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POLAR LIPIDS AND NET PHOTOSYNTHESIS POTENTIAL OF SUBARCTIC *DIAPENSIA LAPPONICA*

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Abstract—The overall net photosynthesis potential of *Diapensia lapponica* was very low. It was highest at the end of August (11.1 μmol CO_2 (μmol chlorophyll)$^{-1}$ hr^{-1}) and decreased during the autumn and winter, reaching a minimum in February (0.6 μmol CO_2 (μmol chlorophyll)$^{-1}$ hr^{-1}). In March, with increased light in the subarticum, the potential rose temporarily, and after another minimum at the beginning of May, the potential slowly rose to the summer level (2.3–6.5 μmol CO_2 (μmol chlorophyll)$^{-1}$ hr^{-1}). The seasonal fluctuation pattern of the potential of net photosynthesis was the same when calculated on a dry weight basis. Seasonal changes also occurred in the chlorophyll content and in the contents of polar lipids, particularly DGDG (digalactosyl diacylglycerols) and PC (phosphatidyl choline) and less clearly in MGDG (monogalactosyl diacylglycerols), PE (phosphatidyl ethanolamine) and PG (phosphatidyl glycerols). The contents of chlorophylls, DGDG and PC increased during autumn and early winter during the hardening process and decreasing light and temperature. Their contents decreased in late winter and spring in response to dehardening and increased light and temperature in the subarcticum. Thus, the molar ratios of MGDG:DGDG and PE:PC varied throughout the year, being lowest in winter. In addition, fatty acids of individual lipid classes showed seasonal fluctuation. In both MGDG and DGDG, the proportion of linolenic acid was higher in summer than in winter and that of linoleic acid was *vice versa*. In both PC and PE, the proportion of palmitic acid was highest in summer and lowest in winter and, particularly in PC, this variation was compensated for by changes in linoleic acid and less clearly in linolenic acid, and, in PE, by the long-chain behenic acid. In PG, the proportions of *trans*-16:1 and oleic acid were higher in summer than in winter, whereas the proportions of palmitic and linoleic acids were higher in winter than in summer. Thus, there was not a clear increase in the degree of unsaturation of fatty acids during winter-time. © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Diapensia lapponica is an evergreen chionophobous cushion plant growing in subarctic, arctic and alpine tundra regions in the northern hemisphere. In its natural habitat, the plant is exposed to strong winds and frost, and it is extremely frost resistant. The schlerephyllous leaves last for two to three years, surviving in winter at -60 to $-70°$, in summer at -7 to $-9°$ [1, 2]; the plant is clearly extremely hardy according to the classification by Levitt [3]. Frost-resistance is acquired rapidly in October and it remains high until the beginning of March [2]. Hardening can occur both in short- and long-day conditions [4].

To understand the survival mechanisms of *Diapensia*, we undertook a series of studies and described seasonal differences in the shape and ultrastructure of chloroplasts in mesophyll and palisade cells of the leaves [5, 6], in mitochondria [7] and in lipid globules [8, 9]. In the present paper, we report on the seasonal changes in the contents of chlorophyll, polar lipids and in the potential for net photosynthesis.

RESULTS

CO_2 *fixation*

Seasonal variation in the potential of net photosynthesis of *Diapensia* was clear (Table 1). It was highest at the end of August and decreased slowly during autumn and winter reaching a minimum in February. In March, the potential was higher than in February. After another minimum at the beginning of May, the

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Table 1. Seasonal variation in chlorophyll content and CO_2-fixation at optimum temperature in saturating light, in leafy tops of *Diapensia lapponica*. Nd, not determined; $\pm$, s.e.; Chl, chlorophyll

Date	mg CO_2 (g dry wt)$^{-1}$ hr^{-1}	μmol CO_2 (μmol Chl)$^{-1}$ h^{-1}	μmol Chl (g dry wt)$^{-1}$
June 1	nd	nd	2.8 ± 0.2
June 30	nd	nd	1.7 ± 0.2
June 30 (new leaves)	nd	nd	3.2 ± 0.2
August 4 (old leaves)	nd	nd	3.0 ± 0.2
August 4 (new leaves)	nd	nd	3.5 ± 0.1
August 31	nd	nd	1.6 ± 0.1
September 29	0.26 ± 0.05	2.7 ± 0.5	2.2 ± 0.2
November 3	0.33 ± 0.10	2.3 ± 0.7	3.4 ± 0.2
December 1	0.15 ± 0.01	0.9 ± 0.1	3.8 ± 0.5
February 2	0.09 ± 0.02	0.6 ± 0.1	3.5 ± 0.1
March 2	0.21 ± 0.02	1.6 ± 0.2	3.1 ± 0.3
March 30	0.16 ± 0.01	1.3 ± 0.1	2.8 ± 0.2
May 3	0.07 ± 0.01	0.8 ± 0.2	2.1 ± 0.1
May 12	0.21 ± 0.07	2.8 ± 0.9	1.7 ± 0.1
June 1	0.28 ± 0.04	2.3 ± 0.4	2.8 ± 0.3
June 29	0.35 ± 0.07	6.5 ± 1.2	1.3 ± 0.1
August 11	0.32 ± 0.07	nd	nd
August 31	0.79 ± 0.06	11.1 ± 0.8	1.7 ± 0.1
September 14	0.44 ± 0.09	6.0 ± 1.2	1.7 ± 0.1

potential slowly rose to the higher summer level. The results were calculated on both a dry weight and a chlorophyll basis; the pattern was similar (Table 1).

Chlorophyll

The seasonal changes in the chlorophyll content of the leafy tops of *Diapensia* were clear (Table 1). The content clearly increased during the autumn and early winter, reaching a maximum in December. Thereafter, the chlorophyll content decreased and reached a minimum in May. During summer also, the developmental stage of the leaves affected the chlorophyll values. The growing and greening new leaves (June 30, August 4) had a high chlorophyll content, and so had also the old leaves on August 4. At the end of August, when all leaves of the green top were mature, the chlorophyll content had dropped to the low summer level again.

Glycolipids

Seasonal variation in the fatty acid content of glycolipids calculated on a dry weight basis was most prominent in DGDG and it followed the same pattern as that of the chlorophyll content; low values in summer were followed by increases in autumn and early winter, and by decreases later in winter and in spring (Table 2). The content of MGDG varied only slightly; a somewhat higher level was found in the beginning (June) and at the end (August–September) of the growing season than during the rest of the year. The contents of other glycolipids were low and without substantial seasonal fluctuations (data now shown).

The molar ratio of MGDG to DGDG was low in winter (0.40–0.58) due to the high content of DGDG in winter (Table 2). In summer and in autumn (June–September), the ratio varied between 0.82 and 2.02. The molar ratio of MGDG to chlorophyll was lowest during winter, varying between 0.89 and 1.07 from November to March (Table 2). It fluctuated between 1.49 and 3.03 from May to October, showing the highest ratio at the end of August. The seasonal fluctuations in the ratios of MGDG to chlorophyll are mostly due to the extensive fluctuations in chlorophyll content (Table 1) and less to the relatively slight changes in the content of MDGD. The seasonal pattern of the molar ratios of DGDG to chlorophyll was different; the ratio increased from 1.75 in September to 3.28 in May (Table 2) and then decreased. In autumn and early winter, the increase is attributed to more extensive accumulation of DGDG than chlorophyll, in late winter and spring, to greater decreases in chlorophyll than in DGDG.

During summer also, the developmental stage of the leaves affected the results. In August 4, when the growth and greening of the new leaves were occurring, the ratios of MGDG and DGDG to chlorophyll were exceptionally low compared with other summer values in both new and old leaves, due to their high chlorophyll contents. Also, the ratio of MGDG to DGDG was exceptionally low in the new leaves due to their elevated content of DGDG.

The major fatty acids of MGDG and DGDG were 18:3, 18:2 and 16:0, accounting for 88–97% of the total fatty acids in both glycolipids (Table 3). In MGDG and in DGDG, the proportion of 18:3 acid ranged between 65 and 90% and between 47 and 70%, respectively. The proportion of 18:2 varied from 3 to 34% in MGDG and from 8 to 24% in DGDG. In both MGDG and DGDG, the proportion of 18:3 was higher in summer than in winter and that of 18:2 was lower in summer than in winter. The proportion of

Table 2. Seasonal variation of glycolipid contents and molar ratios of glycolipids in leafy tops of *Diapensia lapponica*. ±, s.e.; Chl, chlorophyll

Date	mg Fatty acids (g dry wt)$^{-1}$ MGDG	DGDG	Molar ratio MGDG:Chl	DGDG:Chl	MGDG:DGDG
June 1	2.23±0.14	2.41±0.13	1.49±0.10	1.61±0.08	0.93
June 30	1.95±0.08	2.02±0.13	2.08±0.08	2.15±0.13	0.97
August 4 (old leaves)	1.88±0.06	2.16±0.18	0.56±0.02	0.79±0.12	0.72
August 4 (new leaves)	1.59±0.18	2.47±0.07	0.41±0.02	0.77±0.03	0.53
August 31	2.60±0.20	1.29±0.34	3.03±0.24	1.50±0.49	2.02
September 29	1.84±0.26	2.09±0.48	1.54±0.22	1.75±0.40	0.88
November 3	1.94±0.06	3.33±0.35	1.07±0.03	1.84±0.19	0.58
December 1	1.80±0.09	3.66±0.19	0.89±0.04	1.81±0.10	0.49
February 2	1.80±0.06	4.31±0.07	0.94±0.03	2.26±0.03	0.42
March 2	1.69±0.06	4.25±0.18	1.01±0.03	2.54±0.11	0.40
March 30	1.57±0.03	3.67±0.44	1.05±0.01	2.46±0.29	0.43
May 3	2.14±0.23	3.74±0.26	1.87±0.20	3.28±0.23	0.57
May 12	1.75±0.11	2.86±0.09	1.87±0.12	3.05±0.10	0.61
June 1	2.45±0.12	2.98±0.32	1.60±0.08	1.95±0.21	0.82
June 29	1.73±0.09	1.96±0.03	2.53±0.13	2.86±0.04	0.88
August 3	1.68±0.06	1.62±0.04	1.79±0.07	1.72±0.05	1.04
August 31	2.60±0.10	2.72±0.06	2.88±0.11	3.01±0.06	0.96
September 14	2.40±0.09	2.72±0.24	2.58±0.10	2.93±0.26	0.88

Table 3. Seasonal variation in percentage by weight composition of major fatty acids of MGDG and DGDG in leafy tops of *Diapensia lapponica*: s.e. varies between 0.5 and 5%

Date	MGDG 16:0	18:2	18:3	16:0+18:2+18:3	DGDG 16:0	18:2	18:3	16:0+18:2+18:3
June 1	3	5	89	97	19	12	61	92
June 30	3	5	89	97	17	8	67	92
August 4 (old leaves)	2	7	88	97	17	13	60	90
August 4 (new leaves)	3	4	90	97	16	8	70	94
August 31	12	16	65	93	20	15	55	90
September 29	5	14	77	96	20	22	50	92
November 3	4	16	73	93	19	19	51	89
December 1	5	22	66	93	19	24	47	90
February 2	3	22	70	95	19	23	51	93
March 2	4	24	68	96	19	23	48	90
March 30	3	21	70	94	19	23	50	92
May 3	5	20	67	92	19	22	50	91
May 12	5	5	80	90	19	15	54	88
June 1	3	3	90	96	18	13	59	90
June 29	3	3	91	97	17	10	65	92
August 3	2	4	89	95	17	10	66	93
August 31	3	8	85	96	18	13	61	92
September 14	3	8	85	96	18	15	59	92
October 5	2	10	84	96	18	18	56	92

16:0 was relatively constant in both MGDG and DGDG throughout the year, ranging usually from 2 to 5% in MGDG and from 16 to 20% in DGDG.

The developmental stage of the leaves in summer had little affect on the fatty acid profiles of glycolipids. The main difference of the new developing leaves compared with the old leaves, was a high proportion of 18:3 in DGDG.

Phospholipids

The fatty acid content of phospholipids was high in autumn and winter and low in summer (Table 4).

Table 4. Seasonal variation of phospholipid contents in leafy tops of *Diapensia lapponica*. ±, s.e.

Date	PC	PE	mg Fatty acids (g dry wt)$^{-1}$ PG	PA	U*	Total
June 1	1.37±0.02	1.03±0.09	0.44±0.02	0.23±0.03	0.20±0.04	3.27
June 30	1.03±0.09	0.79±0.05	0.34±0.01	0.18±0.03	0.26±0.06	2.60
August 4 (old leaves)	1.20±0.14	0.76±0.13	0.25±0.02	0.10±0.02	0.26±0.09	2.57
August 4 (new leaves)	0.63±0.04	1.52±0.09	0.39±0.03	0.32±0.01	0.42±0.01	3.28
August 31	1.27±0.09	0.73±0.10	0.11±0.01	0.10±0.01	0.24±0.04	2.45
September 29	0.95±0.13	0.36±0.07	0.21±0.06	0.19±0.04	0.60±0.18	2.31
November 3	1.19±0.19	0.94±0.15	0.57±0.05	0.20±0.05	0.53±0.08	3.43
December 1	1.17±0.12	0.62±0.05	0.38±0.04	0.16±0.02	0.57±0.00	2.90
February 2	1.56±0.20	0.79±0.08	0.43±0.04	0.13±0.01	0.47±0.04	3.38
March 2	1.56±0.15	0.72±0.14	0.46±0.08	0.14±0.03	0.47±0.11	3.35
March 30	2.06±0.07	0.65±0.04	0.63±0.06	0.11±0.01	0.48±0.07	3.93
May 3	1.58±0.07	0.56±0.06	0.52±0.09	0.19±0.04	0.59±0.09	3.44
May 12	1.08±0.12	0.54±0.02	0.53±0.04	0.27±0.05	0.78±0.04	3.20
June 1	1.55±0.18	0.43±0.04	0.45±0.02	0.13±0.01	0.41±0.09	2.97
June 29	1.04±0.12	0.32±0.03	0.22±0.02	0.13±0.01	0.36±0.03	2.07
August 3	0.97±0.09	0.28±0.04	0.24±0.02	0.11±0.01	0.54±0.09	2.14
August 31	1.73±0.13	0.40±0.05	0.42±0.04	0.19±0.01	0.59±0.09	3.33
September 14	2.13±0.06	0.59±0.06	0.56±0.06	0.26±0.03	0.68±0.05	4.22
October 5	2.30±0.20	0.70±0.07	0.53±0.03	0.18±0.03	0.66±0.06	4.37

* U = unknown phospholipid.

Fluctuation was mostly due to the changes in the content of PC, the major phospholipid of the leafy tops of *Diapensia*, and less to PE, PG and an unidentified phospholipid. PC did not, however, behave in a similar way during the two succeeding years; the considerable accumulation which was evident during the second autumn was less pronounced in the first autumn. Similarly, great differences were found in the seasonal pattern of lipid phosphorus accumulation during the experiments carried out during a three year period (data now shown). The content of PA was low in all seasons.

In PC, PE and PG, the seasonal fatty acid pattern was slightly different. In both PC and PE, the proportion of 16:0 was highest in summer and lowest in winter (Tables 5 and 6). The proportions of 18:2 and less clearly that of 18:3 in PC were lowest in summer, whereas in PE these acids did not show clear seasonal variation. In PE, the high 16:0 proportion in summer was compensated for by the low proportion of the long-chain fatty acid, 22:0. PG (Table 7) differed from PC and PE in having a low proportion of 16:0 in summer. This was, however, compensated for by a higher proportion of *trans*-16:1, a unique fatty acid in PG of chloroplasts. The proportion of 18:1 in PG was higher in summer than in winter, whereas 18:2 showed the opposite behaviour. The 18:3 acid of this lipid class did not fluctuate seasonally. In PA, 16:0 and 18:2 were the major fatty acids whereas 18:0, 18:1, 18:3 and 22:0 were minor components. None of these acids showed seasonal fluctuations (data now shown).

The development of new leaves in summer had some effects on phospholipids (Tables 4–7). The major difference was the exceptional low content of PC and the exceptional high content of PE in the new developing leaves. Only slight differences were found in the fatty acid profiles of the phospholipids between the new and old leaves. PC was slightly more saturated in

Table 5. Seasonal variation in percentage by weight composition of major fatty acids of PC in leafy tops of *Diapensia lapponica*: s.e. varies between 0.5 and 5%

Date	16:0	18:1	18:2	18:3	22:0
June 1	19	7	52	18	2
June 30	23	7	48	18	1
August 4 (old leaves)	23	5	50	19	1
August 4 (new leaves)	26	5	42	15	4
August 31	20	7	55	14	1
September 29	19	5	56	14	2
November 3	17	4	52	19	2
December 1	14	4	53	19	3
February 2	15	4	55	20	2
March 2	16	3	55	20	2
March 30	13	4	57	21	2
May 3	15	4	57	18	2
May 12	19	6	50	18	2
June 1	22	6	47	18	1
June 29	24	6	45	20	1
August 3	22	6	50	18	0
August 31	22	4	52	17	1
September 14	19	3	56	18	1
October 5	17	3	58	17	2

Table 6. Seasonal variation in percentage by weight composition of major fatty acids of PE in leafy tops in *Diapensia lapponica*: s.e. varies between 0.5 and 5%

Date	16:0	18:1	18:2	18:3	22:0	24:0
June 1	19	3	52	11	5	2
June 30	23	3	53	11	2	2
August 4 (old leaves)	24	3	53	12	3	1
August 4 (new leaves)	23	5	51	10	3	1
August 31	21	4	54	9	4	1
September 29	20	4	47	8	10	3
November 3	16	2	49	11	11	2
December 1	15	3	48	12	9	2
February 2	18	3	48	13	8	2
March 2	17	3	48	12	10	2
March 30	16	3	50	13	9	2
May 3	24	5	35	16	6	2
May 12	20	7	44	12	6	2
June 1	27	5	41	10	4	2
June 29	27	4	44	11	5	2
August 3	26	4	45	14	3	1
August 31	21	4	43	9	4	3
September 14	22	4	47	10	7	2
October 5	23	3	48	11	7	1

Table 7. Seasonal variations in percentage by weight composition of major fatty acids of PG in leafy tops of *Diapensia lapponica*: s.e. varies between 0.5 and 5%

Date	16:0	*trans*-16:1	18:0	18:1	18:2	18:3	16:0 + *trans*-16:1
June 1	33	8	1	12	20	16	41
June 30	32	12	1	11	19	20	44
August 4 (old leaves)	37	9	2	13	21	12	46
August 4 (new leaves)	30	13	2	8	21	15	43
August 31	24	3	5	8	19	18	27
September 29	29	1	3	6	27	21	30
November 3	39	2	1	7	28	15	41
December 1	31	2	2	5	31	17	33
February 2	39	2	2	6	31	15	41
March 2	38	2	2	6	29	14	40
March 30	39	2	2	6	31	16	41
May 3	34	3	2	7	28	18	37
May 12	32	3	3	13	25	17	35
June 1	34	8	2	12	22	14	42
June 29	34	7	2	12	20	17	41
August 3	32	8	2	10	21	19	40
August 31	33	7	2	9	24	18	40
September 14	37	4	2	7	27	18	41
October 5	41	3	1	6	28	14	44

the new leaves than in the old leaves, and, in PG, the proportion of *trans*-16:1 was higher in the new leaves than in the old leaves, whereas in the case of 16:0, the situation was *vice versa*.

DISCUSSION

Characteristic of the potential for net photosynthesis of subarctic *Diapensia lapponica* is the overall low level (Table 1), which is lower than those found in evergreen tundra dwarf shrubs, being similar to mosses and lichens [10]. An unexpected feature was also the relatively high winter-time capacity compared with the summer-time capacity. The winter values, calculated on a dry weight basis, were *ca* 10% of the maximum value at the end of August and over 20% of late June and early August values. Thus, *Diapensia* is an example of an extremely hardy plant with LT at −58° in winter [2], whose photosynthetic capacity is not completely blocked during winter, as is usually the case in extremely hardy plants [3, 11], such as evergreen Scots pine and spruce [12] or evergreen herbaceous, *Pilosella officinalis* [13]. The decline in *Diapensia* is, however, greater on a chlorophyll basis due to an increase in the chlorophyll content during autumn and early winter (Table 1). The increase in the chlorophyll content is apparently a response of the photosynthetic apparatus to decreased light intensity and day length [10, 14, 15], but it may also be a response to cold acclimation and acquirement of considerable freezing-tolerance [2, 16]. In *Diapensia*, the increases in the amount of chlorophyll are apparently used to compensate for the winter inhibition of photosynthesis. Such a mechanism is not found in the needles of Scots pine [17] or arctic sedge (*Eriophorum vaginatum*) [18]; both photosynthetic activity and chlorophyll content decline in autumn.

From November to February, during the darkest period of the year, the net photosynthesis potential decreased, although no losses occurred in chlorophyll content (Table 1). Inactivation of photosynthesis was accompanied by a reduction in the size of the chloroplasts and by the transformation of chloroplasts from an ellipsoid to a spherical shape, as well as by changes in thylakoid organization [5, 6]. Although some disintegration of chloroplasts were visible in the mesophyll cells [5], the damage was apparently not very great, because no decreases were found in chlorophyll content. Therefore, it is unlikely that the decreases in net photosynthesis potential could be attributed to the disintegrated chloroplasts, but more likely to inactivation of light-activated genes of photosynthesis [19] during the darkest season in the subarcticum. This concept is further supported by the observation that the potential is at a higher level at the beginning of March than at the beginning of February (Table 1), apparently in response to increased light in March in the subarcticum [20]. Later, however, the potential drops again and since this occurs concomitantly with a decrease in the chlorophyll content, the photosynthetic apparatus has apparently been subjected to photo-oxidative damage [21]. It is well known that light at low temperature can easily cause photo-oxidative damage [22] and, in a cold climate, this occurs frequently in late winter and early spring, as evidenced by the partial chlorophyll bleaching of conifer needles [17, 23]. In *Diapensia*, this damage was reparable as indicated by an increase in the chlorophyll content in the beginning of June followed by an increased potential of net photosynthesis at the end of June (Table 1).

A priori, it could be thought that lipids are of particular importance for the survival of arctic, subarctic and alpine plants. The polar lipid and fatty acid composition of *Diapensia* (Tables 2–7) does not, however, similarly to alpine plants [24], deviate from plants grown in warmer conditions or regions [25]. The survival of plants under harsh environmental conditions has often been attributed to the functioning of the membranes and, here, the degree of fatty acid unsaturation has been considered one of the most important factor controlling fluidity and membrane functioning [26]. In addition, phase separation of the lipids causes damage in biological membranes, and the polar moieties of the lipids have a great influence on the phase separation of the membranes [27]. In *Diapensia*, seasonal variation is found both in the contents of polar lipids of the membranes and their degree of fatty acid unsaturation (Tables 2–7).

In the leafy tops of *Diapensia*, seasonal fluctuations were most prominent in DGDG and PC, and their patterns were similar to those of chlorophyll; their contents increased in autumn and early winter and decreased in late winter and spring. In DGDG, the fluctuation was similar in both years, and in PC the increase was more prominent during the second year than in the first autumn and winter. Accordingly, during the coldest period of the year, the contents of DGDG and PC were high and the ratios of MGDG:DGDG and PE:PC low. Also, in evergreen, extremely hardy pine and spruce (one-year-old needles), the winter-time values for DGDG are high [28–30] due to increases in DGDG or, as in the case of the subarctic moss *Dicranum elongatum*, due to decreases in MGDG in autumn and winter [31]. In addition to *Diapensia*, lowered winter-time PE:PC values have been observed in other hardy evergreens, such as spruce [28]. Experimentally it has been shown that hardening [32, 33], as well as freezing temperatures [34], result in lowered MGDG:DGDG ratios. Increased levels of phospholipids, particularly PC, have been found either during winter or experimentally-induced hardening [35–37]. Hardiness-related changes in polar lipids caused by low temperature have been studied intensively and cited, e.g. in Sakai and Larcher [38] and Alberdi and Corcuera [11]. Accordingly, the level of lipids (DGDG and PC) forming the lamellar phase [39–42] was high during the coldest period of the year or in cold growth temperatures. For plants, like *Diapensia*, growing in a cold climate this may be of adaptational value in stabilizing the lipid bilayer against the influence of temperature fluctuations on the phase behaviour of membrane lipids [27, 40, 43].

In late winter and spring, not only the chlorophyll content decreased but also the contents of membrane lipids, particularly those of DGDG and PC (Tables 2 and 4). Most of these decreases occurred during the dehardening of *Diapensia* [2], a phenomenon which is well documented [38]. In *Diapensia*, the content of DGDG continued, however, to decrease beyond the dehardening time and it decreased during the whole summer. Interestingly, in the growing and greening new leaves (August 4) the DGDG content was high, however. Modifications occurred also in the contents of MDGD, a concomitant and temporary increase along with the chlorophyll content at the beginning of summer (June 1) and another increase at the end of August by the time of the highest potential for net photosynthesis (Table 1). These modifications may be due to several environmental factors, such as increasing/decreasing light intensities, day-length and drought, in addition to changes in temperature (cf [44–48]).

Recently, the unsaturation of membrane lipid fatty acids, particularly the introduction of the second double bond, have been shown to be crucial in protecting the photosynthetic machinery of cyanobacteria from photoinhibition under cold conditions [49]. In higher plants, the situation is more complicated, involving regulation of fatty acid unsaturation up to three or sometimes up to four double bonds. An increase in unsaturation often parallels hardiness, although this does not hold true for all plants [38]. In *Diapensia*, the fatty acids of MGDG and DGDG, the major thylakoid lipids, undergo changes but in an unexpected direction; the proportion of 18:3 acid was lower and, that of 18:2, higher during winter (Table 3). *Diapensia* is a plant with a long life-span and Kuiper [50] has suggested that correlation between the degree of unsaturation and cold-resistance might be characteristic of plants with a short life span but not of those with a long life span. Interestingly, induction of the second and third double bond into the fatty acids does not notably change their fluidity parameters or transition temperatures [51, 52]. Therefore, in the thylakoids of *Diapensia*, which has to tolerate temperatures below -30 or even $-40°$, a more important survival mechanism must be the decrease in the MGDG:DGDG ratio; for MGDG, the transition temperature is $-30°$, for DGDG $-50°$ [53].

A priori, it could be assumed that the degree of fatty acid unsaturation might be exceptionally high in plants growing in a cold climate. The fatty acid composition of various phospholipid fractions from *Diapensia* (Tables 5–7) do not, however, similarly to the alpine plants [24], deviate from plants grown in warmer conditions or regions [25]. The proportion of saturated fatty acids (16:0, the major) in the phospholipids of *Diapensia* is relatively high, ranging within the values found in alpine plants [24]. A similar trend is also found in the maritime antarctic, *Deschampsia antarctica* [54], in Chilean woody evergreens [55, 56] and Alaskan *Carex aquatilis* [57], where the proportion of 16:0 and/or 18:0 is surprisingly high at the expense of 18:2 and 18:3. Thus, highly unsaturated phospholipids seem not to be a requirement for plants growing in a cold climate.

The phospholipid fatty acids of *Diapensia* fluctuated seasonally (Tables 5–7). In PC, the proportion of 16:0 was lowest during the coldest period of the year and it was compensated for by a higher proportion of 18:2 and less clearly of 18:3. This is a typical cold-induced response [38] which is, however, not found in PE of *Diapensia*. Here, the lower wintertime values of 16:0 were compensated for by 22:0. The proportion of 18:2 in PE also fluctuated, but a clear pattern is not evident because the summer-time values of two subsequent summers differred so much relative to the winter-time values.

In PG, three fatty acids fluctuated seasonally (Table 7); for 16:0, the highest values were found in winter, the lowest in summer, for *trans*-16:1, the lowest values in winter, the highest in summer and for 18:2, the highest values in winter, the lowest in summer. In *Diapensia*, the overall proportion of *trans*-16:1 is conspicuously low, considerably lower than the values found, e.g. in alpine plants [24] or in evergreen woody plants from cold regions [30, 56] or in temperate-zone evergreens [58]. Hardening effects the content of *trans*-16:1, decreasing its amount in rye [59] and in both cold-sensitive and cold-resistant wheat varieties [60]. In pine needles, the proportion of *trans*-16:1 was, however, the same in unhardened and cold-hardened needles [30]. The percentage of 16:0 in PG of *Diapensia* was higher than the values found in alpine plants or evergreen Chilean conifers [24, 56], but within the same range as the values for temperate-zone evergreens [58]. Hardening of *Diapensia* in the autumn and winter increased the proportion of 16:0 in PG but not so markedly as in the case of pine needles [30] and a cold-resistant wheat variety [60]. Thus, there appears to be only slight seasonal modification of PG fatty acids in *Diapensia*. This is understable because *Diapensia* has to cope with low temperatures even in summer and the plants also develop under cold conditions. 16:0 and *trans*-16:1 have often been considered as saturated fatty acids, although the phase transition temperature of the latter is *ca* 10° lower than that of the former [61]. This does not, however, explain the lower level of *trans*-16:1 in winter. The low wintertime values, as well as the overall low level in *Diapensia*, might be explained by the finding that low temperatures which induce a decrease in *trans*-16:1 in cereals also influences LHCII organization and freezing-tolerance [59, 62].

Diapensia is a plant which is moderately hardy even in summer, i.e. it survives at $-9°$ [2], and hardening in autumn and early winter results in an extremely hardy plant. The sustaining of a hardened state, even in summer, explains the low net photosynthesis potential, as well as the relatively slight seasonal variations of individual fatty acids. Hardening from a moderately hardy state to an extremely hardy state induces accumulation of chlorophylls, DGDG and PC; these increases occur in response to lowered temperature and decreased light. The opposite occurs in late winter and early spring in response to increased temperature and light in the dehardening of *Diapensia*, and probably also partly in response to photoinhibition and to photo-oxidation which appears, however, to be reversible.

EXPERIMENTAL

Cushions of evergreen *D. lapponica* L. were collected in winter from the outdoor gardens of the Kevo Research Station (69°45′N, 21°0′E), where the plants were transplanted from northern Finland and Norway. From May to October, the plants were gathered directly from the growing site near the Kevo Research Station. During transportation by air to

Turku, the plants were kept cool (summer) or frozen (winter) and, upon arrival, the leafy tops were immediately subjected to lipid analysis. Lipids were sepd and analysed as described earlier [63, 64]. In brief, a few leafy tops (0.5 g) were boiled for 30 s in $CHCl_3$–*iso*-PrOH (2:1) and after cooling, homogenized in the dark in an ice-bath with a glass homogenizer. Lipids were first extracted with $CHCl_3$–MeOH (2:1) and then with $CHCl_3$, followed by MeOH, at room temp. Lipids were fractionated using CC (SilicAR CC-4, Mallinckrodt). Lipids of increasing polarity were eluted with $CHCl_3$ (neutral lipids), Me_2CO (glycolipids) and MeOH (phospholipids). Glycolipids and phospholipids were further sepd into classes by TLC (silica gel 60G) using $CHCl_3$–MeOH–HOAc–H_2O (68:12:8:2.8). Fatty acids in the frs were analysed by GC after extraction and saponification as Me esters and quantified using 17:0 as int. standard. Chlorophyll (a + b) contents were measured according to ref. (65). For CO_2-fixation, leafy tops were moistened with H_2O and kept in darkness at 5° for 20–24 hr. Thereafter, CO_2-exchange was measured with IRGA at saturating light (Osram HQI-E 400 W/DV) and at optimum temp. for net photosynthesis.

For dry wt determination, leafy tops were kept at 80° overnight. All values presented are means of at least three independent experiments ± standard error (se).

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