniana*. A single aldehyde was identified in *P. fawcettiae* ($C_{32}H_{64}O$). However, several aldehydes were present in the other alpine species, *P. costiniana* (38% $C_{26}H_{52}O$, 13% $C_{28}H_{56}O$, 28% $C_{30}H_{60}O$ and 21% $C_{32}H_{64}O$). Three types of acetates were identified in *P. trivialis* (the only species to contain acetates): $C_{26}H_{52}O_2$ (19% of total acetates), $C_{29}H_{58}O_2$ (32% of total acetates) and $C_{31}H_{60}O_2$ (49% of total acetates).

2.1.3. Amount of leaf waxes per unit leaf area in the six *Poa* species

Table 2 demonstrates that there was substantial variation in the total amount of leaf cuticular wax per unit leaf area between the species. While there were no clear systematic altitudinal differences between the species, the lowest concentration of leaf wax was found in the alpine species, *P. fawcettiae*. Moreover, the fastest growing lowland species, *P. trivialis*, also exhibited low concentrations of wax per unit leaf area. Clearly, the hypothesis that leaf wax concentrations would be greatest in the alpine species is not supported by our results.

Table 3
Effect of UV-B treatment and UV-A control treatment on the specific leaf area (SLA, ± s.e.) and total amount and chemical composition of cuticular leaf waxes in four *Poa* species. Wax properties are expressed as concentration per unit leaf dry mass (DM) basis and as a percentage of total basis (values in brackets)

Species	Origin	Treatment	SLA $m^2\,kg^{-1}$	Total wax concentration $\mu g\,g^{-1}$ DM	Alkanes $\mu g\,g^{-1}$ DM(%)	Primary alcohols $\mu g\,g^{-1}$ DM(%)	Acetates $\mu g\,g^{-1}$ DM(%)	Aldehydes $\mu g\,g^{-1}$ DM(%)	Triterpenoid $\mu g\,g^{-1}$ DM(%)	Long chain esters $\mu g\,g^{-1}$ DM(%)	Not identified $\mu g\,g^{-1}$ DM(%)
P. costiniana	alpine	UV-A control	26.6 ± 1.2	214	134 (63)	—	—	12 (6)	—	9 (4)	59 (27)
		UV-B treatment	28.4 ± 1.2	257	105 (41)	—	—	23 (9)	—	6 (2)	122 (48)
P. alpina	sub-alpine	UV-A control	32.8 ± 1.9	1281	34 (3)	1044 (81)	—	—	—	39 (3)	164 (13)
		UV-B treatment	31.4 ± 1.3	1497	32 (2)	1255 (84)	—	—	—	58 (4)	152 (10)
P. compressa	lowland	UV-A control	41.5 ± 1.0	1626	32 (2)	1467 (90)	—	—	55 (3)	17 (1)	55 (4)
		UV-B treatment	40.7 ± 1.5	952	45 (5)	654 (69)	—	—	86 (9)	85 (9)	82 (8)
P. trivialis	lowland	UV-A control	42.7 ± 3.3	883	78 (9)	626 (71)	44 (5)	—	21 (2)	82 (9)	32 (4)
		UV-B treatment	41.4 ± 3.3	794	115 (14)	555 (70)	38 (5)	—	8 (1)	36 (5)	42 (5)

2.2. Effects of UV treatment

2.2.1. Cuticular wax characteristics for the UV-treated Poa species

We measured the concentration and composition of the cuticular waxes in four *Poa* species exposed to UV-B/(UV-A) radiation ('UV-B treatment') and control plants, only irradiated with UV-A ('UV-A control treatment'). There was no general response observed, either in terms of changes in the total amount of wax per unit leaf area, or in the chemical composition of the waxes (Table 3).

For example, UV-B treatment had little effect on the chemical composition or total amount of wax in *P. trivialis* or *P. alpina*. In contrast, UV-B radiation did alter the chemical composition of cuticular waxes in *P. costiniana* and *P. compressa*. In the UV-B treatment, *P. costiniana* exhibited a decrease in the absolute amount and proportion of total leaf waxes present as alkanes, as compared to the UV-A control condition (UV-A control = 63% of total waxes; UV-B treatment = 41% of total waxes). Moreover, an unknown compound (23.7% of total waxes) with a high RT value (46.42 min.) (data not shown) did emerge. The UV-B treatment had little effect on the concentration of waxes per unit leaf mass or area in *P. costiniana*. In *P. compressa*, exposure to UV-B radiation resulted in a lower concentration of total wax per unit leaf area and mass (Table 3). This was due to a lower absolute amount of alcohols per unit leaf mass. The proportion of total waxes present as alcohols also decreased in *P. compressa* due to a maintenance or slight increase in the absolute amount of waxes present as alkanes, triterpenoids and LCE-s (Table 3).

2.2.2. UV absorbing compounds in leaf extracts and leaf morphological characteristics

The concentrations of UV absorbing compounds in the leaves of four of the six *Poa* species are shown in Fig. 4. Significant differences in leaf UV absorbing compounds were observed between the four species. However, there was no systematic trend with regard to altitudinal origin. Moreover, while UV treatment tended towards slightly higher concentrations of UV absorbing compounds in *P. trivialis* and *P. compressa*, the differences were only statistically significant for *P. compressa*.

Exposure to UV-B radiation also had no effect on SLA (Table 3) or its underlying components (leaf thickness and density; data not shown) in any of the four *Poa* species. Taken together, it appears that exposure to the applied UV-B radiation did not have a substantial impact on leaf characteristics of *Poa* species.

3. Discussion

The results of our study demonstrate that the chemical composition of leaf cuticular waxes is inherently different in slow-growing alpine and fast-growing lowland *Poa* species (Table 2). The true alpine species (*P. costiniana* and *P. fawcettiae*) exhibit cuticular waxes that are deficient in alcohols and high in alkanes, relative to their sub-alpine and lowland counterparts. The differences in leaf wax composition are maintained both under conditions with and without UV radiation (Table 3). To our knowledge, no other study has compared the wax properties of congeneric species from

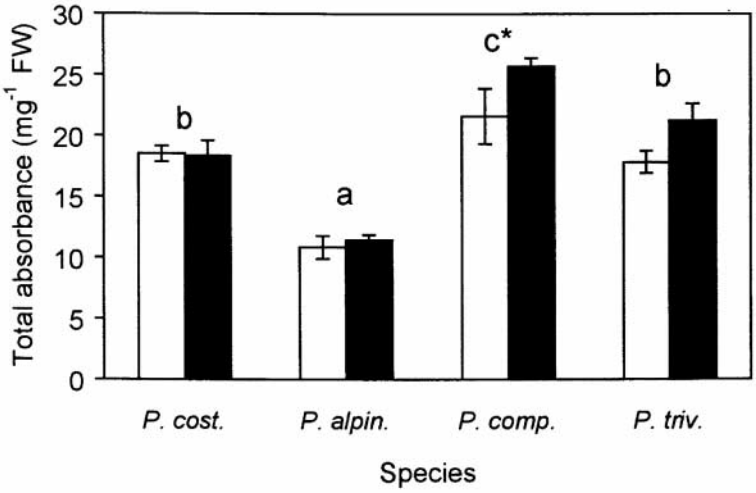

Fig. 4. Effect of UV-B treatment on concentration of total UV absorbing compounds in whole shoot extracts of four *Poa* species (*P. cost.*: *P. costiniana*, *P. alpi.*: *P. alpina*, *P. comp.*: *P. compressa*, *P. triv.*: *P. trivialis*). Shown are mean ± standard error. Open bars represent the UV-A control treatment, while the closed bars represent the UV-B treatment. Effect of species and treatment was tested in a two-way ANOVA. Significant differences between the species are indicated with a different letter ($p < 0.05$). Significant differences between the treatments are indicated with an * ($p < 0.05$).

contrasting environments under identical environmental conditions. While our results cannot be used to make generalised statements on the wax properties of alpine versus lowland plants, or slow- versus fast-growing plants, they do suggest that adaptation to contrasting environments may have resulted in contrasting wax properties.

Previous studies have also suggested that plants from contrasting environments have contrasting wax properties. For example, the proportion of leaf waxes present as alkanes is higher in Arctic plants exposed to high degrees of desiccation, low temperatures and low soil moisture than in Arctic plants growing in more sheltered areas (Rieley, Welker, Callaghan & Eglinton, 1995). Other species from harsh environments (e.g. dry, high-altitude areas) exhibit lower proportions of leaf wax alkanes than species characteristic of more favourable environments (e.g. Vioque, Pastor & Vioque, 1994). Productive clones of *Salix* sp. (presumably characteristic of more favourable environments) also exhibit higher proportions of alkanes in their waxes and greater amounts of leaf cuticular waxes than less productive clones (Hietala, Laakso & Rosenqvist, 1995). While the above studies are not all in agreement, they indicate that various environmental factors may, either combined or as single factors, influence the cuticular wax composition.

We had expected that the alpine *Poa* species would exhibit inherently higher concentrations of wax per unit leaf area. This hypothesis was based on the assumption that high leaf wax concentrations might assist in increasing reflection and/or absorptive screening of the incoming radiation (e.g. see Premachandra, Saneoka, Kanaya & Ogata, 1991 and because some high-altitude species exhibit highly reflective waxy layers in the field Thomas & Barber, 1974). High concentrations of cuticular waxes appear to protect some leaves from high irradiances (Robinson et al., 1993) including UV-B radiation (Caldwell, 1981). Moreover, the quantity of leaf wax increases with increasing environmental harshness (e.g. soil moisture, Hunt & Baker, 1982; altitude, DeLucia & Berlyn, 1983; Gunthardt & Wanner, 1982) in some species. Clearly, our results do not support the hypothesis that alpine species exhibit greater wax concentrations per unit leaf area. If anything, alpine *Poa* species possess less wax per unit leaf area, not more. Thus, if alpine species do possess an inherently more effective reflective/absorptive surface, then such a surface that is associated with leaf waxes must be derived from differences in wax chemical composition and structure, rather than the amount of wax *per se*. Under field conditions, however, the alpine *Poa* species might exhibit greater concentrations of wax per unit leaf area than the lowland species do.

We extracted cuticular waxes from intact whole shoots (i.e. leaves plus 'stems'), without distinguishing between the adaxial and abaxial leaf surfaces. Gonzalez et al. (1996) recently reported that wax concentrations per unit leaf area are higher on the abaxial surface of several pea lines differing in leaf surface wax. However, in their study, the ratio of adaxial to abaxial wax was not constant: this suggests that it is not possible to predict the concentrations of adaxial and abaxial wax in our six *Poa* species based on their results and our whole shoot extraction results. Information on the concentration of wax on both leaf surfaces would be useful in assessing whether alpine species really do not have higher concentrations of leaf wax, compared to lowland species.

Our results indicate that exposure to UV-B radiation does not increase leaf wax concentrations of whole shoot extracts in any of the four investigated species (Table 3). Gonzalez et al. (1996) reported that exposure to UV-B radiation increased the concentration of leaf wax on both the adaxial and abaxial leaf surfaces in four of their five pea lines. However, exposure to UV-B radiation decreased wax content on both surfaces in the pea line with the highest wax concentration. UV-B radiation also resulted in a decrease in wax concentration in *P. compressa* in our study (Table 3). Taken together, it seems clear that exposure to UV-B does not *necessarily* result in an increase in wax concentrations in all species.

Changes in plant morphological parameters (increased leaf angle, decreased SLA) could alter the impact of UV-B radiation on leaf cells. At the same time increased leaf angle and/or decreased SLA also negatively influence photosynthesis. In contrast to the significant increase in SLA, as demonstrated in *Phaseolus vulgaris* (Rozema, Van de Staaij & Tosserams, 1997), we did not see changes in SLA for the *Poa* species exposed to UV-B.

The fact that exposure to UV-B radiation did not result in a significant increase in UV absorbing compounds in any of the investigated *Poa* species (Fig. 4) could have been due to several factors. Firstly, it may be that there were constitutive levels of UV absorbing components that could not be increased as has also been found for several other species (Tosserams, Magendans & Rozema, 1997), or that our UV-B level was not high enough to stimulate the synthesis of such compounds. Alternatively, it may be that the UV-B treatment induced the synthesis of such compounds in the adaxial epidermal cells of old or young leaves only (e.g. Poorter, 1994). If this had occurred, then it is quite likely that we would not have been able to detect any changes in UV absorbing compounds of whole leaf extracts. We are therefore unable to conclusively state that UV-B treatment did not alter the concen-

tration of UV absorbing compounds in the selected *Poa* species.

UV-B treatment altered the chemical composition of *P. costiniana* and *P. compressa*, with the proportion of wax present as alkanes and alcohols being lower under UV-B radiation in *P. costiniana* and *P. compressa*, respectively (Table 3). However, we found no evidence that exposure to UV-B radiation results in a shortening of chain lengths, such as that reported for esters by Day *et al.* (Day, Howells & Ruhland, 1996, and papers cited therein). The functional relevance, if any, of the UV-induced changes in wax composition in *P. costiniana* and *P. compressa* remains unknown.

In conclusion, our results suggest that that the chemical composition of the cuticular waxes is inherently different in the alpine and lowland *Poa* species. There are clearly inter-specific differences in the response to UV-B radiation in terms of leaf wax content and chemical composition. However, there does not appear to be a generalized alpine or lowland species response to UV-B radiation. We also found no evidence that alpine species have higher constitutive or inducible levels of UV absorbing compounds.

4. Experimental

Six *Poa* species were chosen for investigation. These included three lowland (*P. pratensis* L., *P. compressa* L., *P. trivialis* L.) one sub-alpine (*P. alpina* L.) and two alpine species (*P. costiniana* J. Vickery, *P. fawcettiae* J. Vickery). All plants were grown hydroponically from seed under controlled-environment conditions (constant 20°C temperature; 14 h day photoperiod; 520 ± 24 μmol photons $m^{-2} s^{-1}$ (PAR, 600–700 nm), 70% relative humidity). Full details on the growth conditions and growth analysis procedures are given by Atkin et al. (1996a,b).

Leaf cuticular waxes were analysed for all six *Poa* species grown under conditions without UV radiation (see also Table 1). Four of the six *Poa* species (*P. costiniana, P. alpina, P. compressa* and *P. trivialis*) were also exposed to UV-B/(UV-A) radiation (referred to as 'UV-B treatment'), while the control plants were irradiated only with UV-A (referred to as 'UV-A control treatment'). In both treatments the plants were exposed to UV radiation for 4 h per day in the same growth cabinet used to grow the plants without UV radiation (see also Table 1). UV-B radiation was generated by Philips TL 40W/12 tubes suspended 70–90 cm above the hydroponically grown plants. As plants grew, the height of the lamps was adjusted in such a way that the desired UV-B radiation was kept constant. For the UV-B treatment, radiation transmitted by the TL tubes was filtered by using 0.1 mm cellulose diacetate foil (thickness: 0.10 mm thick, Tamboer & Co. Chemie B.V., Haarlem, The Netherlands), which absorbs radiation below 290 nm. For the UV-A control treatment, Mylar sheets (thickness: 0.13 mm; Dupont Industries, USA) were used, which absorb all radiation below 313 nm. Because of photodegradation, the cellulose diacetate sheets were replaced twice a week, while the Mylar foil was replaced weekly. Suspension of the UV lamps and filters above the plants had little effect on the amount of incident PAR radiation received by the plants. UV treatment resulted in a daily UV-B radiation of 7.5 kJ m^{-2} day^{-1} UV_{BE}, weighted according to the generalised plant action spectrum of Caldwell (1971), normalised to 300 nm. The spectral irradiance was measured with a portable UVX radiometer equipped with a UVX 31 sensor (San Gabriel, CA, USA), that was calibrated by using a double-monochromator spectroradiometer (Optronic Model OL 752, Orlando, FL, USA). Before the calibration, absolute responsivity of the double-monochromator spectroradiometer was checked against a 200 W tungsten-halogen standard lamp (Optronic Model OL 752-10, Orlando, FL, USA). Furthermore, the wavelength accuracy was checked by scanning a low-pressure mercury vapour lamp with a known peak emission at 312.9 nm.

The effect of UV-B radiation on RGR and its components was assessed comparing the growth response of plants exposed to UV-treated plants to those of the UV-A control plants.

To determine the specific leaf area (SLA: the ratio of leaf area to leaf dry mass) of the four species in the UV-B experiment, sampling began when individual plants had reached approximately 150 mg total fresh mass. The species were harvested 7–8 times over different periods (21–35 days, depending on their growth rate). On each harvest day, eight plants were randomly sampled and the fresh and dry mass (following freeze-drying; Virtus Unitop, Model 600 SL, Gardiner, NY, USA) of the leaves recorded. Leaf areas were determined using a LiCOR inc. 3100 leaf area meter (Lincoln, NE, USA). Correction factors (established using transverse sections) were used to establish the true leaf area of the two alpine species as both exhibited cylindrical leaves (Atkin et al., 1996b). Per species and per treatment the mean value of the SLA was calculated for the entire growth period.

4.1. Extraction and analysis of UV absorbing compounds

For each species and treatment, the first mature leaf of six individual plants was collected at day 21 (after transferring the seedlings to hydroponic solution). For the analysis of UV absorbing compounds, approximately 15 mg fresh mass was heated (90°C) for 1 hour in acidified methanol (methanol:water:hydrochloric

acid; 79:20:1). Subsequently the UV absorbing capacity was determined spectrophotometrically (Hitachi U-2000, double beam spectrophotometer) by scanning the extract between 280–320 nm. The UV absorbing capacity of the leaves was expressed as the total absorbance per nm and per mg FW in the scanned wavelength range.

4.2. Extraction and analysis of cuticular waxes:

4.2.1. Plant material

The ontogenetic changes in wax properties were studied in a lowland (*Poa compressa*) and a sub-alpine (*Poa alpina*) grass species. Three times during growth (day 10, day 21 and day 36 after transferring the seedlings to hydroponic solution) one to three complete shoots were analysed for wax content and composition. In a second experiment the inherent differences between the cuticular waxes of six *Poa* species (*P. pratensis, P. compressa, P. trivialis, P. alpina, P. costiniana, P. fawcettiae*) were studied in the absence of UV radiation. In a third experiment the effect of UV-B radiation on the wax content and composition of four *Poa* species (*P. costiniana*, *P. alpina*, *P. compressa*, *P. trivialis*) was studied. For each species a complete and mature shoot was used at day 21 after transferring the seedlings to the hydroponic solution. All plant material was grown in a climate chamber under the conditions as described earlier.

4.2.2. Wax extraction

Complete shoots were immersed in a 100–300 ml chloroform/methanol (2:1) for 1 min. During the extraction the shoots were slightly moved around in the organic solutes to optimise the physical contact between the plant tissue and the extraction mixture. As an internal standard 100 μl of a 5 α-cholestane solution (1 mg/ml diethylether [DEE]) was added to the extract. To remove small tissue particles, the extract was filtered over Whatman filter paper and dried in a conical flask, using a rotation evaporator. Subsequently, the extract was transferred quantitatively with diethylether to a small vial with a known weight. After drying the DEE-extract with a stream of nitrogen gas in a heating block at 40°, the total weight of the cuticular waxes was determined by using an analytical balance. Finally the waxes were dissolved in 250 μl DEE and stored in a refrigerator at 5° until further analysis.

4.2.3. GC-analysis

To separate the various components present in the cuticular waxes of the *Poa* species a Hewlett Packard 5890 gas chromatograph was used. The injector and the FID splitness detector were connected by a Chrompack fused silica capillary column: CP-SIL 5CB, 25 m, 0.32 mm i.d., film thickness: 0.12 μm. The GC was performed at the following conditions: initial temperature was 250° for 45 min, followed by a temperature increase to 300° (at 50°/min), with the end temperature maintained for 100 min, injector temperature: 280°, FID temperature: 280°. Carrier gas and flow: N_2 at 6 psi column head pressure, make-up gas flow rate: 30 ml/min. Injection volume: 1–3 μl DEE-extract, 2 μl PE. Split ratio: 1:10. The separated wax components were identified by comparing retention times and performing GCMS under the following conditions: GCMS: Hewlett Packard 5992B with a CP-SIL 5CB column, 25 m, 0.32 mm i.d., film thickness: 0.12 μm. Ionisation took place at 70 eV. Temp. prog.: initial temp. at 250° for 2 min, followed by a temperature increase to 280° at 4°C/min, end temperature: 280° for 32 min. Carrier gas and flow: N_2 at 6 psi. Injector and transfer line: 280°.

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