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EPICUTICULAR WAXES OF TWO ARCTIC SPECIES: COMPOSITIONAL DIFFERENCES IN RELATION TO WINTER SNOW COVER

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Key Word Index—*Dryas octopetala*; Rosacea; *Saxifraga oppositifolia*; Saxifragacea; Arctic plant ecology; climate change; epicuticular wax; Svalbard.

Abstract—The leaf wax characteristics of *Dryas octopetala* and *Saxifraga oppositifolia*, collected from the high Arctic semi-desert of Svalbard, Norway (79° N, 13° E), were compared and differences in their wax composition related to winter snow cover. The leaf wax composition of the winter-green *D. octopetala* differed from that of the herbaceous *S. oppositifolia* in that high abundances of the triterpenoids, ursolic acid, oleanoic acid and uvaol, were observed in *D. octopetala* extracts but not in *S. oppositifolia* extracts. *D. octopetala* leaf waxes were consistently lower in *n*-alkanes and in *n*-alkanols compared to the leaf waxes of *S. oppositifolia*. Leaf waxes of both species from snow-free, wind-swept microsites had significantly higher abundances of *n*-alkanes than in those plants growing in adjacent, swale areas where snow accumulates in winter. It is hypothesized that this higher abundance of *n*-alkanes may be due to a response to a greater degree of dessication, lower temperatures and lower soil moisture experienced by plants on the snow-free ridge microsites during leaf expansion. In order to test whether these biochemical and anatomical attributes might change in response to short term alterations in winter climate, snow fences were erected on ridge sites. The wax attributes of ridge plants exposed to a single year of increased winter snow cover were examined and the *n*-alkane composition of leaf waxes were observed to be more like those of plants growing in adjacent swale areas than for those of ridge plants growing in unmanipulated areas. This shift in leaf wax composition implies that environmental differences during leaf development can have an influence on final leaf wax composition.

INTRODUCTION

The climate of Arctic regions is predicted to be strongly influenced by elevated trace gases and associated changes in temperature, moisture and cloud cover [1]. However, whether these changes will be manifested during winter, summer or all seasons is unclear [2]. Earlier studies have indicated that increases in summer temperature and/or moisture will have significant consequences for plant performance [3–6]. The repercussions of changes in winter precipitation (snowfall) are less well known, though snowpack variation appears to significantly alter the structure and function of tundra ecosystems [5, 7, 8].

In the high Arctic, snow blown across the landscape by strong winds accumulates in small depressions or swales, while adjacent ridges or raised beaches are blown free of snow [9–11] (Fig. 1). Consequently, a very heterogeneous environment exists in Arctic settings during winter with uneven snow distribution and large variations in summer and winter micro-environmental conditions [11]. In winter, snow-free areas experience the coldest temperatures and encounter the largest variations in temperature because the insulating properties of snow are absent or very low [12]. Such variations in winter snow cover subsequently affect growing season length and soil moisture in the different microsites, with shorter growing seasons and wetter soils in swale sites relative to the ridge sites.

Arctic plant communities are composed of numerous species and multiple life forms, ranging from the prostrate growing evergreen species, *Dryas octopetala*, to cushion and spreading herbaceous plants, like *Saxifraga oppositifolia* [9, 13]. This diversity of life forms suggests that an array of growth strategies exists for coping with the harsh conditions of winter and summer in these polar settings [14]. Indeed, individualistic properties of growth and physiology have been shown to be important in modifying whole system responses to annual variation in climate [3] and to adjusting to seasonal changes in soil and atmospheric drought [15]. However, less is known about the similarities or differences in the biochemical and anatomical attributes of Arctic plant species, espe-

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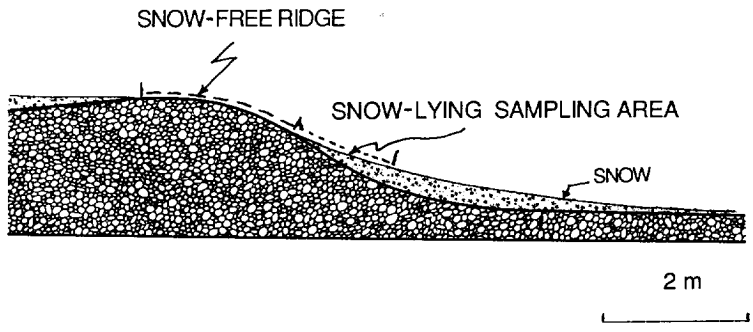

Fig. 1. Schematic of ridge and swale system under study. Note the occurrence of snow lying in the swales and the snow-free nature of the ridges.

cially leaf waxes which act as the interface between plants and their environment [16, 17]. This wax layer is vital as a barrier against mechanical and biological damage to the plant and is also important in controlling cuticular water loss [16, 18–20]. The compositional attributes of leaf waxes may be sensitive to divergent environmental conditions [21–23] and changes in composition may act to curtail cuticular water loss which may be especially important during winter conditions and also may affect the susceptibility of leaves to ice-damage [24]. However, the extent to which microscale differences in snow cover and subsequent differences in growth conditions affect leaf wax attributes of Arctic plants has yet to be examined. In light of this, the objectives of this study have been to (1) quantify the organic constituents of leaf waxes in two Arctic plant species representing divergent life forms, (2) examine the extent to which leaf wax constituents of these two species differ in plants found in snow-free as opposed to snow accumulating microsites, and (3) ascertain whether leaf wax attributes of plants from areas which typically experience thin snow cover in winter, change when snow cover is increased by artificial manipulation.

RESULTS

Absolute concentration of leaf waxes

Epicuticular wax extraction was undertaken on two Arctic plant species, *Saxifraga oppositifolia* and *Dryas octopetala*, collected from two different years (1990 and 1992) and from three different microsites as detailed in the Experimental. All leaves were harvested at the end of the respective plants' growing season. Total concentration of surface wax extracted from the different leaf collections are presented in Table 1. The largest wax abundance was observed for *S. oppositifolia* leaves at 6% of dry leaf weight, *D. octopetala* leaf wax abundance averaged at 2% of dry leaf weight, a similar amount to that observed by Luetz and Guelz (2.3%) [25]. No significant trends were observed between absolute amounts of leaf waxes extracted and microsite (Table 1).

Table 1. Leaf wax abundances of plants collected from different microsites in the Arctic (mg wax g^{-1} dry leaf wt)

	Swale	Ridge	Manipulated ridge
S. oppositifolia			
1990	58(4)	61(2)	—
1992	66(8)	61(5)	66(9)
D. octopetala			
1990	27(8)	33(5)	—
1992	22(3)	20(1)	20(5)

Figures in parentheses are standard deviations of replicate analyses.

Species traits and differences in relation to natural winter snow cover: 1990 collection

Initial investigations upon the relative abundances of leaf wax compositions in relation to environment were undertaken using leaves from plants on ridge sites and in swales collected in November 1990 (Table 2; Figs 2 and 3). A series of homologous *n*-alkanes were the main components of *S. oppositifolia* epicuticular leaf waxes, representing between 31% and 47% of the total wax extracts (Table 2). Plants from the ridge sites (snow-free during the Arctic winter) exhibited significantly ($P < 0.01$) higher proportions of *n*-alkanes in their leaf waxes than did those plants from the swale sites (snow-covered during the Arctic winter), the wax of the former comprising an average of 41% of the total wax, the latter 36% (Table 2). However, the distribution of the *n*-alkane homologues did not show significant differences between the two sites (Fig. 2). In all plant extracts examined, the C_{31} *n*-alkane was the most abundant homologue followed in decreasing abundance by C_{29}, C_{33}, and C_{27} *n*-alkanes, these homologues constituting >95% of the *n*-alkanes in all cases (Fig. 2). Other hydrocarbons present in all extracts in low abundances (<0.5% of total wax) were a monounsaturated C_{31} alkene and the isoprenoids, kaurene and squalene.

Table 2. *Saxifraga oppositifolia* leaf wax compositions of plants collected in November 1990. Figures given are the amount of each lipid class expressed as a percentage of the total leaf wax extract

Microsite sample number	%*n*-alkanes/ total wax	%wax esters/ total wax	%t.t. esters*/ total wax	%*n*-acids/ total wax	%*n*-alkanols/ total wax	%sterols/ total wax	%triterpenoids/ total wax
Swale							
1	36.0	31.7	8.3	3.7	1.3	0.6	0.3
2	37.8	30.2	11.8	3.3	1.5	0.6	0.1
3	37.1	29.1	7.2	5.2	1.2	1.3	0.3
4	36.4	27.8	8.0	4.7	1.5	0.8	0.6
5	31.0	33.3	8.0	1.8	1.1	0.9	0.2
6	39.8	34.6	11.0	2.1	1.7	0.4	0.3
$\bar{x}(\sigma_{n-1})$	36.4(2.8)	31.1(2.6)	9.1(1.9)	3.5(1.3)	1.4(0.2)	0.8(0.3)	0.3(0.2)
Ridge							
1	40.9	25.6	7.8	5.8	1.3	0.4	0.1
2	41.9	27.1	8.0	4.4	1.4	0.5	0.2
3	40.0	26.8	8.4	4.7	0.6	1.6	0.2
4	40.5	25.1	3.6	4.8	0.6	1.2	0.2
5	42.6	28.2	5.2	5.4	0.8	1.2	0.2
6	38.6	28.4	7.7	4.4	1.3	0.8	0.4
7	39.4	33.1	10.4	1.7	0.9	0.7	0.1
8	43.6	32.3	7.5	2.0	1.1	0.8	0.2
9	46.6	23.1	4.7	2.3	1.0	0.9	1.0
10	36.7	30.1	7.8	2.3	1.1	0.7	0.2
11	40.9	23.5	9.5	2.3	0.8	1.0	0.2
$\bar{x}(\sigma_{n-1})$	41.1(2.6)	27.6(3.3)	7.3(2.0)	3.7(1.5)	1.0(0.3)	0.9(0.3)	0.3(0.2)

*Triterpenoid esters.

Wax esters (*n*-alkyl alkanoates) from C_{34}–C_{44} were the second most abundant component of *S. oppositifolia* leaf waxes with a large even over odd carbon number predominance (Fig. 3). The wax esters constituted between 25 and 35% of the total wax extracted (Table 2). In the snow-lying sites, wax esters represented 31% of the total wax which was significantly ($P<0.05$) greater than that on the snow-free sites where wax esters represented 27% of the total wax (Table 2). The C_{42} *n*-alkyl ester was the most abundant homologue, followed by the C_{40}, C_{44} and C_{38} homologues (Fig. 3), In general, the wax esters consisted of palmitic acid (C_{16}) esterified to an *n*-alkanol. As observed for the *n*-alkanes, no consistent differences were observed for the distributions of individual homologues (Fig. 3).

Triterpenoid esters identified in *S. oppositifolia* were β-amyrin, α-amyrin and friedelin esterified to short chain saturated fatty acids (C_{10}–C_{20}), β-amyrin being the most abundant triterpenoid alcohol and palmitic acid being the most abundant fatty acid. This group formed a major proportion of the waxes and represented *ca* 5–10% of the total wax (Table 2). As with the wax esters, the proportion of triterpenoid esters relative to total wax was significantly ($P<0.01$) greater in the plants from snow-lying sites (9% of total) than that in the plants from snow-free sites (7% of total) (Table 2).

A series of *n*-alkanoic acids was present in the waxes of *S. oppositifolia* ranging from C_{14}–C_{32}, though these components were minor constituents of the leaf waxes (Table 2) and in total only represented between 2 and 5% of the total wax (Table 2). No significant difference was noted between the *n*-alkanoic acid composition of snow-free plants and snow-lying plants. A slight increase in the proportion of *n*-alkanols (C_{28} and C_{30}) was observed in plants from snow-free ridges though the difference was not significant (Table 2). Sterols identified were 24-ethylcholesterol, 24-methylcholesterol and 24-ethylcholesta-5,24(28)*Z*-dienol. Free triterpenoids identified were β-amyrin, α-amyrin and friedelin. No change in the abundances of the sterols or triterpenoids were observed between microsites (Table 2).

The leaf wax composition of *D. octopetala* was dominated by three main classes of compounds, triterpenoids, *n*-alkanes and wax esters (Table 3). Triterpenoid acids predominated, especially ursolic acid (3β-hydroxy-urs-11-en-28-oic acid) which constituted up to 43% of the wax; other triterpenoids present were oleanoic acid (3β-hydroxy-olean-11-en-28-oic acid) and uvaol (28-hydroxy-urs-11-en-3β-ol). A much greater abundance of ursolic acid was observed in the leaf waxes of those plants growing in ridge sites, 43% vs 32% for plants from the swales.

A series of wax esters, represented up to 27% of the *D. octopetala* leaf waxes examined, C_{42} and C_{44} homologues being the most abundant components (Table 3). The abundance of total wax esters was greater at 27% for leaf waxes of plants collected from the ridge sites, than for swale plants at 23% (Table 3).

n-Alkanes represented up to 12% of total leaf wax of *D. octopetala*, C_{29} and C_{27} homologues being the major

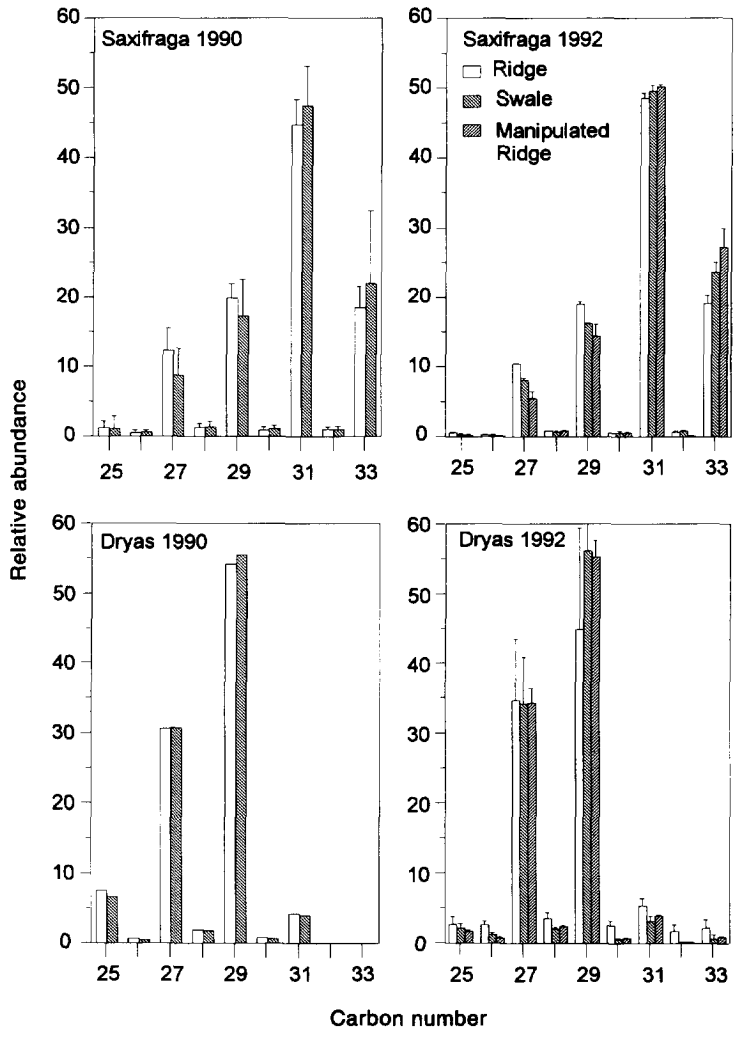

Fig. 2. *n*-Alkane distributions of Arctic plant leaf waxes, as percentage of total *n*-alkanes identified, against homologue carbon number. Dryas = *Dryas octopetala*, Saxifraga = *Saxifraga oppositifolia*. Error bars are one standard deviation.

components (Table 3). A slightly greater abundance of *n*-alkanes was observed in the ridge plant leaf waxes relative to those of swale plants, 12% for the former and 10% for the latter (Table 3). Minor leaf wax constituents present were *n*-alkanols at *ca* 2% of total wax, *n*-alkanoic acids at *ca* 1% of total wax, triterpenols (α-amyrin and taraxasterol) at *ca* 1%, sterols (24-ethylcholesterol and 24-methylcholesta-5,22-dienol) at *ca* 0.5%, triterpenoid esters at *ca* 1.5% and hexoses at *ca* 2% of total leaf wax (Table 3). As observed for *S. oppositifolia*, no major differences in the relative abundance of the homologous components of the major lipid classes of *D. octopetala* were observed (Figs 2 and 3).

Species traits and differences in relation to natural and manipulated winter snow cover: 1992 collection

As found with the November 1990 collection, *n*-alkanes were the most abundant lipid class in the leaf waxes of *S. oppositifolia*, representing up to 51% of total leaf waxes (Table 4). Again, *n*-alkanes were more abundant in plants growing in the ridge sites (51%) than in the swale sites (43%). The abundance of *n*-alkanes in plants growing in the 'manipulated' ridge sites (46%) were intermediate between those of swale plants and unmanipulated ridge plants (Table 4). Wax esters constituted between 38% of total leaf wax for swale plants and 30% for ridge plants. 'Manipulated' ridge plants had leaf waxes with 36% of total leaf wax being wax esters (Table 4). The other major constituent of *S. oppositifolia* leaf waxes were triterpenoid esters, constituting 9% of swale plant leaf waxes, 10% of the ridge plant leaf waxes and 11% of the 'manipulated' ridge plant leaf waxes (Table 4). Other constituents each represented less than 1% of the total waxes. All components identified in the 1990 collection were identified in the 1992 collected plants. *n*-Alkanes, wax esters and triterpenoid esters were more abundant in the leaf waxes from the 1992 plants than those collected in 1990 (Tables 2 and

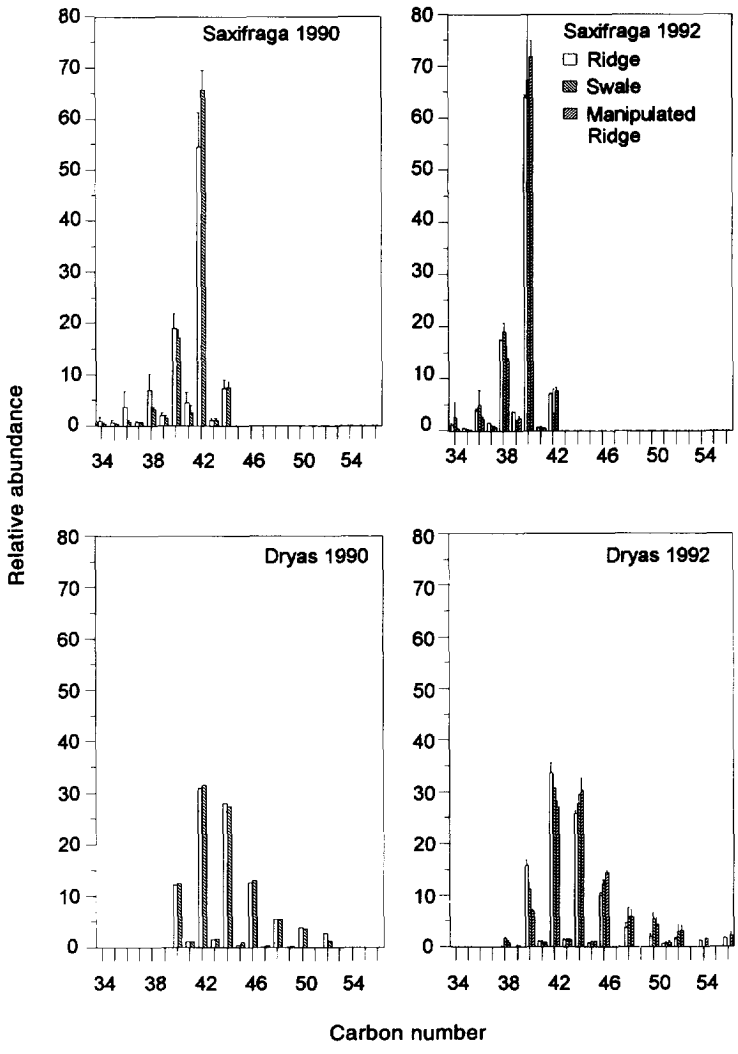

Fig. 3. Wax ester (*n*-alkyl alkanoate) distributions of Arctic plant leaf waxes, as percentage of total wax esters identified, against homologue carbon number. Dryas = *Dryas octopetala*, Saxifraga = *Saxifraga oppositifolia*. Error bars are one standard deviation.

Table 3. *Dryas octopetala* leaf wax compositions of plants collected during November 1990

Constituent	Swale	Ridge
n-Alkanes	9.7	11.5
n-Alkanols	1.9	2.3
n-Alkanoic acids	0.4	1.1
Wax esters	23.2	27.0
Uvaol	2.4	2.4
Oleanoic acid	9.7	8.8
Ursolic acid	42.5	31.5
Triterpenols	1.0	1.1
Triterpenoid esters	0.9	1.7
Sterols	0.3	0.4
Hexoses	1.1	2.4

Plant material was collected from raised beaches or ridges and from swales in between the ridges. Numbers given are percentage of total leaf wax lipid extracted and represent values obtained by the analysis of combined plant material from 11 sub-samples for ridge sites and 6 for swale sites.

4). The reverse trend was observed in other components, such as *n*-alkanols and *n*-alkanoic acids (Table 5, 2 and 4).

The major constituents of the *D. octopetala* leaf waxes of the 1992 collection were the same as those for the 1990 collection, being ursolic and oleanoic acids, wax esters and *n*-alkanes (Table 5). Ursolic acid represented 40% of the swale, 34% of the ridge and 32% of the 'manipulated' ridge plant leaf waxes. Oleanoic acid abundances were 8%, 6% and 6%, respectively, for the three sites studied (Table 5). Wax ester abundances were greatest in the leaf waxes of 'manipulated' ridge plants at 29% and virtually identical in ridge and swale plants at 26% (Table 5). *n*-Alkane abundances were greatest in the leaf waxes of ridge plants at 14%, lowest in swale plants at 12% and intermediate in 'manipulated' ridge plants at 13% (Table 5). Of the other constituents present in the leaf waxes of *D. octopetala*, triterpenoid esters were more abundant in swale than ridge plants, the opposite trend being observed for *n*-alkanols and *n*-alkanoic acids (Table 5).

Table 4. *Saxifraga oppositifolia* leaf wax compositions of plants collected in July/August 1992 from three different microsites; ridges, swales in between ridges and ridge sites upon which snow fences were constructed in order that snow would lie during the Arctic winter (manipulated ridges)

Constituent	Swale		Ridge		Manipulated ridge	
n-Alkanes	43.0	(1.1)	51.3	(0.1)	45.8	(5.5)
n-Alkanols	0.3	(0.1)	0.4	(0.1)	0.3	(0.1)
n-Alkanoic acids	0.8	(0.01)	0.4	(0.3)	0.1	(0.1)
Wax esters	38.0	(1.1)	30.0	(1.0)	35.7	(2.0)
Triterpenoid esters	9.4	(2.5)	9.9	(0.8)	11.1	(0.1)
Triterpenoids	0.2	(0.1)	0.1	(0.1)	0.2	(0.1)

Figures in parentheses are standard deviations of the mean of analyses on plant material from two different locations.

Table 5. *Dryas octopetala* leaf wax compositions of plants collected in July/August 1992 from different microsites in the Arctic

Constituent	Swale		Ridge		Manipulated ridge	
n-Alkanes	11.5	(0.8)	13.9	(0.8)	12.8	(0.6)
n-Alkanols	1.4	(0.3)	3.3	(1.0)	2.0	(0.6)
n-Alkanoic acids	0.5	(0.1)	1.0	(0.1)	0.8	(0.1)
Wax esters	25.6	(1.6)	25.5	(3.3)	29.0	(3.9)
Ursolic acid	39.9	(1.6)	33.7	(2.9)	32.0	(0.02)
Oleanoic acid	7.7	(0.6)	6.2	(1.0)	6.3	(0.2)
Uvaol	1.5	(0.1)	1.6	(0.5)	1.5	(0.03)
Triterpenoid esters	2.0	(0.4)	0.8	(0.2)	0.5	(0.2)
Triterpenoids	0.6	(0.3)	0.8	(0.1)	0.7	(0.2)
Hexoses	0.1	(0.1)	0.2	(0.1)	0.3	(0.3)

Figures in parentheses are standard deviations of the mean of analyses on plant material from two different locations.

DISCUSSION

Species differences

No leaf wax components were observed which were peculiar to plants growing in any of the microsites examined, which would have supported site-specific synthesis of particular leaf wax components in response to divergent growth conditions. However, certain compounds were observed which were characteristic of the individual plant species examined. For example, ursolic acid, oleanoic acid and uvaol were only observed in the *D. octopetala* samples, and may be an adaptation particular to the growth habit of this species in the Svalbard region.

Microsite differences

The ridge sites examined were distinguished from the swale sites by a general lack of snow-cover throughout the Arctic winter and a much deeper permafrost layer throughout the year [9]. The result of these factors leads to an earlier growing season for ridge plants than for those in the swale sites; the ridge sites being much drier and colder than the swale sites during leaf expansion and throughout the growing season [5, 26]. For both Arctic plant species analysed in this study, the leaf waxes of those plants growing in ridge sites had consistently greater *n*-alkane concentrations than those of plants growing in swale sites (Tables 2 to 5). The greater relative abundance of *n*-alkanes in the leaf waxes of the ridge plants compared to that in the swale plants may lead to the ridge plant leaf waxes being more hydrophobic than those of the swale plants and, thus, help to prevent cuticular water loss [20]. Therefore, differences in the composition of the leaf waxes of plants growing in the dry ridge sites may help reduce cuticular water loss and curtail winter and summer dessication which are greater in the ridge sites compared to the swale sites [5]. In addition, it should be noted that the differences in leaf wax compositions observed are also likely to affect the crystallization properties of the wax on the surface of the cuticle and may allow for protection against abrasion damage from wind blown ice crystals in the exposed ridge sites [25].

Field manipulations

Leaf waxes of plants from ridge sites where snow-cover was increased using snow fences, generally had compositions (those of *n*-alkanes in particular) intermediate be-

tween those of unmanipulated ridge sites and swales. This similarity was most marked in *S. oppositifolia* leaf waxes (Table 5). The shift in composition, as compared to that of plants growing in unmanipulated sites and associated with increases in winter snow cover, indicates a link between snow conditions and subsequent effects on summer conditions (shorter growing season, wetter soils, higher plant water potentials) and the leaf wax compositions of *D. octopetala* and *S. oppositifolia* in the high Arctic. Changes in plant biochemical and anatomical attributes due to alterations of environmental conditions in winter have seldom been reported for tundra plants, though changes in Arctic plant performance due to manipulation of summer conditions have been extensively investigated [3–6].

Ontogenetic and environmental effects

Baker and Hunt [27] demonstrated how leaf wax compositions can vary considerably during early leaf development and concluded that "ontogenetic variation in surface [wax] distributions result largely from the changes in wax production occurring during the critical three-week period following leaf emergence". In the light of such observations, it could be argued that the leaf wax compositional differences we present are a 'snapshot' of variations inherent during leaf development. However, all collections were made at the end of the growing season of the respective species (several months) by which time leaf wax composition would have been fixed for some time [27]. Therefore, a more likely interpretation of the results presented is that environmental differences during leaf expansion have affected the final leaf wax compositions of the Arctic plant species under study.

Overview

An array of growth forms and species exist in the high Arctic, ranging from dwarf shrubs, such as *D. octopetala*, to herbaceous species, like *S. oppositifolia* [9]. Inherent differences in species attributes visually manifested in growth form differences, are also expressed in species specific leaf wax properties, such as observed in this study. While the leaf wax composition of individual species appears to be unique, differences in relative abundances of individual compounds (and especially *n*-alkanes) follow consistent patterns from moist swales to dry ridges across the polar semi-desert landscape. Relative abundances of leaf wax components, while being microsite dependent, also appear to be sensitive to simulated changes in winter conditions, as exemplified by alterations in snow cover in this study, where consistently lower relative *n*-alkane contents were observed in plants growing in sites covered in snow during the Arctic winter, irregardless of species. Overall, our findings indicate that environmental differences during leaf development can play a significant role in affecting the final leaf wax composition of individual plant species. Further analyses of the wax compositions of leaves of Arctic plants during leaf development and beyond under different environmental conditions will help to confirm this conclusion.

EXPERIMENTAL

Study Site. The studies reported were carried out on the archipelago of Svalbard, Norway, near the settlement of Ny Alesund, situated on the southern coast of Kongsfjorden (78°56.12′N, 11°50.4′E). The study sites were located *ca* 2 km NW of Ny Alesund on the Broggerhalvoya peninsula. The area is dominated by polar desert vegetation with undulating topography creating ridges which are free of snow in winter adjacent to snow lying areas where snow accumulates in winter (Fig. 1).

Plant collection. All leaf collections were undertaken at the end of the leaf growing season so that a 'snapshot' effect which reflected variations of leaf wax compositions during leaf expansion was avoided, and that leaf wax compositions reported would reflect a whole season of development. In November 1990, plant samples were collected from 4 representative snow-free and adjacent snow-lying microsites over a 2 day period. Every 5 m along a 25 m transect, a 10 cm diameter core of vegetation was collected, separately bagged, sealed and kept frozen until sorting and analysis in the U.K. In the snow-lying areas, snow averaging 25 cm in depth was removed down to plant surfaces for sample collection. For this study, 11 samples were examined from snow-free sites and 6 samples from snow-covered sites. During the autumn of 1991, snow fences were constructed on 2 ridge sites, in order that snow would accumulate behind the fences during the following winter. Plant material was harvested from behind the snow fences, from unmanipulated ridge sites and from snow-lying sites in July and August of 1992. The material from the two sampling dates was bulked.

Extraction. Whole green leaves from one seasons growth (*ca* × 100 mg) were dried at 60° for 24 hr and the surface wax extracted using the method of ref. [25], but using CH_2Cl_2 instead of CH_2Cl_3 to remove surface waxes [28]. Total lipid extracts were fractioned by silica gel CC sequentially eluting with hexane, hexane–CH_2Cl_2 (1:1) and CH_2Cl_2–MeOH (1:1) in order to obtain frs of differing polarity to aid lipid identification and quantification [28, 29].

Identification of wax constituents. After derivatization with *bis*(trimethylsilyl)trifluoroacetamide (BSTFA) or *bis*(trimethylsilyl)acetamide (BSA), the total wax extracts and individual frs were analysed by GC on a 25m HT-5 (SGE) fused silica capillary column; 0.1 μm film thickness, 0.32 mm i.d. H_2 was used as carrier gas. The temp. program used was 50°–180° @ 12° min^{-1} then 180°–350° @ 4° min^{1} and then isothermal for 25 min. Lipid identification was made by MS with the GC system as described above, but using He as carrier gas. Compounds were identified by lit. comparison and coinjection techniques. Differences in leaf wax attributes between microsites were tested using Student's *t*-test.

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