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EFFECTS OF TEMPERATURE AND LIGHT ON THE LIPIDS OF *SPHAGNUM MAGELLANICUM*

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Key Word Index—*Sphagnum magellanicum*; mosses; growth; polar lipids; fatty acids; temperature; light.

Abstract—The growth, lipid, protein and chlorophyll contents and fatty acid composition changes in polar lipids were studied in the moss *Sphagnum magellanicum* cultured aseptically under different light and temperature conditions. In dim light, changes in growth and the amounts of the studied organic components were larger than in darkness. The lipid and chlorophyll *a* and *b* amounts increased in dim light, while the actual growth declined. The chlorophyll *a*/*b* ratios increased slightly when the light decreased.

In normal light, temperature decrease was accompanied by a decrease in the amounts of linolenic acid and a corresponding increase in the amounts of palmitic acid in the glycolipids. When the change in temperature was combined with a change in light, decreasing light and temperature was accompanied by a decrease in linolenic and a reverse reaction for palmitic acids in the glycolipids. Often no linear pattern of change in the glycolipid fatty acids could be seen, and no profound unsaturation/saturation changes occurred.

When the temperature change was combined with a corresponding change in the length of the light period (in normal light), the optimum conditions for the formation of linolenic acid appeared to be 15° and 12 hr/light, although no clear patterns of change were seen regardless of the glyco- or phospholipid studied. The largest deviations from the 'normal' fatty acid composition under these conditions were seen generally at lower temperatures (0–5°) and shorter periods of light (3–6 hr light/day).

INTRODUCTION

The changes in growth and lipid composition of mosses caused by light and temperature conditions have been studied to some extent using material collected from natural habitats [1–3], but little work has been done using long-term aseptic moss cultures [4]. The effect of light and especially temperature on the lipid profiles of higher plants has been the subject of numerous investigations, mainly because of the connections which these features have with the temperature tolerance and hardening of economically important plants [5–12]. Some higher plant studies have also been done using cell cultures [13, 14].

In the present study, we have tried to evaluate the responses in growth, chlorophyll and lipid formation and lipid composition to both simultaneous and independent temperature and light changes in the moss *Sphagnum magellanicum* cultured aseptically, to make up, to some extent, for the lack of moss studies of this type. This moss species is common in Finland, especially in raised bogs. It is ecologically tolerant and grows mainly in nutrient-poor bogs, where it contributes to peat formation.

In our experiments, we tried to 'simulate' natural conditions, where light and temperature changes simultaneously and continuously interact with other ecological factors. Light and temperature are known to alter the lipid composition of many plants, but not many studies on *Sphagnum* exist [3]. The lipid compositions of various *Sphagnum* species are known [4, 15–17]. The fatty acids of *S. magellanicum* have, to a limited extent been studied [15, 17]. The fatty acid pattern of mosses is usually fairly complex [18], but this does not necessarily always apply to *S. magellanicum*. Earlier studies also demonstrate that caution should be exerted when interpreting the fatty acid patterns of mosses, as they obviously are a result of many genetic and environmental factors, and, thus, experiments are difficult to repeat successfully.

RESULTS

Growth, lipid, protein and chlorophyll contents of S. magellanicum *at various light/temperature conditions*

The results are presented in Table 1. At 25°, a remarkable similarity in result was observed between those mosses cultured at 'normal' light (75 μE/m^2/sec 18 hr/day) and those grown in complete darkness. The main differences were instead seen under 'dim' light conditions (1% of 'normal' light', where, particularly, the lipid level and chlorophyll (Chl) a concentration in the mosses rose markedly. A slight increase was also observed in the amount of chlorophyll (Chl) *b* and, accordingly, in the Chl *a*/*b* ratio. Under 'dim' light, the moss protein content did not change, but a decrease ($< 50\%$) was observed in both fr. and dry wts.

At 15° the mosses grew much more slowly (Table 1). In other respects the results resembled those obtained at 25°, but the difference between 'dim' light and 'normal' light/darkness was much greater, and not all factors returned to 'normal' light levels in darkness. Under 'dim'

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Table 1. Results of the growth experiments on *S. magellanicum* at 25° and 15° (absolute values)

Temp (°C)	Light intensity: 75 μE/m²/sec	0.75 μE/m²/sec	0 μE (darkness)	
25	10.8***	6.4***	8.9***	fr. wt
15	3.6	1.0***	1.3***	(g)
25	275***	146	238***	dry wt
15	136	20***	47***	(mg)
25	2.7**	6.3*	1.6***	lipid
15	4.4	9.3***	5.5	(% dry wt)
25	24.6**	20.0**	21.6*	protein (mg/g
15	36.9	92.7***	84.1***	dry wt)
25	0.21***	0.47***	0.19***	Chl *a* (mg/g
15	1.95	10.68***	6.06***	dry wt)
25	0.19***	0.30***	0.16***	Chl *b* (mg/g
15	1.16	5.98***	2.78***	dry wt)
25	1.09*	1.58	1.19	Chl *a*/Chl *b*
15	1.68	1.78	2.17*	

Average results based on 6–9 independent samples. All results were statistically tested with Student's *t* test: * = $p \leq 0.05$. ** = $p \leq 0.01$. *** = $p \leq 0.001$. Results at 15° 75 μE/m²/sec were always used as a standard for the *t* test.

light the Chl *a* level rose five fold, and in darkness remained at a level three times that seen under normal light. Chl *b* showed a similar response.

At 15° the lipid and protein levels also increased, but the fr. and dry wts decreased under dim light, and the dry wt remained at a lower level also in darkness. It seems that the 'dim' light intensity caused larger changes than darkness in the limited time space used. Darkness would certainly cause more profound changes, if present for a longer time.

Fatty acid compositions of S. magellanicum *polar lipids*

The results are presented in Table 2. When compared with the earlier incomplete surveys [15, 17] both similarities and differences can be found. As to the glycolipids, the results obtained at 65 μE/m²/sec are closer to earlier results concerning *Sphagnum* glycolipid fatty acids. It was found that the main fatty acids in both monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG) were palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2ω6) and α-linolenic acid (18:3ω3), the last named being the most important of these, although the amount found was surprisingly low. In MGDG, small amounts of arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and nervonic acid (24:1) were also found. The amounts of C_{20} acids were quite small in both glycolipids, and this was also true of the unsaturated C_{16} acids. The *S. magellanicum* glycolipid fatty acid composition appears to be quite simple and is similar to that found in *Ceratodon purpureus* [19], except for the scarcity of 16:3 in *S. magellanicum*. There seemed to be some clear differences between MGDG and DGDG. MGDG contained more 18:0, 18:1, 20:4ω6, 20:5ω3 and 24:1, while the amounts of 16:0 and 18:3ω3 were larger in MGDG.

The fatty acids of the phospholipids of *S. magellanicum* which we studied, phosphatidyl choline (PC) and phosphatidyl glycerol (PG), were quite different from the glycolipids. The most important acids were, to some extent, identical with those of the glycolipids, but the phospholipids contained much more 18:0, 18:1 and C_{20}

Table 2. Fatty acid composition of *S. magellanicum* polar lipids cultured at 25° in 65 μE/m²/sec light (MGDG, DGDG) and 55 μE/m²/sec light (PC, PG) 18 hr/day

Fatty acid	MGDG	DGDG	PC	PG
14:0	0.5	tr	1.9	tr
14:1	tr	—	—	tr
14:3+16:1	0.8	—	4.6	tr
15:0	tr	0.5	2.1	tr
16:0	11.7	17.7	24.8	17.6
tr-16:1ω13	—	—	—	4.0
16:2	tr	tr	1.7	tr
16:3	1.0	0.5	—	—
17:0	0.6	0.5	1.2	1.4
18:0	3.8	1.2	7.5	11.8
18:1	5.7	1.1	21.5	13.0
18:2ω6	18.1	16.3	11.9	11.2
18:3ω3	33.9	60.7	5.9	6.9
18:3ω6	1.1	tr	tr	0.7
20:0	tr	tr	0.5	tr
20:1	tr	—	0.7	1.3
20:2ω6	tr	tr	1.4	2.1
20:3ω6	tr	tr	1.2	6.1
20:4ω6	4.1	0.7	1.6	3.4
20:5ω3	3.2	1.7	0.6	6.4
22:2	tr	—	–	—
23:0	—	tr	2.0	—
24:0	—	tr	2.4	0.7
24:1	3.6	—	1.4	—

Average of 4–6 independent determinations.
tr = amount < 0.5%.

acids, while the 18:3ω3 amounts were relatively small. The amount of *trans*-hexadecenoic acid (16:1ω13), an acid peculiar to PG, was quite low. This feature, however, perhaps is not uncommon in mosses [19].

Except for 16:1ω13, there were also other differences between PC and PG. PC contained more C_{14}–C_{15} acids, 16:0, 18:1 and 24:0, while PG was richer in 18:0 and the C_{20} unsaturated acids, of which especially 20:3ω6 and 20:5ω3 should be mentioned. All of the lipids studied also contained small amounts of γ-linolenic acid (18:3ω6).

Effects of different temperatures on the glycolipid fatty acid compositions in S. magellanicum *in normal and dim light*

The results are presented in Tables 3 and 4 (normal light), 5 and 6 (dim light). It should be noted that the results at 25° were obtained in normal light and thus are identical to those at 25° in Tables 3 and 4; the results in Tables 5 and 6 show a gradual transition from dim to normal light. Of the different temperatures used, 15° was regarded as 'normal', considering the usual growth period temperatures in Finland.

In normal light (65 μE/m²/sec 18 hr/day), the main effects of temperature seemed to be shown in the amounts of 16:0 and 18:3ω3 (Tables 3 and 4). Particularly in DGDG, the amounts of 16:0 increased wiuh decreasing temperature, while the reverse pattern was seen in the amounts of 18:3ω3. In MGDG, 16:0 seemed to be at a maximum at 10° and a corresponding minimum of 18:3ω3 was observed. In MGDG, the amount of 16:3 increased with temperature; the same result was obtained for 18:2ω6 in both glycolipids. As to the reported unsaturating effect of low temperatures, only 20:5ω3 seemed to conform slightly to that concept. However, no large desaturation changes were seen in either direction.

In the transition from very dim to normal light, accompanied by rising temperature (Tables 5 and 6), the amounts of 16:0 decreased markedly with increasing light and temperature; in MGDG, this applied also to the amounts of 18:0. A reverse reaction was obtained for

Table 3. Composition of *S. magellanicum* MGDG fatty acids at different temperatures and in 65 μE/m²/sec 18 hr/day light ('normal light'). Only the most important fatty acids are included

	Temperature (°)					
Fatty acid	0	5	10	15	20	25
14:0	0.7	1.3	2.1	0.6	tr	0.5
16:0	20.4	22.3	24.9	23.9	20.8	11.7*
16:3	3.0	5.9	3.2	5.0	5.1	4.7
18:0	3.3	4.6	3.7	5.5	2.2***	3.8
18:1	7.8	7.2	8.0	7.0	5.7	5.7
18:2ω6	16.0	14.8	12.7	13.0	19.6*	18.1*
18:3ω3	29.4	25.4	18.5	26.6	29.7	33.9
20:4ω6	tr	1.6	1.0	tr	0.5	4.0**
20:5ω3	0.7	4.7*	2.7	1.4	2.1	3.2
23:0	1.4	2.2	2.4	2.3	tr	—

Average of 4–6 independent samples. For the *t* test, results at 15° were held as a standard. tr = amount < 0.5%.

Table 4. Composition of *S. magellanicum* DGDG fatty acids at different temperatures and in 65 μE/m²/sec light 18 hr/day ('normal light'). Only the most important fatty acids are included

	Temperature (°)					
Fatty acid	0	5	10	15	20	25
14:0	0.8	0.7	0.8	0.6	—	—
16:0	21.3	19.2	19.1	15.3	13.4	17.7
16:3	—	0.8	0.5	tr	0.5	0.5
18:0	2.2	3.4	2.3	4.0	1.3***	1.2*
18:1	3.0	3.9	2.8	2.7	1.5	1.1*
18:2ω6	10.6	8.0***	7.8**	10.9	13.9**	16.3***
18:3ω3	47.7	48.5	49.9	54.8	61.5	60.7
20:4ω6	2.1	1.2	0.9	1.3	0.8	0.7
20:5ω3	3.1	3.5	1.8	—	—	1.7
23:0	3.1	4.4	1.7	1.3	—	tr

Average of 4–6 independent samples. For the *t* test, results at 15° were held as a standard. tr = amount < 0.5%.

Table 5. Composition of *S. magellanicum* MGDG fatty acids at different temperatures and during a gradual transition from dim to normal light (0–10°: 0.65 $\mu E/m^2/sec$, 15–20°: 11 $\mu E/m^2/sec$, 25°: 65 $\mu E/m^2/sec$). Only the most important fatty acids are included

	Temperature (°)					
Fatty acid	0	5	10	15	20	25
14:0	1.6	0.7	tr	tr	tr	0.5
16:0	22.9	15.3	7.7*	5.9*	6.3**	11.7*
16:3	2.7	5.7	8.0	8.7*	7.7	4.7
18:0	4.6	2.3**	1.4***	1.2***	1.8***	3.8
18:1	6.6	4.1	2.2**	3.5	2.8*	5.7
18:2ω6	15.8	20.9**	17.9*	19.1**	18.4*	18.1*
18:3ω3	22.7	35.9	45.3**	43.8**	40.7*	33.9
20:4ω6	3.3**	3.3**	5.5***	4.2**	4.2**	4.1**
20:5ω3	2.4	1.4	2.4	tr	2.2	3.2
23:0	tr	tr	0.9	tr	5.3*	—

Average of 4–6 independent samples. For the *t* test, MGDG results at 15° and 65 $\mu E/m^2/sec$ were held as a standard (Table 3).
tr = amount < 0.5%.

Table 6. Composition of *S. magellanicum* DGDG fatty acids at different temperatures and during a gradual transition from dim to normal light (0–10°: 0.65 $\mu E/m^2/sec$, 15–20°: 11 $\mu E/m^2/sec$, 25°: 65 $\mu E/m^2/sec$). Only the most important fatty acids are included

	Temperature (°)					
Fatty acid	0	5	10	15	20	25
14:0	0.5	tr	tr	tr	tr	—
16:0	18.8	21.9*	14.9	13.2	14.4	17.7
16:3	tr	0.5	1.1	tr	tr	0.5
18:0	1.5**	1.2**	1.2**	1.3*	1.2***	1.2*
18:1	1.9	1.5	0.9***	1.7	1.6*	1.1*
18:2ω6	19.0***	19.7***	19.1***	19.7***	16.3***	16.3***
18:3ω3	47.2	46.1	52.6	56.6	52.1	60.7
20:4ω6	tr	1.3	1.3	0.7	0.6	0.7
20:5ω3	tr	1.2	0.7	tr	0.6	1.7
23:0	tr	tr	1.4	tr	2.2	tr

Average of 4–6 independent samples. For the *t* test, DGDG results at 15° and 65 $\mu E/m^2/sec$ were held as a standard (Table 4).
tr = amount < 0.5%.

18:3ω3 in both glycolipids. For many fatty acids, however, no linear response to light and temperature changes could be observed.

As an overall picture of the differences in results between the normal and dim light conditions, it can be stated that the combined changes of light and temperature seemed to influence the fatty acid compositions more than temperature changes alone. However, even the combinations failed to produce the features regarded as typical for low temperature effects (powerful unsaturation, increase of especially 18:3ω3 and decrease of 16:0) described in other studies [7, 20]. The results presented here clearly show that unsaturation at low temperatures cannot be regarded as a universal feature. The results stress both the complicated nature of fatty acid formation and the differences between different plant species and types. The features which we observed are, however, by no means unique and will be discussed later (see Discussion).

Combined effects of different temperatures and illumination periods on S. magellanicum *polar lipid fatty acid compositions*

The results are presented in Tables 7 (MGDG), 8 (DGDG), 9 (PC) and 10 (PG). As to the statistical calculations, 15° and a 12 hr light period per day were regarded as 'normal' conditions.

In the glycolipids it can be observed that the degree of unsaturation of both MGDG and DGDG was highest under the above mentioned 'normal' conditions. In spite of the alleged tendency towards unsaturation at lower temperatures, no great differences in unsaturation were

Table 7. Composition of *S. magellanicum* MGDG fatty acids in mosses cultured at different temperatures and light periods at a light intensity of 55 $\mu E/m^2/sec$. Only the most important fatty acids are included. T = temperature (°), L = length of light period per day

Fatty acid	T25/L18	T20/L15	T15/L12	T10/L9	T5/L6	T0/L3
14:0	2.0*	2.1**	0.6	1.5	1.3	1.1
16:0	22.3	20.3	18.6	21.2	24.1**	18.0
16:3	0.8	1.6**	0.5	1.4	4.2**	2.6***
18:0	10.8	10.5	10.7	14.5	19.2*	13.5
18:1	11.6	8.0*	11.0	13.9	9.8	9.3
18:2ω6	9.6	9.1	14.3	7.3	7.4*	7.7
18:3ω3	16.8	25.1	24.4	16.4	12.0**	26.4
20:4ω6	1.6	1.7	2.0	2.1	1.0**	3.1
20:5ω3	—	—	tr	tr	tr	tr
23:0	2.9	0.6	1.8	0.6	0.6	1.0

Average of 4–6 independent samples. For the *t* test, results of 'T15/L12' were held as a standard.

tr = amount < 0.5%.

Table 8. Composition of *S. magellanicum* DGDG fatty acids in mosses cultured at different temperatures and light periods at a light intensity of 55 $\mu E/m^2/sec$. Only the most important fatty acids are included. T = temperature (°), L = length of light period per day

Fatty acid	T25/L18	T20/L15	T15/L12	T10/L9	T5/L6	T0/L3
14:0	1.3***	1.2**	tr	1.6***	1.5**	0.8
16:0	24.0***	16.5	16.9	21.8**	20.4*	18.9
16:3	—	—	—	—	—	—
18:0	6.7	5.8	4.8	8.5**	11.3***	8.1*
18:1	5.7	5.6	3.9	6.6*	8.5**	5.2
18:2ω6	11.4	10.9	11.7	8.3***	7.6***	9.7**
18:3ω3	39.2**	48.8	52.0	36.0**	30.7***	44.2
20:4ω6	1.0	0.9	1.5	1.4	3.2	1.3
20:5ω3	—	2.0	—	—	2.0	—
23:0	tr	1.0	0.7	0.6	0.6	0.5

Average of 4–6 independent samples. For the *t* test, results of 'T15/L12' were held as a standard.

tr = amount < 0.5%.

Table 9. Composition of *S. magellanicum* PC fatty acids in mosses cultured at different temperatures and light periods at a light intensity of 55 $\mu E/m^2/sec$. Only the most important fatty acids are included. T = temperature (°), L = length of light period per day

Fatty acid	T25/L18	T20/L15	T15/L12	T10/L9	T5/L6	T0/L3
14:0	1.9	1.6	0.9	1.5	1.5	2.2
16:0	24.8	22.7	27.8	20.2	24.5*	27.3
16:1+14:3	4.6	2.7	2.7	2.4	3.4	4.0
18:0	7.5	10.0	6.2	6.7	5.7	6.7
18:1	21.5	14.2	12.7	11.8	11.5	12.1
18:2ω6	11.9*	11.0*	22.3	16.5	16.8*	13.9*
18:3ω3	5.9*	7.4	11.5	11.5	10.4	9.5
20:4ω6	1.6*	1.6**	3.6	3.0	2.9	2.4
20:5ω3	1.1*	2.0*	tr	0.6*	1.2*	tr
23:0	2.0	2.6*	0.8	tr	1.2	—

Average of 4–6 independent samples. For the *t* test, results of 'T15/L12' were used as standard.

tr = amount < 0.5%.

Table 10. Composition of *S. magellanicum* PG fatty acids in mosses cultured at different temperatures and light periods at a light intensity of 55 $\mu E/m^2/sec$. Only the most important fatty acids are included. T = temperature (°), L = length of light period per day

Fatty acid	T25/L18	T20/L15	T15/L12	T10/L9	T5/L6	T0/L3
14:0	0.3	1.1	1.1	1.1	1.2	0.5
16:0	17.6	20.7	20.0	18.8	12.2	16.1
*tr*16:1ω13	4.2	3.4**	5.7	6.7	1.9***	3.9*
18:0	11.8*	12.9*	5.2	8.7	9.1*	7.4
18:1	13.0	14.7	10.5	12.3	15.4	8.7
18:2ω6	11.2	11.1	8.1	9.6	7.1	8.5
18:3ω3	6.9	3.9*	8.9	4.2*	2.6**	8.7
20:4ω6	3.4	tr*	6.7	2.0	5.9	8.6
20:5ω3	6.4*	0.9	2.4	tr	0.9	3.4
23:0	—	—	1.4	0.7	1.4	5.1*

Average of 4–6 independent samples. For the *t* test, results of 'T15/L12' were used as standard.

tr = amount < 0.5%.

found. The most common fatty acids in MGDG and DGDG, 16:0 and 18:3ω3, showed no clear maxima under any conditions tested. Although the 16:0 levels were high at 25°/18 hr, they were also high at 5°/6 hr and 10°/9 hr in both glycolipids. The amounts of 18:3ω3 were largest at 20°/15 hr and 15°/12 hr, but also at 0°/3 hr; perhaps the amount would have been larger at this temperature, but the short light period prevented this. The amounts of 18:0 were slightly elevated at 10°/9 hr and 5°/6 hr in both glycolipids. The 18:2ω6 amounts showed only minor changes. The fatty acids of DGDG were always slightly more unsaturated than those of MGDG.

The phospholipids investigated, PC and PG, were also only moderately unsaturated. The changes caused by the different light/temperature conditions tested were not very dramatic and no linearity could be found. At 0°/3 hr, the PG fatty acid levels were fairly unusual, which perhaps indicates that these conditions are enough to affect even *S. magellanicum*, which is in many respects hardy. In PG, the higher temperatures and longer light periods seemed to favour the formation of 16:0 and 18:0, but this was not found in PC. Instead, in PC the maximum level of 18:2ω6 occurred at 15°/12 hr. In PG, 18:3ω3 and 20:4ω6 showed two maxima, at 15°/12 hr and 0°/3 hr. The acid 23:0 behaved quite oddly: in PC larger amounts were found at long light periods/high temperatures, but in PG the reverse occurred. *Trans*-16:1ω13 was found in PG in the largest amounts at 15°/12 hr and 10°/9 hr. Its small amount in PG indicate that the molecular species of PG, PG-16:0/16:1, which is common in many plants, is not very prominent in *S. magellanicum* [21].

The amount of 18:0 was higher at higher temperature/longer light period conditions in both PC and PG. In most cases it seemed that the extreme conditions used (25°/18 hr and 0°/3 hr) caused larger deviations from the chosen 'normal' (15°/12 hr), which is closest to Finnish summer conditions. The results found here were similar to those in Tables 3–6 in not exhibiting any linear trends or extensive saturation or unsaturation. In our opinion, this reflects the good tolerance of *S. magellanicum* to different light and temperature conditions. It also shows that its adaptation is not primarily due to direct changes in its polar lipid fatty acid composition.

MGDG/DGDG ratios

The ratios determined at different temperature/light period conditions are presented in Table 11. They remained between 1.0–1.7 in all experiments, but rose slightly during lower temperatures/shorter light periods. The values obtained are close to those observed earlier in *Ceratodon* and *Pleurozium* [2]. The rise in the MGDG/DGDG ratio in mosses in winter conditions has also been mentioned earlier [22].

DISCUSSION

The fr. and dry wt determinations of *S. magellanicum* at 25 and 15° showed that the former temperature supports a *ca* three-fold growth compared with the latter at 75 $\mu E/m^2/sec$ light. Dim light (0.75 $\mu E/m^2/sec$) or darkness changed the ratio to a six- or eight-fold one, respectively; growth is thus clearly suppressed. At 15°, the lipid, protein and chlorophyll levels are markedly larger than at 25°. This a well-documented phenomenon; lipids often accumulate at lower temperatures in plants [3, 23].

Table 11. MGDG/DGDG ratio in *S. magellanicum* cultured at different temperatures and light periods at a light intensity of 55 $\mu E/m^2/sec$. T = temperature (°), L = length of light period per day

T/L	MGDG/DGDG
T25/L18	1.0
T20/L15	1.2
T15/L12	1.0
T10/L9	1.4
T5/L6	1.7
T0/L3	1.5

Average of 4–6 independent determinations.

Probably no large changes in photosynthesis occurred as a result of temperature change, since the Chl *a*/*b* ratios remained quite stable under all conditions.

The effect of light intensity is more interesting. It was observed that dim light (0.75 $\mu E/m^2/sec$) caused an increase, particularly in the lipid and chlorophyll levels, at both temperatures and also in the amounts of protein at 15°. It is known that high light intensities lower the amount of chlorophyll in mosses [1], and perhaps the dim light was favourable for chlorophyll formation. This phenomenon has also been observed in *Euglena* [24]. Higher light intensities are also known to enhance lipid decomposition in aging cells [25]. The results of the present work clearly showed that dim light produced surprisingly far-reaching effects on aseptically grown *S. magellanicum* and that these effects are even more pronounced at 15 than at 25°. The lower temperature is closer to the actual growth season temperatures under Finnish climatic conditions.

The work of Corrigan *et al.* [15] describes the total fatty acid profile of *S. magellanicum* collected from a bog. Their results differ to some extent from those presented in this work (Table 2) for polar lipids. Unfortunately, they do not state the month of collection of the moss, and no separation of polar lipids has been made. For this reason, the results are not directly comparable. The total lipid fatty acid composition of aseptically cultured *S. magellanicum* has been reported [17]. The profound differences in the lipid profile given in the two mentioned works [15, 17] is perhaps best explained as a result of differences in growth conditions. Corrigan *et al.* [15] reported a very low amount of 18:3ω3 in *S. magellanicum*, and the present study shows the same result. Both contrast with earlier results [17]. The heterogeneity of the moss strains studied may partly explain these differences. To our knowledge, the present paper is the first one where the polar lipid fatty acid compositions of *S. magellanicum* have been surveyed separately.

The polar lipid fatty acid compositions of *S. magellanicum* observed here (Table 2) show a quite marked saturation grade. The amounts of 18:3ω3 were fairly low. The scarcity of *trans*-16:1ω13 in PG is noteworthy: usually its amounts are larger as a result of the common occurrence of the molecular species 16:0/*tr*16:1-PG and C18/*tr*16:1-PG [21]. Chilling-resistant species are reported to be low in 16:0/16:0-PG and 16:0/*tr*16:1-PG [11]. The formation of *tr*16:1ω13 is strongly increased by light, so in darkness or in dim light its amounts usually are low [26]. As to the acids 20:4 and 20:5, they were not measured in high amounts in this study compared with those previously reported [17]. Generally, a more saturated fatty acid pattern emerged from this study.

The large amounts of 18:0, 18:1 and particularly of 16:0 observed in *S. magellanicum* glycolipids in dim light and at low temperatures (0.65 $\mu E/m^2/sec$, 0–5°) are not very easily explained. It may be that dim light suppressed the formation of 18:2 and 18:3 and that, in this way, it reversed the unsaturating effect of the low temperature. No overall desaturation was observed even at 0–5°. On the contrary, the unsaturation appeared to be somewhat greater at 10–15° and in fairly in dim light (11 $\mu E/m^2/sec$). It seems that the statement of ref. [14] is true, that no single physical feature can totally control the ability of plant cells to desaturate fatty acids. Much more important in aseptic cultures is the oxygen concentration in the culture medium and in the vessel [5, 13, 27, 28]. In this study, however, the mosses grew on an agar medium, while a liquid medium was used in [17]. It was assumed that neither of them restricted the oxygen supply to the mosses.

The concept that unsaturation invariably results from a decrease in temperature seems to be questionable; sometimes it has been observed that the amount of 18:3ω3 in plants decreases at low temperatures and that different plant species may respond in opposite directions to the same external variation in culture conditions [28]. In *S. magellanicum*, our results seem to be in accord with this; no tested temperature/light intensity combination was able to cause very profound desaturation in the fatty acid compositions of MGDG and DGDG. The oleyl-CoA-desaturase seems to be enhanced in cold and in dim light, and perhaps this is reflected in the results we observed in the glycolipid 18:1 [5, 13, 27, 28]. Trémolières *et al.* [28] state that the tendency observed sometimes in cultures towards increased desaturation of polar lipids at low temperatures seems to be a result of genetic factors in the plant cells and of elevated oxygen concentration in the cultures rather than a direct result of low temperature. The necessary oxygen is perhaps produced by continuing photosynthesis [5]. It is difficult to say whether the oxygen concentrations were sufficient in our cultures, as no gas exchange was provided for. This could perhaps partly suppress the unsaturation at low temperatures.

Temperature/light conditions may also either accelerate degradation and/or decrease synthesis of some fatty acids [2]. In *Pleurozium* and *Ceratodon* the changes in glycolipid fatty acid compositions were likewise quite small in response to light conditions [2].

If we examine the changes in polar lipid fatty acid compositions of *S. magellanicum* in varying temperature/light period combinations while the light intensity itself was left unchanged (Tables 7–10), it can be seen that the above comments generally also apply to these results. No tested condition was able to change the fatty acid composition radically in the studied polar lipids, MGDG, DGDG, PC and PG. This is reminiscent of some earlier results, e.g. those on alpine plants [12], where, likewise, no large changes could be found compared with plants grown in more favourable climates. Of course, *S. magellanicum* must be regarded as a hardy species and not directly comparable to higher plants. The hardiness does not, however, seem to be simply a result of a capacity to adjust the fatty acid compositions in response to changing climatic conditions. This characteristic has earlier been observed in many plants, e.g. sycamore cells in culture [14], wheat [29, 30], and the bark of poplar and black locust trees [31, 32]. These features are probably genetically determined, and it can be stated that the lipid fatty acid compositions in plants are affected both by the genotype and the environment [33]. The adaptive value of environment-induced lipid changes may often be doubtful [11]. More attention has been directed lately towards the effects of temperature on the membrane fluidity of plants. This is a wide field which, for the most part, is poorly investigated, but it can be assumed that in *S. magellanicum* at least, partial fluidity is maintained even under very severe natural conditions.

EXPERIMENTAL

Plant material. Aseptic gametophyte cultures of *S. magellanicum* Brid. were used, grown on a 0.9% solid synthetic agar

medium [17, 34]. This strain originates from surface-sterilized moss spores [35]. In nature, *S. magellanicum* is abundant in the drier parts of raised nutrient-poor bogs and in forest hollows in Finland. In bogs, it contributes to peat formation. The moss was cultured in 100 ml Erlenmeyer flasks stoppered with cotton and aluminium foil. The flasks were cultured in climate chambers under Airam L 40 W Floralux tubes for one month. After this, the initial light/temp. conditions were changed, and samples were taken usually once a week, simultaneously with the change in the light/temp. conditions.

Light and temperature conditions. Three different light/temp. combination types were used. Combination 1. A temp of 15 or 25° and a light intensity of 75 $\mu E/m^2/sec$, 18 hr/day for one month was used initially. Then the light intensity was decreased either to 0.75 $\mu E/m^2/sec$ or to darkness, while a third part of the cultures were allowed to grow continuously in 75 $\mu E/m^2/sec$. The temp. and the length of light period were not changed. The cultures were grown for one month under the changed conditions. Combination 2. An initial temp. of 25° and a light intensity of 65 $\mu E/m^2/sec$, 18 hr/day, for one month. Then both temp. and light intensity were changed at one week intervals as follows: 20° and 10 $\mu E/m^2/sec$, then 15° and 0.65 μE, then 10° and 0.65 μE, then 5° and 0.65 μE, then 0° and 0.65 μE. The light period was kept at 18 hr/day. Combination 3. An initial temp. of 25° and a light intensity of 55 $\mu E/m^2/sec$, 18 hr/day, for one month. Then both the temp. and the length of light period were changed at one week intervals as follows: 20° and 15 hr/day, then 15° and 12 hr/day, then 10° and 9 hr/day, then 5° and 6 hr/day, then 0° and 3 hr/day. The light intensity was kept at 55 $\mu E/m^2/sec$. Samples from the cultures were always harvested when conditions were changed.

Growth and chemical analyses. Cultures of type 1 were analysed to determine fr. and dry (lyophilized) wt, total lipid amount [17], total proteins and Chl *a* and *b*. Proteins were extd with 50 mM NaOH and amounts were determined spectrophotometrically using the Folin–Ciocalteu reagent (Orion). Chlorophylls were extd with Me_2CO–H_2O (9:1, with 0.01% w/v $MgCO_3$), and their amounts determined spectrophotometrically according to ref. [36]. The Chl *a*/*b* ratios were calculated.

Lipids were extd from lyophilized samples of type 2 cultures with $CHCl_3$–MeOH (2:1), fractionated on a silicic acid column and the glycolipids sepd by TLC [37]. The MGDG and DGDG fatty acids were converted to Me esters [38] and subjected to GC/MS analysis.

Lipids were extd from fr. samples of type 3 cultures as above and the polar lipids MGDG, DGDG, PC and PG subjected to fatty acid analysis on GC/MS as above. The MGDG/DGDG ratios were determined spectrophotometrically [37].

GC and GC/MS analysis. Me esters were analysed on a WCOT capillary column Silar 10C 30 m (ID 0.3 mm) with an injection vol. of 2 μl, temp. prog. 120–185° at 3°/min with a total run time of 1 hr. Injection and detector heater temps were 210°. N_2 at 30 ml/min was used as carrier gas. For MS samples were run on a SP-1000 WCOT glass capillary column of 50 m (ID 0.33 mm) with an initial temp. of 60°. After solvent elution the column was rapidly heated to 150°, then the temp. was prog. to 210° at 4° min. The interface temp. was 260°, the ionization current 300 μA and voltage 30 eV. FID was used in all GC [37]. ECL values were used to determine the double bond positions in the polyunsaturated fatty acid Me esters [37, 38].

Statistics. Replicates in culture type 1 analyses numbered 6–9; in culture types 2 and 3, 4–6 (independent samples). The fatty acid compositions are expressed as % of total fatty acids. The statistical significance of all results were tested with Student's *t* test.

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