

Seasonally Induced Changes in Acyl Lipids and Fatty Acids of Chloroplast Thylakoids of *Pinus silvestris*¹

A CORRELATION BETWEEN THE LEVEL OF UNSATURATION OF MONOGALACTOSYLDIGLYCERIDE AND THE RATE OF ELECTRON TRANSPORT

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ABSTRACT

Current-year needles were sampled regularly from an approximately 20-year-old natural stand of *Pinus silvestris*. Chloroplast thylakoids were isolated. The electron transport capacities of photosystem II + photosystem I, as well as of the partial photoreactions, were measured. The amounts and the fatty acid compositions of monogalactosyldiglyceride, digalactosyldiglyceride, and sulfolipid of the thylakoids were analyzed. The fatty acid composition of the phospholipids (total) was also determined.

There was a 2-fold increase in the content of the galactolipids and the sulfolipid in the thylakoid preparations during the autumn. Except for monogalactosyldiglyceride that decreased in content during early winter, the content of the other two lipids remained high during the winter, until spring when their content fell toward a low summer level. The ratio of monogalactosyldiglyceride to digalactosyldiglyceride was lower in thylakoids isolated from frosthardened (autumn, winter) than in those isolated from unhardened (summer) needles.

Only monogalactosyldiglyceride showed pronounced and significant seasonal variations in the molar ratio of unsaturated to saturated fatty acids. It was low in the winter, and it increased strongly during the spring. The highest ratio was observed in September, prior to the first night frosts, after which a steady decrease toward the low winter value occurred. This decrease is thought to be a reflection of membrane damages (photooxidation of membrane components, including polyunsaturated fatty acids), as earlier seen reflected in chlorophyll destruction, electron transport inhibition, and structural changes.

There was very good correlation between the seasonal variations in the capacity for electron transport from water to NADP and the level of unsaturation of monogalactosyldiglyceride ($r = 0.93$). It has been shown earlier that the winter inhibition of electron transport preferentially occurs at the lipophilic electron transport carrier plastoquinone and that the free miscibility of plastoquinone and monogalactosyldiglyceride is prevented in saturated monogalactosyldiglyceride. Therefore, it is hypothesized that the decreased level of unsaturation of monogalactosyldiglyceride reduces the mobility of plastoquinone in the thylakoids, resulting in an inhibition of the electron transport by way of plastoquinone.

photosynthesis (22). Studies of pine seedlings exposed to artificial frosthardenening (photoperiod, 8 h; 3°C) followed by winter stress in light at sub-zero temperature (photoperiod, 8 h; -5°C) showed that the decay and final winter inhibition of net photosynthesis was paralleled with an inhibition of the photosynthetic electron transport, whereas the activity of extracted ribulose-1,5-bisphosphate carboxylase was much less inhibited (18). It has also been shown (11, 13, 19) that the primary site of winter-induced inhibition of electron transport in pine is at the site of plastoquinone, although the capacities of the partial reactions of the two photosystems also decayed during prolonged winter stress. Senger and Beck (25) have also reported winter inhibition of electron transport in chloroplasts isolated from *Picea abies*.

Seasonal variations in the Chl content of pine and other conifers have been dealt with in other publications (17). Analyses of Chl-protein complexes have shown that the winter-induced destruction of Chl is much more severe in the reaction center Chl *a* antennae of the two photosystems than it is in the light-harvesting Chl *a/b* antenna, which lacks reaction centers (20). However, the distribution of the major polypeptides, as separated and identified by SDS polyacrylamide gel electrophoresis, did not vary significantly over the year, except that the proteins were a little less soluble in winter than they were in summer and that the apoprotein of the reaction center Chl antenna of PSI appeared when its Chl was destroyed during the winter (21).

Seasonally induced changes of the chloroplast ultrastructure have also been frequently studied in conifers (17). Our analyses of pine essentially support earlier observations. The onset of frost hardening during autumn was accompanied by swelling of the chloroplasts; the following winter stress-induced effects on thylakoid membrane function and content of Chl-protein complexes were paralleled with a gradual disorganization of the thylakoid membrane structure, resulting in mostly randomly oriented membrane fragments by the end of the winter (13). The described winter inhibition and destruction in pine chloroplasts are most likely caused by photooxidation (17). This is supposed to occur when the Chl antennae absorb excess light, because photosynthesis is largely inhibited by low temperatures. The damage of the thylakoids observed during winter stress should not be confused with the initial induction of frost hardiness, which occurs prior to the winter stress damage (18).

Low temperature acclimation and adaptation of plants very often result in an increased unsaturation of the membrane acyl lipids; this change has been correlated with a decrease in the phase transition temperature of the lipids from a liquid-crystalline to a solid state (10). The low temperature-induced accumulation of polyunsaturated fatty acids has been ascribed to an activation of the desaturation system by increased concentration of oxygen (6) or to an activation of the desaturase activity when the membrane

We have published a series of papers on climatically induced changes of the function and organization of the chloroplast thylakoids of *Pinus silvestris*. The studies recently have been reviewed (17). Prolonged exposure of conifers to near- and sub-zero temperatures causes a well-known depression of the rate of net

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fluidity decreases at low temperatures (26). Whether an increased level of unsaturation in the chloroplast thylakoids also are of importance for the survival at sub-zero temperatures of freezing-tolerant plants can be questioned, as the chloroplast thylakoids of higher plants are so fluid that, even in the tender state, they do not undergo phase transitions before well below 0°C (15). However, there are reports that hardening and dehardening affects the proportion between MGDG² and DGDG in *Pinus silvestris* (2) as well as the lipid unsaturation of chloroplast thylakoids of *Pinus strobus* (4).

To investigate whether such changes can be correlated with changes in the function and organization of the chloroplast thylakoids of pine, we show how the amount of acyl lipids and their distribution of fatty acids vary over the year. Special attention is paid to the good correlation found between the seasonal variations in the rate of electron transport and the level of unsaturation of MGDG.

MATERIALS AND METHODS

Current-year needles (1–2 m above ground) were regularly sampled during a period of 1 year (1978–1979), and on four different occasions during 1980, from one individual of an approximately 20-year-old, natural stand of *Pinus silvestris* L. (Umeå; N63°50' E20°20'; altitude, 15 m). Chloroplasts were isolated from 1.5 g of needles, as described earlier (11). The chloroplasts were ruptured by suspension in 10 ml hypotonic buffer (0.05 M Hepes, pH 7.6), after which the thylakoids were centrifuged at 3,000g for 15 min. During initial experiments, the thylakoids were then fractured in a French pressure cell (0.05 M Hepes [pH 6.8], 0.15 M KCl, 0.01 M NaCl, 0.005 M MgCl₂; 1,400 kg cm⁻²), and the bulk green band of a five-step discontinuous sucrose gradient (3) was, after 1-h centrifugation at 30,000g in a SW-27 rotor, taken for lipid analysis. As this band of presumably the grana fraction contained lipid and fatty acid compositions similar to the chloroplast thylakoid preparation (data not shown), these tedious fractionation steps were abandoned, and the isolated chloroplast thylakoids were extracted for lipids immediately after isolation.

The total lipid content of the thylakoids was first extracted with 20 ml ice-cold chloroform:methanol (2:1, v/v), and the extracted thylakoids were centrifuged at 27,000g for 5 min. The extraction of the pellet was then repeated with 20 ml chloroform:methanol (1:1, v/v) and finally with 20 ml chloroform:methanol (1:2, v/v), to which was added 6 ml 0.73% NaCl to extract the most polar lipids. The supernatants after each extraction were combined and evaporated to dryness at 40°C. The residue was dissolved in chloroform and applied to a silicic acid column (Silicar CC7, Mallinckrodt). Lipids with increasing polarity were eluted with chloroform (neutral lipids); chloroform:acetone (1:1) followed by pure acetone (galactolipids + sulpholipid); and methanol (PLs). The second and third fractions were evaporated to dryness, and the lipids were separated by TLC (Silica Gel H, Merck) developed in chloroform:methanol:acetic acid:H₂O (85:15:10:3.6). The plates were dried in N₂ atmosphere. The lipids were located by I₂ vapor, and bands with MGDG, DGDG, SL, and PL (total) were scraped off and the lipids eluted with chloroform:methanol (2:1, v/v). For details, see (Refs. 16, 23, and 24). The fatty acids of the lipids were converted to methyl esters using Sigma's 14% boron trifluoride in methanol (14). The methyl esters were then separated and identified by GLC (Perkin-Elmer 900) equipped with a 3.5-mm × 2-m glass column packed with 4.5% butane-1,4-diol succinate (HI-EFF 4BP; Applied Sciences Lab., Inc.) on Chromosorb W AW-DMCS 80/100 (Applied Sciences Lab., Inc.). The column

temperature was 180°C. Heptadecanoid acid (17:0) was used as internal standard.

The electron transport reactions H₂O → NADP (PSII + PSI), H₂O → DPIP (PSII), and Asc/DPIP → NADP (PSI) were measured in isolated pine chloroplasts, as described elsewhere (11). When NADP reduction was studied, rate-saturating amounts of NADP-reductase and plastocyanin were added.

RESULTS

During the experimental year of 1978 to 1979, the first night frost appeared in late September (Fig. 1). The temperatures varied around 0°C during October and November and stayed constantly below zero from late November until March. The frost-free period began in the middle of May. The ground was snow-covered from November to the end of April.

The overall electron transport capacities from water to NADP (PSII + PSI) and from water to DPIP (PSII) decreased steadily from September, when the first night frosts occurred (Fig. 2). The rates decreased relatively less during the cold midwinter, and the lowest activities were obtained in April. The electron transport

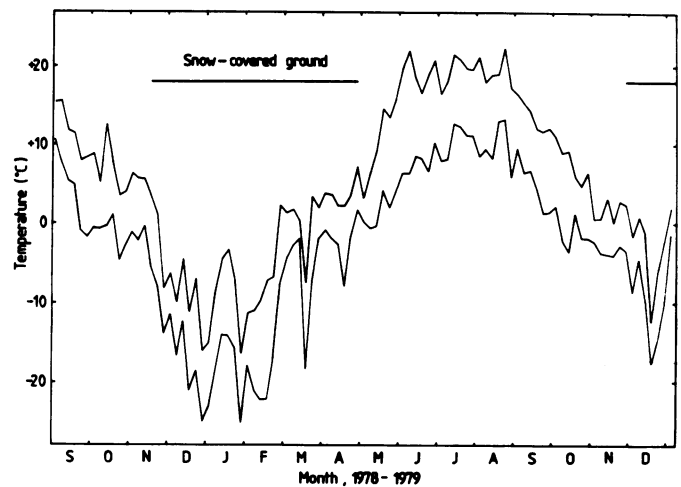


FIG. 1. The seasonal variations in maximum and minimum temperatures during 1978 to 1979 in Umeå (63°50' N and 20°20' E), Sweden. The mean temperatures over a period of 5 d are presented. The snow-covered period is indicated.

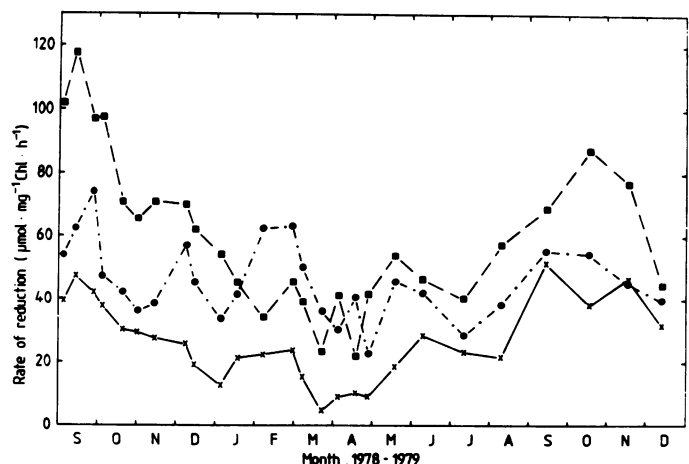


FIG. 2. The seasonal variations of PSII (■—■; H₂O → DPIP), PSI (●—●; Asc/DPIP → NADP), and PSII + PSI (×—×; H₂O → NADP) of chloroplasts isolated from current-year needles of a naturally grown *Pinus silvestris*.

² Abbreviations: MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; SL, sulfolipid; PL, phospholipid; Asc, Na-ascorbate; DPIP, 2,6-dichlorophenol indophenol.

rates recovered steadily after the last frost nights in May, and the highest activities were measured in September before the autumn decay started again. The PSI reaction ($\text{Asc/DPIP} \rightarrow \text{NADP}$) showed much less seasonal variation, but the lowest activities were also in this case, measured in April before the spring recovery began. These data essentially confirm the results of similar electron transport measurements performed with pine chloroplasts during 1976 to 1977 (12). The winter inhibition was, however, more severe in all three photoreactions in 1976 to 1977 than it was in 1978 to 1979. This was especially marked for the overall electron transport from water to NADP, which was totally inhibited between November, 1976 and May, 1977. The quantitatively different extents of winter inhibition between the two experimental years are most likely explained by the different temperature characteristics of the two years; stable winter temperatures were below zero in 1978 to 1979 (Fig. 1), whereas the winter temperatures in 1976 to 1977 were variable, with frequent fluctuations between low temperatures of -15 to -30°C and high temperatures around 0°C (12). We believe that the more severe winter inhibition of the electron transport in 1976 to 1977 was caused by the stress induced by the repeated freezings and thawings of the needles during the course of the winter. These results give a good example of how climatic variations from year to year can induce quantitatively different physiological changes. It stresses the importance of studying seasonally induced changes of physiological parameters for more than 1 year before general conclusions are finally drawn.

The chloroplast thylakoid acyl lipids were quantitatively determined relative to the needle fresh weight at four different occasions during 1980 (Table I). The needle fresh weight-to-dry weight ratio did not vary by more than 8% between the times of sampling. The total lipid content of the membrane preparation was lowest in July. It increased during the autumn and stayed 2 to 3 times higher during the course of the winter than it did during the summer. Although quantitatively different, similar trends in seasonal variations were observed for the individual lipids, except for MGDG, in which the content had dropped to a minimum in December.

In view of the usually low relative content of PL in chloroplast thylakoids (7), it is unexpected to find such high contents of PL (near 50%) in the chloroplast thylakoids isolated during autumn and winter (Table I; the same result was obtained during the experimental year of 1978 to 1979, but this is not shown). Isolated pine chloroplast thylakoids have a tendency to aggregate during this period of the year (G. Öquist, unpublished observation), and it is, therefore, quite possible that the high content of PL is due to an increased proportion of membranes other than thylakoids in the preparations made during autumn and winter. The fatty acid trans-3-hexadecanoic acid (16:1) is specific for the dominating PL, phosphatidyl glycerol, in the thylakoids (5). This acid might, therefore, be used as a marker for the origin of extracted PL. The relative content of 16:1 in the extracts of PL was about 3 times lower in the winter than in the summer (Table II; see below), which finding also is a strong indication that the fluctuations in

the amount of extracted PL is, to a significant extent, due to seasonal variations in the purity of the isolated chloroplast thylakoid preparations. Hence, possible seasonal effects on the PL content of pine chloroplast thylakoids are not considered in this study, which, as far as the lipid content is concerned, concentrates on the plastide-specific lipids MGDG, DGDG, and SL.

Analyses of the pine chloroplast thylakoid acyl lipids MGDG, DGDG, and SL during the course of 1978 to 1979 (Fig. 3) revealed that the relative content of MGDG stayed low during the winter (about 27 mol %) and that it increased steadily from the middle of April to the middle of August (maximum 43%), after which month it started to decrease towards the low winter level. The changes of DGDG were less pronounced, and they occurred in reverse to those of MGDG; the mol % of DGDG was about 56% during the winter but went down to a minimum of 45% in August. The relative content of SL also tended to be a little higher in the winter (about 17%) than it was in the summer (about 13%).

The seasonal variations in the relative mol % of the fatty acids were also determined for the acyl lipids (Table II). In order to visualize these data, we have plotted, in Figure 4, the seasonal variations of the mol ratio of unsaturated to saturated fatty acid. Only MGDG showed pronounced seasonal fluctuations in this ratio. The ratio decreased steadily during the autumn, reached a low, stable value of about 6 during midwinter, and started to increase again after a minimum in the middle of April. The ratio then increased to a peak value of 38, which was reached in September. From then on, it decreased toward the low winter level. A closer look at Table II reveals that the variations in the unsaturation/saturation ratio of MGDG were mediated, first of all, by changes in the relative amounts of the saturated palmitic acid (16:0) and the polyunsaturated linolenic acid (18:3). The unsaturation-to-saturation ratio of DGDG stayed at 6 during most of the year, although it increased to 9 during the autumn. PL and SL showed no variations in their unsaturation-to-saturation ratios during the course of the year. The ratios were about 3 and 1 for PL and SL, respectively.

During the fatty acid determinations of the individual lipids, we usually noted traces of compounds (2 to 3 peaks) with longer retention time than 18:3 (Table II; denoted >18). In PL, however, these components made up some 10 to 15% of the total fatty acid content. They did not vary in any significant pattern over the year, and we have not, therefore, made any attempt to identify them. Similar long chain fatty acids have been observed in pine by others (2, 4).

DISCUSSION

Amount of MGDG, DGDG, and SL. The approximately 2-fold increase of MGDG, DGDG, and SL of the chloroplast thylakoids of pine during the autumn (Table I) agrees with the well-known phenomenon that the lipid content of different species and plants increases during frost hardening conditions (8). A large number of correlative studies have indicated that an increase in lipid content is related to acclimation to tolerance of freezing temperatures.

Table I. Quantitative Expressions of the Contents of MGDG, DGDG, SL, and PL of Chloroplast Thylakoid Preparations Obtained from Naturally Grown *Pinus silvestris* at Four Representative Times of the Year 1980

SD are given for $n = 3$.

| Day | Total | MGDG | DGDG | SL | PL |
|---|-------|----------------------------|----------------|---------------|----------------|
| <i>nm of thylakoid lipids per g fresh wt of needles</i> | | | | | |
| April 22 | 2,043 | 291 ± 16 (14) ^a | 565 ± 22 (28) | 216 ± 30 (11) | 973 ± 100 (48) |
| July 30 | 866 | 248 ± 41 (29) | 206 ± 69 (24) | 97 ± 33 (11) | 315 ± 131 (36) |
| October 13 | 2,250 | 473 ± 18 (21) | 434 ± 126 (19) | 231 ± 22 (10) | 1112 ± 93 (49) |
| December 16 | 1,574 | 218 ± 60 (14) | 561 ± 27 (36) | 155 ± 14 (10) | 640 ± 75 (41) |

^a Number in parentheses, relative percentages of the different lipids.

Table II. Seasonal Variations of Relative Mol Percent of Fatty Acids of MGDG, DGDG, SL, and PL of Chloroplast Thylakoid Preparations Obtained from Current-Year Needles of Naturally Grown *Pinus silvestris*

The experiment was performed between October 19, 1978, and December 17, 1979. SD is given for $n = 3$, but it was not calculated in the case when $n = 2$. Less than 2% is shown by tr (traces). 16:0, palmitic acid; 16:1, trans-3-hexadecanoic acid; 18:0 stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid.

| Day | 16:0 | 16:1 | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 | >18 |
|--------------|------------|-----------|------------|-----------|-----------|------------|------------|-----|
| MGDG | | | | | | | | |
| October 19 | 4.4 ± 1.2 | tr | 9.3 ± 1.4 | tr | tr | 5.5 ± 1.3 | 75.1 ± 7.8 | tr |
| October 31 | 6.1 | tr | 7.7 | tr | tr | 5.4 | 74.3 | tr |
| November 27 | 7.9 | tr | 8.3 | tr | tr | 8.5 | 73.0 | tr |
| December 18 | 9.0 | tr | 8.0 | tr | tr | 8.6 | 67.1 | 5.4 |
| January 18 | 11.3 | tr | 12.3 | 2.3 | 2.6 | 9.3 | 62.4 | tr |
| March 6 | 11.5 ± 1.2 | 2.6 ± 1.0 | 11.1 ± 0.7 | 2.1 ± 0.2 | 3.3 ± 0.2 | 11.4 ± 0.4 | 58.9 ± 0.7 | tr |
| March 28 | 12.2 ± 0.5 | tr | 6.5 ± 2.4 | tr | 3.0 ± 0.2 | 11.9 ± 3.9 | 64.6 ± 6.3 | tr |
| April 17 | 16.1 ± 1.6 | 3.5 ± 0.4 | 6.6 ± 0.8 | 2.1 ± 0.5 | 3.2 ± 0.3 | 7.4 ± 4.1 | 61.2 ± 2.9 | tr |
| May 2 | 8.6 ± 0.8 | 4.4 ± 0.3 | 10.5 ± 0.9 | tr | tr | 7.5 ± 1.0 | 68.2 ± 2.5 | tr |
| May 18 | 6.0 ± 1.8 | 2.4 ± 0.7 | 11.6 ± 1.2 | tr | 2.4 ± 0.8 | 6.3 ± 1.4 | 70.6 ± 3.7 | tr |
| June 5 | 5.4 ± 0.6 | tr | 14.4 ± 1.6 | tr | tr | 7.5 ± 0.4 | 68.9 ± 2.7 | tr |
| July 9 | 2.9 ± 0.5 | tr | 17.6 ± 2.0 | tr | tr | 3.4 ± 0.4 | 75.7 ± 2.8 | tr |
| August 21 | 3.0 ± 0.2 | tr | 7.8 ± 0.6 | tr | tr | 4.4 ± 0.6 | 85.0 ± 1.1 | tr |
| September 21 | 2.5 ± 0.2 | tr | 7.4 ± 0.3 | tr | tr | tr | 88.4 ± 1.9 | tr |
| October 18 | 4.1 ± 0.7 | tr | 8.5 ± 0.4 | tr | tr | 4.0 ± 0.6 | 83.5 ± 0.9 | tr |
| November 21 | 5.8 ± 1.2 | tr | 6.8 ± 0.3 | tr | tr | 6.7 ± 0.8 | 80.7 ± 1.8 | tr |
| December 17 | 4.5 ± 2.9 | tr | 8.0 ± 1.3 | tr | tr | 5.1 ± 1.2 | 82.3 ± 2.8 | tr |
| DGDG | | | | | | | | |
| October 19 | 11.4 ± 0.7 | 0 | 2.4 ± 0.3 | tr | tr | 2.4 ± 0.3 | 79.3 ± 1.8 | tr |
| October 31 | 12.1 | tr | 2.2 | tr | tr | 2.6 | 78.1 | tr |
| November 27 | 11.7 | tr | tr | 2.2 | tr | 3.4 | 79.2 | tr |
| December 18 | 14.0 | tr | 2.9 | 2.5 | tr | 3.0 | 74.7 | tr |
| January 18 | 12.9 | tr | 6.1 | 2.8 | tr | 2.9 | 74.2 | tr |
| March 6 | 13.4 ± 1.1 | tr | 5.3 ± 0.7 | tr | tr | 3.9 ± 0.4 | 76.6 ± 0.1 | tr |
| March 28 | 14.4 ± 0.5 | tr | 2.6 ± 0.8 | tr | tr | 3.3 ± 0.2 | 77.2 ± 1.3 | tr |
| April 17 | 19.7 ± 0.6 | tr | 2.4 ± 0.4 | tr | tr | tr | 77.3 ± 3.3 | tr |
| May 2 | 16.8 ± 2.2 | tr | 3.2 ± 0.1 | tr | tr | tr | 79.4 ± 3.2 | tr |
| May 18 | 15.0 ± 1.0 | tr | 3.9 ± 0.1 | tr | tr | 2.2 ± 0.3 | 79.0 ± 0.6 | tr |
| June 5 | 14.8 ± 1.4 | 0 | 6.4 ± 0.5 | 2.6 ± 0.9 | tr | 4.4 ± 0.3 | 70.9 ± 4.0 | tr |
| July 9 | 12.7 ± 0.9 | 0 | 9.5 ± 1.4 | tr | tr | 3.5 ± 0.6 | 72.4 ± 0.3 | tr |
| August 21 | 11.6 ± 0.5 | 0 | 3.3 ± 0.5 | 2.5 ± 0.4 | tr | 4.9 ± 0.2 | 77.8 ± 0.5 | tr |
| September 21 | 10.9 ± 0.5 | 0 | 1.8 ± 0.3 | tr | tr | 3.3 ± 0.5 | 82.8 ± 1.9 | tr |
| October 18 | 10.9 ± 0.5 | tr | 2.3 ± 0.2 | tr | tr | 2.8 ± 0.1 | 82.4 ± 0.7 | tr |
| November 21 | 9.6 ± 0.5 | tr | tr | tr | tr | 3.0 ± 0.4 | 84.6 ± 1.1 | tr |
| December 17 | 10.6 ± 0.7 | tr | tr | tr | tr | 3.0 ± 0.2 | 85.0 ± 2.0 | tr |
| SL | | | | | | | | |
| October 19 | 49.0 ± 4.3 | 0 | 0 | 2.2 ± 0.2 | tr | 5.8 ± 0.3 | 41.7 ± 3.9 | 0 |
| October 31 | 42.1 | 0 | 0 | 2.6 | tr | 5.7 | 47.0 | 0 |
| November 27 | 43.0 | tr | tr | 2.8 | 2.4 | 6.9 | 45.1 | 0 |
| December 18 | 44.6 | tr | 2.8 | 2.3 | tr | 6.4 | 44.1 | 0 |
| January 18 | 45.9 | tr | 7 | 3.4 | 2.1 | 5.9 | 35.5 | 0 |
| March 6 | 39.3 ± 2.8 | tr | 5.8 ± 1.4 | 3.9 ± 0.5 | 3.2 ± 0.6 | 5.6 ± 0.8 | 44.5 ± 3.1 | 0 |
| March 28 | 42.6 ± 1.8 | tr | tr | 3.4 ± 0.5 | 2.8 ± 0.2 | 4.7 ± 0.3 | 44.9 ± 3.6 | 0 |
| April 17 | 57.7 ± 3.3 | tr | 5.1 ± 0.4 | tr | 3.9 ± 0.5 | tr | 32.2 ± 6.5 | 0 |
| May 2 | 44.2 ± 1.6 | 0 | 7.7 ± 3.9 | 3.0 ± 0.6 | 5.8 ± 0.1 | tr | 39.4 ± 3.3 | 0 |
| May 18 | 46.3 ± 1.2 | 0 | 3.0 ± 0.4 | 2.6 ± 0.2 | 7.5 ± 0.8 | 6.1 ± 0.3 | 34.5 ± 0.9 | 0 |
| June 5 | 46.9 ± 2.4 | 0 | 6.0 ± 1.5 | 3.1 ± 0.8 | 2.4 ± 0.6 | 9.6 ± 0.4 | 32.0 ± 1.4 | 0 |
| July 9 | 37.4 ± 0.6 | 0 | 19.3 ± 1.0 | 3.1 ± 0.5 | tr | 6.5 ± 0.5 | 33.3 ± 2.1 | 0 |
| August 21 | 39.8 ± 3.8 | 0 | 7.8 ± 0.8 | 4.5 ± 0.7 | tr | 10.7 ± 0.5 | 36.5 ± 3.0 | 0 |
| September 21 | 40.5 ± 1.7 | 0 | 0 | tr | tr | 7.8 ± 0.3 | 49.8 ± 0.2 | 0 |
| October 18 | 41.5 ± 0.5 | tr | 0 | tr | tr | 6.4 ± 0.4 | 49.8 ± 2.7 | 0 |
| November 21 | 39.9 ± 2.1 | tr | tr | 2.5 ± 0.1 | tr | 5.6 ± 0.4 | 51.9 ± 2.0 | 0 |
| December 17 | 41.7 ± 1.6 | tr | 0 | 2.8 ± 0.3 | tr | 3.4 ± 0.6 | 50.1 ± 1.9 | 0 |

Table II.—Continued

| Day | 16:0 | 16:1 | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 | >18 |
|--------------|------------|-----------|-----------|------|-----------|------------|------------|------------|
| PL | | | | | | | | |
| October 19 | 25.5 ± 0.6 | 4.1 ± 0.6 | tr | tr | 2.0 ± 0.2 | 23.2 ± 1.0 | 28.3 ± 0.8 | 15.4 ± 0.9 |
| October 31 | 19.9 | 3.1 | tr | tr | 2.7 | 27.0 | 30.9 | 14.3 |
| November 27 | 22.0 | tr | tr | tr | 3.0 | 22.8 | 30.5 | 18.9 |
| December 18 | 26.0 | tr | tr | tr | 2.7 | 21.3 | 34.2 | 13.2 |
| January 18 | 25.3 | tr | 2.6 | tr | 3.4 | 19.6 | 37.1 | 12.1 |
| March 6 | 24.9 ± 0.1 | 2.2 ± 0.5 | 2.4 ± 0.3 | tr | 5.2 ± 0.2 | 21.7 ± 1.2 | 29.1 ± 2.7 | 14.1 ± 0.6 |
| March 28 | 22.2 ± 1.3 | 2.7 ± 0.7 | tr | tr | 5.4 ± 0.3 | 24.5 ± 0.9 | 26.8 ± 0.3 | 16.7 ± 2.0 |
| April 17 | 29.4 ± 1.0 | 2.4 ± 0.2 | tr | tr | 4.8 ± 0.1 | 27.3 ± 1.1 | 28.5 ± 2.1 | 7.6 ± 0.2 |
| May 2 | 24.9 ± 2.0 | 3.4 ± 0.7 | 2.2 ± 0.7 | tr | 4.1 ± 0.3 | 27.9 ± 0.4 | 26.5 ± 0.2 | 9.5 ± 1.2 |
| May 18 | 22.6 ± 1.4 | 5.0 ± 0.1 | tr | tr | 5.5 ± 0.4 | 29.4 ± 1.4 | 24.2 ± 2.6 | 9.9 ± 1.3 |
| June 5 | 24.6 ± 1.2 | 5.8 ± 0.3 | 2.8 ± 0.1 | tr | 5.2 ± 0.2 | 26.0 ± 0.8 | 28.0 ± 1.3 | 6.0 ± 0.4 |
| July 9 | 22.8 ± 1.2 | 6.7 ± 0.2 | 8.5 ± 1.1 | tr | 2.7 ± 0.2 | 23.4 ± 2.4 | 28.5 ± 1.1 | 5.8 ± 0.4 |
| August 21 | 23.5 ± 0.2 | 7.7 ± 0.4 | 2.6 ± 1.0 | tr | 3.1 ± 0.2 | 25.0 ± 2.5 | 27.9 ± 3.3 | 8.3 ± 1.2 |
| September 21 | 24.3 ± 0.4 | 5.8 ± 0.4 | tr | tr | tr | 24.7 ± 0.6 | 31.8 ± 2.3 | 10.9 ± 0.6 |
| October 18 | 25.8 ± 0.5 | 4.0 ± 0.8 | tr | tr | tr | 23.6 ± 0.7 | 34.8 ± 2.9 | 10.1 ± 0.9 |
| November 21 | 23.6 ± 1.5 | 2.1 ± 0.1 | tr | tr | tr | 21.1 ± 1.6 | 37.5 ± 1.9 | 10.1 ± 1.7 |
| December 17 | 27.9 ± 1.1 | 2.0 ± 0.4 | tr | tr | tr | 20.8 ± 0.5 | 38.1 ± 0.6 | 6.6 ± 0.6 |

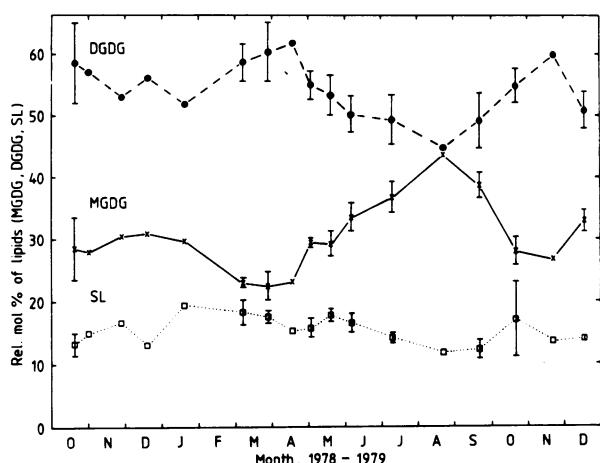


FIG. 3. The seasonal variations of the relative mol percent of MGDG, DGDG, and SL of chloroplast thylakoid preparations obtained from current-year needles of naturally grown *Pinus silvestris*. The vertical bars give the standard deviations for $n = 3$. When no bars are shown, $n = 2$.

The decrease of MGDG and the increase of DGDG during autumn (Fig. 3) are most likely due to the preferential synthesis of DGDG and are accentuated by a drop in the MGDG content from October 13 to December 16 (Table I). These concomitant changes in MGDG and DGDG suggest that the content of DGDG increased as a result of galactosidation of MGDG during late autumn or early winter. Bervaes *et al.* (2) have also observed that hardened pine has a lower MGDG/DGDG ratio than de-hardened pine. Such a change would increase the capacity of the polar heads of the galactolipids to bind water, which might be an acclimation of the thylakoids to increase their resistance to freeze dehydration when water freezes extracellularly during the winter (2, 4).

Very small changes occurred in the relative amounts of MGDG, DGDG, and SL during the winter (Fig. 3). There was, however, a small quantitative decrease of all lipids, except for DGDG, during the winter period (Table I). Freezing injuries are known to be manifested in degradation of lipids, and it has been suggested that the build up of lipids during autumn could create a reserve to replace those degraded by freezing (8). In view of the suggestion that DGDG is of importance for the ability of the thylakoids to tolerate freeze desiccation (see "Discussion" above), it is of interest to note that the content of DGDG did not decrease during the

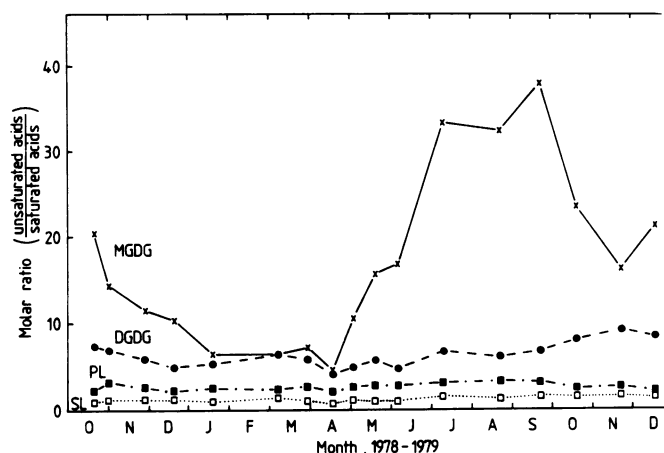


FIG. 4. The seasonal variations of the unsaturated/saturated (16:1 + 16:3 + 18:1 + 18:2 + 18:3/16:0 + 18:0) molar ratio of MGDG, DGDG, SL, and PL of chloroplast thylakoid preparations obtained from current-year needles of naturally grown *Pinus silvestris*. The data were calculated from the data of Table II.

winter (Table I).

During the spring, there was a decrease in the content of DGDG and SL but not of MGDG (Table I). This resulted in a gradual increase of the relative content of MGDG and a decrease of the relative content of DGDG from the middle of April to the middle of August (Fig. 3). A similar response of this ratio in the proportion between the two galactolipids to dehardening conditions has been observed before for pine (2). The ratio MGDG + DGDG/SL (not shown) was highly variable, and no specific conclusions can be drawn from these data.

Level of Unsaturation. The most obvious seasonal variation observed in our analyses of chloroplast thylakoid lipids of pine was that MGDG was fully responsible for the variations in the level of unsaturation of the membranes (Fig. 4). The increase of the unsaturated/saturated fatty acid molar ratio of MGDG during spring and summer must be due to turnover of the MGDG pool, because no net decrease or increase of MGDG was supposed to occur over this period (see "Discussion" above). The drop in the ratio during the autumn was unexpected in view of the generally obtained increase of the level of unsaturation during cold hardening conditions (10), although chilling-sensitive plants also can respond to low temperatures with a decrease in lipid unsaturation

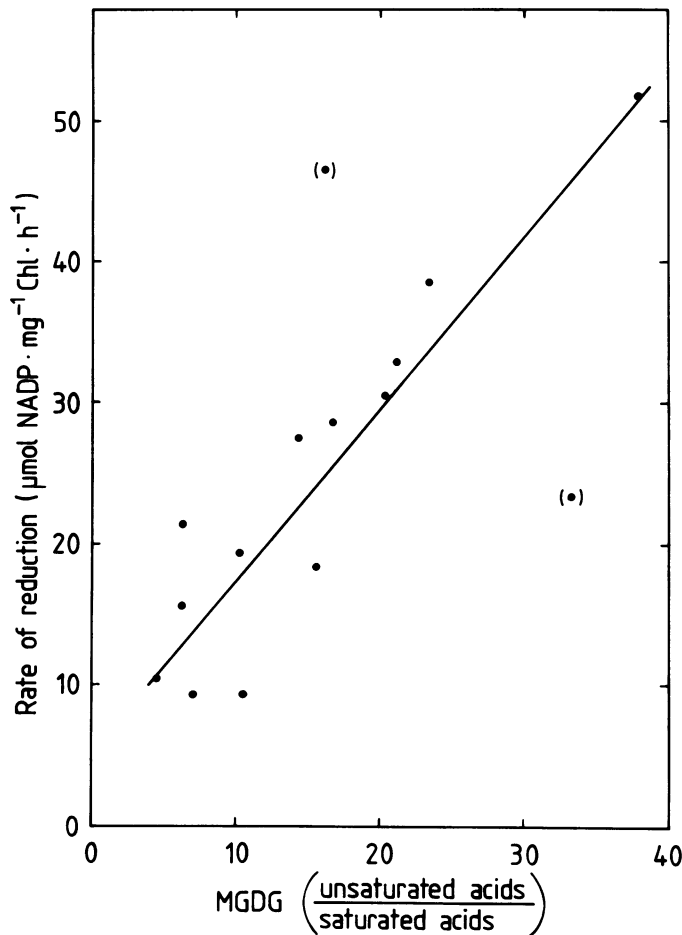


FIG. 5. A linear regression plotting of the rate of overall electron transport ($\text{H}_2\text{O} \rightarrow \text{NADP}$) and the unsaturation-to-saturation M ratio of MGDG in chloroplast thylakoid preparations obtained from naturally grown *Pinus silvestris* during the course of one year. The data were taken from Figures 2 and 4. The values in brackets were excluded for the determination of the regression line: $y = 1.2x + 5.3$; $r = 0.93$.

(29). It is possible that the increase in unsaturation during August and September is a response to frost hardening conditions, *i.e.* to decreasing photoperiod and temperature (8).

The decrease in the level of unsaturation of MGDG (Fig. 4) during autumn and winter occurs parallel with the inhibition of electron transport (Fig. 2; PSII and PSII + PSI) and a partial destruction of Chl, preferentially of Chl *a* in the reaction center antennae of the two photosystems (20). All these changes began and developed further after the first night frosts in September (Fig. 1). Earlier, we gave evidence (17) that this inhibition of electron transport and the Chl destruction are dependent on both light and temperature, so that light causes photooxidation of membrane components under late autumn and winter conditions when photosynthesis is largely inhibited by low temperatures. We believe, therefore, that the decrease in the level of unsaturation of MGDG is another reflection of membrane damage caused by photooxidation of polyunsaturated fatty acids such as 16:3 and 18:3 (Table II). It has been shown (28) that polyunsaturated lipids are very susceptible to photooxidation, when photosynthesis of chilling-sensitive plants has been largely inhibited by temperature below the phase-transition temperature.

Figure 5 presents the data of Figures 2 and 4 in a linear regression plot. The correlation between the changes in the capacity of the overall electron transport from water to NADP with the level of unsaturation in MGDG is striking with an r value of 0.93.

The correlation between the PSII activity and the unsaturation level was less clear ($r = 0.60$; data not shown). We know from earlier studies (11, 12, 18, 19) that the winter inhibition of overall electron transport occurs at the site of plastoquinone, which links the two photosystems. The plastoquinone content of the thylakoids also reaches a minimum level when this inhibition is most severe (12). It is tempting to suggest that the level of unsaturation of MGDG regulates the function of plastoquinone, which is thought to operate as a primarily lipid-associated interface molecule which, possibly by diffusion in the lipophilic matrix of the thylakoids, links the electron flow from PSII to PSI (27). Model experiments with the surface-balance technique (9) have shown that plastoquinone (both reduced and oxidized) has no interactions but has a free molecular miscibility with polyunsaturated MGDG. This free molecular miscibility was prevented in saturated MGDG, and plastoquinone was actually squeezed out of the monolayer at higher pressures. If we assume that there must exist a free molecular miscibility between MGDG and plastoquinone in order for plastoquinone to function as an interface molecule, we hypothesize that the decreased level of unsaturation of MGDG reduces the mobility of plastoquinone (and possibly also the content) in the thylakoid, and the electron transport capacity becomes inhibited. In view of the more and more widely accepted picture that PSII is localized in the grana partition region and PSI in the margins and stroma lamellae (1), plastoquinone must transfer electrons (and protons) laterally over much longer distances than just across the thylakoids. This stresses the feasibility of the suggestion that decreases in the free molecular miscibility between plastoquinone and its environment of galactolipids in the lipid double layer would inhibit the photosynthetic efficiency.

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