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SEASONAL VARIATION OF LIPIDS AND FATTY ACIDS FROM TREE-GROWING LICHENS OF THE GENUS *PHYSCIA*

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Key Word Index—*Physcia dubia*; *P. stellaris*; lichens; tree-growing; lipids; seasonal changes.

Abstract—Lipids, including phospholipids and fatty acids from two lichen species, *Physcia dubia* and *P. stellaris*, collected between March and May, were examined. The seasonal changes occurring in lipid composition due to temperature were identified. Each species had a characteristic temperature-based lipid pattern. *Physcia stellaris* was found to increase its total lipid content from 24.8 in March to 37.5 mg g^{-1} dry wt in May, whereas the lipid content decreased from 63.4 to 51.8 mg g^{-1} dry wt in *P. dubia* from March to May. Both species possessed high neutral lipid contents in March, 70.1 and 56%, respectively, and showed an increase in their glycolipid contents during the same period. Polar and phospholipid contents were also determined, and certain patterns for phosphatidylcholine and diacylglyceryltrimethylhomoserine proportions were observed. Capillary GC-MS demonstrated that seasonal changes also occurred in the fatty acid composition of the two species. Hydroxy acid dynamics were also studied.

INTRODUCTION

The adaptability of lichen organisms to extreme environmental conditions, particularly to temperature, is of great interest [1, 2]. Temperature is one of the basic factors affecting the saturated fatty acids of membranes but, according to Czeczuga [3], sunlight produces a fairly strong effect upon carotenoid composition. In two lichen species, *Xanthoria fallax* and *X. parientina*, it was demonstrated that the amount of mutatoxin found in plants growing in sunlit places was almost triple compared to that detected in shade-grown plants [3]. Dertien *et al.* [4] studied the seasonal lipid and fatty acid composition from five terrestrial and tree-growing lichen species and found some changes in their lipid content.

We have now examined lipids, polar lipids, phospholipids and fatty acids from two species of lichens sampled between March and May when the temperature changed from −7° to +23°. We were able to identify certain patterns of redistribution of polar lipids and fatty acids.

RESULTS

The total lipid contents in each species changed differently. *Physcia stellaris* was found to increase its total lipids from 24.8 to 37.5 mg g^{-1} dry wt (Table 1). Both *Physcia* species decreased their neutral lipid amounts but increased those of glycolipids. The amount of phospholipids in *P. stellaris* increased from 7 to 17.4%, while in *P. dubia* it remained unchanged for March and April with a decrease in May due to sunlight.

The majority of phospholipid classes underwent quantitative changes; the phosphatidylcholine (PC) content in *P. dubia* and *P. stellaris* decreased by 9.1 and 6.7% during the period from March to May, respectively. Similar changes were observed for phosphatidylglycerol (PG) and phosphatidylinositol (PI) (Tables 2 and 3). The greatest quantitative changes occurred in PC and diacylglyceryltrimethylhomoserine (DGTS). It is noteworthy that the March sample of *P. dubia* contained the greatest amount of DGTS among the polar lipids, including phospholipids, except PC and phosphatidylethanolamine (PE), namely 17.8%, which increased towards May to make up 9.1%. At the same time, the PC content was observed to decrease from 29.3 to 20.4% during the same period. These results suggest that there is a correlation between the levels of PC and DGTS in lichens.

The amounts of saturated fatty acids in the neutral lipid fraction increased from 31.8 to 37% in *P. stellaris*, and from 44.5 to 49.9% in *P. dubia* (Table 4). Monoenoic acids were found to increase in a similar way, from 44.9 to 52.4% in *P. stellaris*, and from 36.1 to 40.6% in *P. dubia*. Dienoic and polyenoic acids were observed to decrease. The major fatty acids of the neutral lipids were saturated 14:0, 16:0, 18:0; monoenoic 16:1 (n-9), 16:1 (n-7), 18:1 (n-9) and 18:1 (n-7); dienoic 18:2 (n-6); polyenoic 18:3 (n-6) and 18:3 (n-3). Besides the major fatty acids we also found *iso*-, *anteiso*-, prystanic, phytanic and small amounts of long-chain acids (24:0–28:0). Among the

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Table 1. Composition of total, neutral, glyco- and phospholipids of two lichen species collected during March to May 1992

Collection date	Weather conditions during sampling	*Physcia dubia*				*Physcia stellaris*			
		TL	NL	GL	PL	TL	NL	GL	PL
8 March	Freezing, snow (−7°)	63.4	56.0	27.5	16.5	24.8	70.1	22.9	7.0
23 April	Cool, wet (+7°)	54.9	57.6	25.8	16.6	28.8	56.4	28.7	14.9
28 May	Warm, sunny (+23°)	51.8	53.8	34.8	11.4	37.5	28.2	54.4	17.4

TL, total lipids, mg g^{-1} dry wt. NL, neutral lipids. GL, glycolipids. PL, phospholipids including DGTS. NL, GL and PL as % of TL.

Table 2. Composition of DGTS and phospholipids of *Physcia dubia*

Collection date	DGTS	PC	PG	PI	PS	PE	LPE
8 March	17.8±0.9	29.3±0.8	10.9±0.4	7.7±0.3	7.2±0.4	20.0±0.5	7.1±0.3
23 April	19.7±1.2	22.3±0.9	11.9±0.5	8.1±0.4	8.7±0.3	20.4±0.7	8.9±0.5
28 May	23.0±1.5	20.4±0.7	9.2±0.4	5.6±0.3	8.1±0.3	20.6±0.5	13.1±0.7

Values are means±s.d. (n=3–5).
DGTS, diacylglyceryltrimethylhomoserine. PC, phosphatidylcholine. PG, phosphatidylglycerol. PI, phosphatidylinositol. PS, phosphatidylserine. PE, phosphatidylethanolamine. LPE, lysophosphatidylethanolamine.

Table 3. Composition of DGTS and phospholipids of *Physcia stellaris*

Collection date	DGTS	PC	PG	PI	PS	PE	LPE
8 March	13.5±0.8	23.3±0.8	14.5±0.5	9.1±0.4	11.8±0.5	19.3±0.6	8.5±0.6
23 April	19.3±1.1	27.3±0.9	9.9±0.6	5.5±0.3	11.0±0.5	20.1±0.5	6.9±0.7
28 May	21.7±1.4	16.6±0.8	11.1±0.7	5.6±0.4	7.9±0.6	23.9±0.8	13.2±0.9

Values are means±s.d. (n=3–5).
For abbreviations see Table 2.

monoenoic fatty acids *iso-* and *anteiso-* 17:1 as well as long-chain acids ranging from 24:1 to 30:1 were found. Among the dienoic and polyenoic acids, unusual acids were absent with the exception of 26:3 (n-3) found in *P. dubia*, as well as 24:3 (n-3) found in *P. stellaris*. The number of polyunsaturated acids found in the two lichens sampled during the period of transient temperatures from −7 to +7° changed from 9% (in March) to 19% (in April) to become stable at 9% in May.

Fatty acids from phospholipids differ from those occurring in the neutral lipids by their degree of unsaturation. Fatty acids both from phospholipids and neutral lipids followed a pattern of increased saturation but decreased polyenoic fatty acid proportions when the temperature of the environment increased from −7 to +23°. *Physcia stellaris* increased its saturated acid content from 12.3 to 19.2% but decreased its dienoic and polyenoic acid content from 20.2 to 14.5% and from 32.4 to 12%, respectively. Similar results were obtained for *P. dubia* (Table 5). The number of saturated fatty acids found in phospholipids was less than that among neutral lipids. For instance, long-chain fatty acids having more than 22 carbon atoms were not detected among phospholipids, except in the samples from the transient temperature period. Also absent from the monoenoic acid group were long-chain acids greater than C_{22}. Concurrently, polyunsaturated acids displayed more diversity among phospholipids, as in neutral lipids, they were 16:0, 16:1 (n-9) or 16:1 (n-7), 18:1 (n-9), 18:2 (n-6) and two isomers of 18:3, namely, (n-6) and (n-3). Phospholipids were found to possess higher levels of arachidonic and eicosapentaenoic acids, compared to neutral lipids.

Fatty acids from glycolipids were observed to follow the patterns found for fatty acids from phospholipids and neutral lipids, but, compared to the latter, they also contained hydroxy acids. Monoenoic acids were found to increase from 36.0 to 54.1% in *P. stellaris*, and from 31.9 to 49.2% in *P. dubia* (Table 6). Dienoic and polyenoic acids were observed to decrease. No increase of saturated acids or a decrease of dienoic acids was observed in *P. dubia*. The seasonal distribution of major fatty acids is shown in Fig. 1.

Saturated hydroxy acids displayed the most diversity, ranging from C_{12} to C_{28}. We also detected *iso-* and

Table 4. Neutral lipid fatty acids from two *Physcia* species

	Physcia dubia			*Physcia stellaris*		
Fatty acids	March	April	May	March	April	May
12:0	0.8	0.8	0.9	0.6	0.7	0.7
13:0	0.7	0.8	0.8	0.6	0.7	0.7
14:0	3.7	3.6	4.2	2.2	2.3	2.5
15:0	0.5	0.6	0.6	0.9	1.0	1.1
i-16:0	1.6	1.6	1.8	0.6	0.7	0.7
16:0	18.4	17.2	20.7	12.8	9.2	14.9
17:0	0.5	0.6	0.6	0.8	0.9	0.9
18:0	11.5	10.8	12.9	5.5	5.6	6.4
19:0	0.5	0.5	0.5	0.3	0.4	0.3
20:0	1.7	1.7	1.9	2.3	2.3	2.5
21:0	0.4	0.5	0.5	0.6	0.7	0.7
22:0	0.8	0.9	0.9	1.2	1.3	1.4
24:0	0.5	0.5	0.5	0.7	0.8	0.8
SSMFA	2.9	4.0	3.1	2.8	4.6	3.4
Saturated	44.5	44.1	49.9	31.8	31.2	37.0
14:1 (n-5)	1.0	1.0	1.1	1.5	1.6	1.7
15:1 (n-7)	0.7	0.8	0.8	0.3	0.4	0.3
16:1 (n-9)	11.4	10.4	12.8	3.3	1.3	3.8
16:1 (n-7)	5.0	4.8	5.7	12.4	10.7	14.4
17:1 (n-9)	0.5	0.5	0.5	0.7	0.8	0.8
17:1 (n-7)	0.9	1.0	1.1	1.3	1.4	1.5
18:1 (n-11)	1.2	1.2	1.3	0.6	0.7	0.6
18:1 (n-9)	7.6	7.2	8.5	17.8	17.9	20.7
18:1 (n-7)	1.4	1.4	1.5	1.3	1.4	1.5
20:1 (n-11)	0.6	0.7	0.7	1.1	1.2	1.3
20:1 (n-9)	1.0	1.1	1.1	1.4	1.5	1.6
21:1 (n-8)	0.5	0.6	0.6	0.6	0.7	0.7
24:1 (n-11)	0.7	0.8	0.7	0.3	0.4	0.4
SMMFA	3.6	4.6	4.3	2.3	3.8	3.1
Monoenoic	36.1	36.4	40.7	44.9	43.8	52.4
18:2 (n-6)	13.9	13.0	7.3	13.4	13.5	7.4
SDMFA	0.1	0.5	0.4	0.2	0.6	0.3
Dienoic	14.0	13.5	7.7	13.6	14.1	7.7
18:3 (n-6)	2.8	2.7	0.9	4.6	4.7	1.5
18:3 (n-3)	2.1	2.1	0.5	4.0	4.1	1.1
SPMFA	0.5	1.2	0.3	1.1	2.1	0.3
Polyenoic	5.4	6.0	1.7	9.7	10.9	2.9
TMFA	7.1	10.3	8.1	6.4	11.1	7.1

SSMFA, sum of saturated minor fatty acids.

Saturated minor FAs comprised less than 0.5% of total FAs: 23:0, 25:0, 26:0, 28:0, *i*-12:0, *i*-13:0, *i*-14:0, *i*-15:0, *i*-17:0, *ai*-13:0, *ai*-15:0, *ai*-17:0, *ai*-19:0, pristanic and phytanic acids. SMMFA, sum of monoenoic minor fatty acids. Monoenoic FAs comprised less than 0.5% of total FAs: 12:1 (n-5), 13:1 (n-5), 15:1 (n-9), *trans*-16:1 (n-13), *i*-17:1 (n-9), *ai*-17:1 (n-9), 19:1 (n-8), 22:1 (n-11), 22:1 (n-9), 24:1 (n-9), 25:1 (n-9), 26:1 (n-11), 28:1 and 30:1. SDMFA, sum of dienoic minor fatty acids. Dienoic minor FAs comprised less than 0.5% of total FAs: 14:2 (n-5), 16:2 (n-4), 20:2 (n-6) and 22:2 (n-6).

SPMFA, sum of polyenoic minor fatty acids. Polyenoic minor FAs comprised less than 0.5% of total FAs: 16:3 (n-6), 16:3 (n-3), 20:3 (n-9), 20:3 (n-6), 20:3 (n-3), 22:3 (n-6), 22:3 (n-3), 24:3 (n-3), 26:3 (n-3), 16:4 (n-3), 18:4 (n-3), 20:4 (n-6), 20:4 (n-3), 22:4 (n-3), 20:5 (n-3), 22:5 (n-3) and 22:6 (n-3).

TMFA, total minor fatty acids.

anteiso-hydroxy acids. Twenty-five saturated hydroxy acids and nine monoenoic hydroxy acids including their *iso*-derivatives were identified from March samples (Table 7). Both their number and diversity increased when the environmental temperature rose. April and May samples of *P. dubia* yielded 16 monoenoic hydroxy acids. During the period from March to May, the amounts of hydroxy acids present in *P. stellaris* and *P.*

Table 5. Phospholipid fatty acids from two *Physcia* species

	Physcia dubia			*Physcia stellaris*		
Fatty acids	March	April	May	March	April	May
12:0	0.4	0.5	0.6	0.3	0.4	0.5
13:0	0.6	0.7	0.8	0.1	0.3	0.2
14:0	1.9	1.9	2.5	1.3	1.4	2.0
i-15:0	0.3	0.4	0.4	0.5	0.6	0.7
i-16:0	0.7	0.8	0.9	0.2	0.2	0.3
16:0	8.3	7.9	11.3	0.3	0.4	0.4
18:0	3.1	3.0	4.2	6.3	6.4	9.7
20:0	0.2	0.3	0.3	1.8	1.9	2.7
22:0	0.1	0.2	0.2	0.5	0.6	0.7
SSMFA	0.8	1.4	1.1	1.0	1.9	2.0
Saturated	16.4	17.1	22.3	12.3	14.1	19.2
14:1 (n-5)	0.3	0.4	0.4	0.5	0.6	0.8
16:1 (n-9)	12.5	11.8	17.0	6.1	6.2	9.4
16:1 (n-7)	4.3	4.2	5.9	15.1	15.3	23.4
tr-16:1 (n-13)	1.5	1.5	2.1	1.5	1.7	2.4
17:1 (n-9)	0.5	0.6	0.7	0.9	1.0	1.4
17:1 (n-7)	0.9	1.0	1.2	1.0	1.1	1.6
18:1 (n-11)	1.7	1.8	2.4	2.7	2.6	4.2
18:1 (n-9)	17.6	16.7	24.0	2.1	2.3	3.3
18:1 (n-7)	2.2	2.2	3.0	2.3	0.4	3.5
20:1 (n-11)	0.3	0.4	0.4	1.4	1.5	2.1
20:1 (n-9)	0.2	0.3	0.3	0.5	0.6	0.7
SMMFA	0.6	0.9	0.7	1.0	1.5	1.5
Monoenoic	42.6	41.8	58.1	35.1	34.8	54.3
16:2 (n-4)	0.4	0.5	0.2	0.7	0.8	0.2
18:2 (n-6)	16.2	15.3	10.3	18.6	18.8	13.6
SDMFA	0.6	0.9	0.6	0.9	1.1	0.7
Dienoic	17.2	16.7	11.1	20.2	20.7	14.5
16:3 (n-6)	0.5	0.5	0.2	0.4	0.5	0.1
16:3 (n-3)	0.4	0.5	0.1	1.7	1.8	0.1
18:3 (n-6)	5.0	4.8	1.9	10.7	10.8	4.6
18:3 (n-3)	10.8	10.3	3.4	8.7	6.8	3.1
20:3 (n-6)	0.5	0.6	0.2	0.5	0.6	0.2
20:3 (n-3)	1.2	1.2	0.5	1.2	1.4	0.7
16:4 (n-3)	0.5	0.6	0.2	1.2	1.4	0.5
18:4 (n-3)	0.2	0.3	0.1	1.7	1.8	0.4
20:4 (n-6)	1.0	1.0	0.4	1.8	1.9	0.8
20:4 (n-3)	0.8	0.9	0.2	2.0	1.1	0.5
20:5 (n-3)	1.0	1.1	0.2	1.4	1.5	0.4
SPMFA	1.9	2.6	1.1	1.1	0.8	0.6
Polyenoic	23.8	24.4	8.5	32.4	30.4	12.0
TMFA	3.9	5.8	3.5	4.0	5.3	4.8

For abbreviations see Table 4.

dubia increased from 2.48 to 5.02% (% of total glycolipids), and from 2.01 to 6.78%, respectively.

DISCUSSION

The total lipid content of lichens is subject to seasonal changes [4]. Dertien *et al.* [4] reported that the total lipid content of *Evernia prunastri* increased from 76 to 154 mg g^{-1} wet wt during the period from March to June while that of *Hypogymnia physodes* increased from 54 to 106 mg g^{-1} wet wt during the period from March to May.

Generally, total lipid contents show strong variations in lichens [1, 5]. During cool times many lichen species are known to build up lipid reserves [6]. Of much interest was the examination of phospho- and polar lipids from lichens, since no data concerning their quantitative changes in seasonal dynamics is available [1]. Lichen phospholipids are of interest because they reflect both the mycobiont and phycobiont compositions. According to published data [5, 7–10], the major phospholipid classes present in lichens are PC, PE, PG and phosphatidylserine (PS). Certain species were also found to possess PI, diphosphatidylglycerol (DPG) and some polar lipids identified as DGTS [1]. The summed phospholipid content showed wide variation from 5.6 to 27% of the total lipids [1, 5, 7–10]. Individual phospholipids were also

Table 6. Glycolipid fatty acids from two *Physcia* species

	Physcia dubia			*Physcia stellaris*		
Fatty acids	March	April	May	March	April	May
12:0	0.4	0.5	0.4	0.5	0.6	0.6
14:0	2.8	2.5	2.2	1.6	1.6	1.9
i-15:0	0.2	0.3	0.2	0.5	0.6	0.6
i-16:0	0.8	0.8	0.6	0.4	0.5	0.5
16:0	9.0	9.7	10.2	3.6	4.5	6.0
18:0	8.2	7.8	8.0	1.5	2.4	3.0
20:0	0.5	0.6	0.7	0.8	0.9	1.0
SSMFA	2.9	1.7	2.1	0.7	1.3	0.8
Saturated	24.8	23.9	24.4	9.6	12.4	14.4
14:1 (n-5)	0.3	0.4	0.2	1.2	1.3	1.9
15:1 (n-9)	0.6	0.6	0.7	—	0.1	—
15:1 (n-7)	0.8	0.7	0.6	0.4	0.5	0.5
16:1 (n-9)	4.0	3.5	8.2	3.9	1.9	6.0
16:1 (n-7)	9.8	8.4	11.2	9.3	8.8	14.0
tr-16:1 (n-13)	0.9	0.9	0.7	0.2	0.3	0.3
18:1 (n-11)	0.4	0.5	2.1	2.7	2.6	4.1
18:1 (n-9)	12.4	13.9	19.0	13.9	13.3	20.4
18:1 (n-7)	1.0	1.0	1.7	2.2	2.2	3.4
20:1 (n-11)	0.6	0.2	1.0	0.6	0.7	0.9
20:1 (n-9)	0.6	0.6	1.3	0.7	0.7	1.0
SMMFA	0.5	0.6	2.5	0.9	1.4	1.6
Monoenoic	31.9	31.3	49.2	36.0	33.7	54.1
18:2 (n-6)	10.0	12.4	9.5	19.6	18.5	14.1
SDMFA	0.9	1.2	0.6	0.6	0.7	0.5
Dienoic	10.9	13.6	10.1	20.1	19.2	14.6
16:3 (n-3)	0.3	0.3	0.2	1.2	1.3	0.1
18:3 (n-6)	9.0	8.8	3.7	9.3	8.8	3.2
18:3 (n-3)	7.2	6.2	2.7	14.7	14.0	6.2
20:3 (n-9)	0.6	0.4	0.2	0.2	0.3	0.1
20:3 (n-6)	0.9	0.9	0.2	0.4	0.5	0.2
20:3 (n-3)	2.2	2.0	0.7	0.2	0.3	0.1
16:4 (n-3)	0.4	0.5	0.4	1.5	1.5	0.7
18:4 (n-3)	0.4	0.5	0.1	1.8	1.8	0.4
20:4 (n-6)	1.6	1.5	0.4	0.1	0.1	0.1
20:4 (n-3)	2.0	1.8	0.2	0.6	0.7	0.1
20:5 (n-3)	1.8	1.6	0.2	0.6	0.4	0.2
22:6 (n-3)	0.5	0.5	0.1	1.0	1.0	0.1
SPMFA	1.9	1.8	0.4	0.3	0.6	0.3
Polyenoic	28.8	26.8	9.5	31.9	31.3	11.8
TMFA	6.2	5.3	5.6	2.5	4.0	3.2

For abbreviations see Table 4.

found to vary from family to family [5]. Considerable variations have been reported within a single genus. *Parmelia stenophylla* contained 89.7% PC while *P. sulcata* contained 57.1% PC [5]. The major phospholipid class reported for certain species was PE. The content of PE in *P. tenella* reached 40.8% and up to 53.7% in *Phaeophyscia sciastra* [1, 11]. PG is known to be the third most important phospholipid class after PC and PE. Phospholipid dynamics as a function of temperature change is not well known. Dertien *et al.* [4] does not provide any quantitative phospholipid data.

The presence of one of the major polar lipids, DGTS, in lichens supports the view about the dominating role of the fungal component (mycobiont) in the lichen species examined. It was demonstrated earlier that higher fungi contained fairly large amounts of DGTS [12]. The chemical structure of higher fungi was elucidated using physicochemical methods [12]. As for the phycobiont, it appears impossible to state presently whether it contains DGTS or not due to lack of data. Referring generally to algae, DGTS is known to be widespread in green marine macrophytes [13], as well as in some microalgae [14–20] where it can play the role of a basic polar lipid [21]. The betaine lipids, DGTS as well as DGTA (diacylglycerylhydroxymethyltrimethyl-β-alanine) represent a prominent group of unusual glycerolipids produced by almost all cryptogamic plants [21, 22]. Since the identification of DGTS, this lipid was suggested to play a role similar to

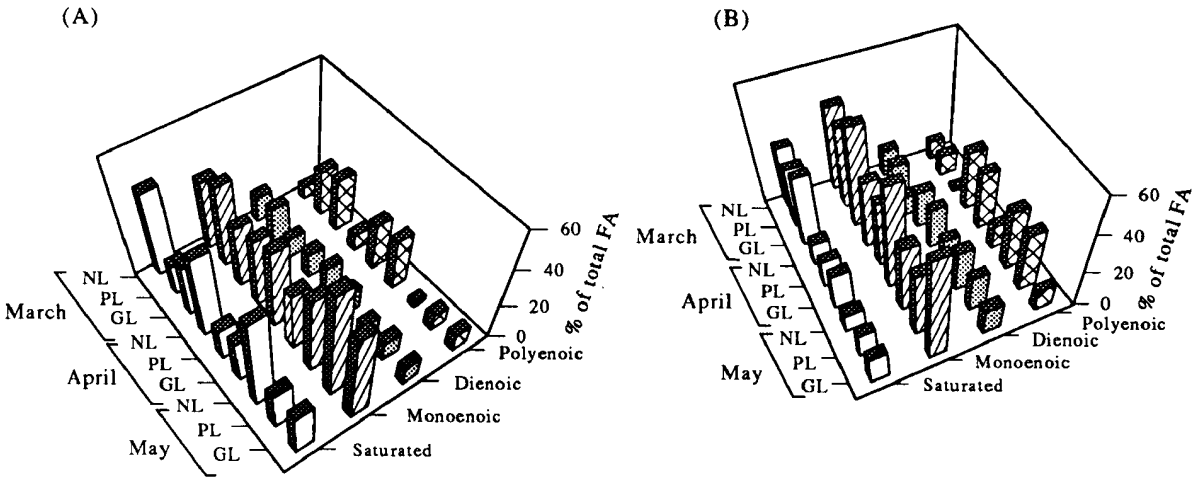

Fig. 1. Seasonal distribution of major fatty acids in different lipid fractions in *Physcia dubia* (A) and *P. stellaris* (B) collected from March to May.

PC, based on the zwitterionic structure which is characteristic of both these lipids.

Fatty acid compositions of various lichen species have been well-studied using packed GC columns [4–7, 10, 23, 24]. However, our recent practice of using capillary columns plus GC-MS methods has proved fairly successful [8, 9, 25, 26].

There are numerous publications dealing with the general idea of temperature-induced changes in the fatty acid composition of plants. Lichens fit in well with this because their total saturated acids increase while their polyunsaturated acids decrease when the ambient temperature rises. The data from our investigation appear to be more important when hydroxy acids are taken into consideration. From literature [27, 28] and our own research results [29–31] it follows that higher fungi, both Basidiomycetes and Ascomycetes, characteristically contain high percentages of hydroxy fatty acids. Recently cyanobacteria were separated and purified from their symbiotic host, *Azolla caroliniana* [32]. Cyanobionts contained *iso*-, *trans*- and hydroxy fatty acids. The presence of hydroxy fatty acids, therefore, would be expected in lichens; Solberg [33–35] reported hydroxy acids in lichens. Our lichen total lipid extracts, however, have not yielded any. Hydroxy acids are not compounds of rare occurrence within the plant kingdom, especially in glycosides and glycolipids [28]. That hydroxy acids in lichens are concentrated only within glycolipids is a matter of interest. Additionally, their relative content, according to our results, increases almost two-fold in all the examined species. It is noteworthy that the sterol compositions of mycobionts and phycobionts are different. According to Lenton *et al.* [36] who studied the sterol composition of *Trebouxia decolorans*, the phycobiont from *Xanthoria parientina* contained two basic sterols, namely ergost-5-en-3β-ol and poriferasterol which were absent from the mycobiont component. The mycobiont, in turn, was found to contain five sterols, namely lichensterol, ergosta-7,22-dien-3β-ol, ergosta-7,22,24(28)-trien-3β-ol and ergosta-7,24(28)-dien-3β-ol, which were absent from the phycobiont component. Such compositions as described suggest different sterol patterns used for sterol biosynthesis of phyco- and mycobionts.

Thus, the experimental data obtained so far on polar lipids, phospholipids and fatty acids including hydroxy fatty acids suggests the existence of interesting symbiotic relations between fungi and algae. We believe that detailed investigations along this line can be expected to throw light upon the distribution of hydroxy acids within various individual classes of glycolipids.

EXPERIMENTAL

Plant material. Physcia dubia (Hoffm.) Lettan. and *P. stellaris* (L) Nyl. were sampled from March to May 1992, in the Zhiguli nature reserve near Togliatti (Russia). Freshly collected lichens were thoroughly cleansed of extraneous matter and homogenized in a high-speed unit.

Lipid extraction and determination. Extraction of lipids, their analysis and quantification, and identification of individual phospholipid classes were carried out as described elsewhere [5, 7, 10]. The total lipid extract was sepd on a silica gel column into the neutral, glyco- and phospholipid frs [7]. DGTS was determined according to the method of ref. [37].

Preparation of fatty acid derivatives. Me esters of corresponding fatty acids (FAME) were prepd by alkaline hydrolysis and reaction of free acids with BF_3–MeOH as described in refs [25, 26]. FAME were sepd by HPLC on a silica gel column (250 × 10 mm, Tessek) with hexane–THF (4:1) as mobile phase. After elution of non-hydroxy FAME, the hydroxy FAMEs were collected and analysed by GC-MS (after derivatization). Picolinyl esters-TMSi ethers were prepd as described in ref [38].

GC-MS analysis of fatty acids. Picolinyl ester-TMSi ethers were identified using a fused silica capillary column (60 m × 0.32 mm i.d.) coated with a 0.25 μm layer of SPB-

Table 7. Hydroxy acids in glycolipid fractions from two *Physcia* species

	Physcia dubia			*Physcia stellaris*		
Fatty acids	March	April	May	March	April	May
OH-12:0	0.09	0.12	0.11	0.31	0.08	0.27
OH-13:0	0.05	0.06	0.07	0.09	0.04	0.09
OH-14:0	0.11	0.43	0.16	0.47	0.11	0.43
OH-15:0	0.23	0.32	0.26	0.19	0.21	0.18
OH-16:0	1.02	0.78	0.89	0.52	0.95	0.45
OH-17:0	0.25	0.25	0.25	0.16	0.23	0.14
OH-18:0	0.21	0.18	0.24	0.01	0.20	0.04
OH-19:0	0.11	0.11	0.17	0.02	0.11	0.07
OH-20:0	0.16	0.14	0.17	0.06	0.15	0.05
OH-21:0	0.06	0.02	0.08	0.02	0.05	0.01
OH-22:0	0.10	0.08	0.11	0.06	0.09	0.05
OH-23:0	—	0.03	0.02	—	—	0.01
OH-24:0	0.22	0.17	0.24	0.03	0.20	0.03
OH-25:0	0.02	0.02	0.02	—	0.01	—
OH-26:0	0.09	0.10	0.11	0.04	0.08	0.04
OH-28:0	0.02	0.04	0.07	—	0.01	—
i-OH-12:0	0.01	0.06	0.11	0.05	0.01	0.11
i-OH-14:0	0.02	0.03	0.39	0.03	0.01	0.12
i-OH-16:0	0.26	0.28	0.90	0.15	0.24	0.96
i-OH-17:0	—	0.03	0.07	0.05	—	0.11
i-OH-20:0	0.07	0.08	0.18	0.02	0.07	0.14
i-OH-21:0	0.02	0.02	0.04	—	0.02	0.04
i-OH-22:0	0.04	0.02	0.10	—	0.03	0.08
ai-OH-13:0	—	0.04	0.05		—	0.03
ai-OH-15:0	0.03	0.07	0.07	0.05	0.02	0.05
ai-OH-17:0	0.09	0.08	0.29	0.04	0.08	0.24
ai-OH-19:0	0.05	0.06	0.14	—	0.04	0.10
Saturated	3.33	3.62	5.31	2.37	3.04	3.84
OH-15:1*	—	0.05	0.28	0.03	—	0.21
OH-9-16:1	0.08	0.13	0.32	0.07	0.08	0.27
OH-10-17:1	—	0.03	0.06	—	—	0.03
OH-11-18:1	—	0.04	0.02	—	—	0.01
OH-9-18:1	0.02	0.02	0.17	—	0.01	0.17
OH-13-22:1	0.02	0.01	0.04	—	0.02	0.03
OH-15-24:1	0.03	0.01	0.17	—	0.03	0.17
OH-16-25:1	—	0.06	0.02	—	—	0.01
OH-17-26:1	—	0.07	0.09	—	—	0.07
OH-28:1*	—	0.06	—	—	—	0.11
i-OH-9-16:1	0.02	0.07	0.13	0.03	0.02	0.09
i-OH-10-17:1	0.03	0.07	0.05	—	0.03	—
i-OH-13-22:1	—	0.03	0.01	—	—	0.02
i-OH-15-24:1	0.10	0.02	0.02	—	0.01	0.03
ai-OH-10-17:1	—	0.01	0.03	—	—	—
ai-OH-12-21:1	—	0.03	0.02	—	—	0.02
Monoenoic	0.18	0.71	1.43	0.13	0.20	1.17
Unknown	—	0.01	—	—	—	—
$>C_{29}$†	—	—	0.04	—	0.13	0.01
Sum of OHFAs‡	3.51	4.34	6.78	2.50	3.37	5.02

*Position of double bond was not determined.

†Probably 2-OH-30:1 and 2-OH-30:0.

‡% of total FAME from total glycolipids, OH-FA not found in other fractions, e.g. neutral lipids and/or phospholipids.

1 (Supelco) using splitless inj. and He as the carrier gas. The oven temp. was progd for 100° to 320° at 4° min^{-1}. HR-MS were determined (as picolinyl ester-TMSi ethers) in the EI mode. Ionization energy was 70 eV, electron multiplier voltage 2.5 kV.

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