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SEASONAL VARIATIONS IN LIPIDS OF *XANTHORIA PARIETINA* GROWING AT HIGH ELEVATIONS

R. PIERVITTORI, F. ALESSIO, L. USAI and M. MAFFEI*

Dipartimento di Biologia Vegetale, Università di Torino, Viale P.A. Mattioli, 25, I-10125 Torino, Italy

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Key Word Index—*Xanthoria parietina*; Theloschistaceae; lichens; fatty acid; seasonal variation; high elevation; multivariate analysis.

Abstract—Seasonal variations (from spring to winter) in the total lipid content, polar, non-polar and neutral lipids were detected in *Xanthoria parietina* growing naturally at high elevation in the northwestern Italian Alps (Piedmont). Various lipid classes were separated and the corresponding fatty acid methyl esters were analysed by GC-MS. The results obtained confirmed a clear response of the lipid metabolism of *X. parietina* growing at high elevations to changing seasonal environmental factors. They also indicated a typical increase in total lipid content from spring to winter and a direct relationship between the degree of fatty acid unsaturation and high temperature and precipitation levels.

INTRODUCTION

The metabolic activity of lichens is characterized, during the changing seasons, by a large variability in their metabolism due to climatic changes [1, 2]. *Xanthoria parietina* has been extensively studied by several authors because of its known characteristics of pollution tolerance [1, 3–9]. Moreover, it is a good experimental organism for studying the processes of adaptation during changing environmental conditions. Variations in primary metabolism are directly correlated with the photosynthetic activity of the photobiont, and the allocation of photosynthates to both photo- and mycobionts may affect the secondary metabolic pathways of both partners [10, 11]. The most notable variations in the secondary metabolism of *X. parietina* relate to general lipid metabolism [12].

Previous studies on *X. parietina* have demonstrated that, in this lichen, fatty acids (FA) are affected by elevation, and that it is not low temperature, typical of high elevations, but other environmental factors which might be involved in FA unsaturation [7]. Various environmental factors are connected with elevation, such as light, temperature and precipitation, as well as UV light and various degrees of stresses caused by anthropic impact. Furthermore, the growing season is shortened and average temperatures are lower. The combination of all these effects exerts pressure on the lipid metabolism of the lichen. For this purpose, we have examined the lipid composition from samples of *X. parietina* growing in a Piedmont valley (northwestern Italy) at elevations which represent the upper limit of the distribution of this species (1300 m).

*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

In order to evaluate the seasonal variations in the FA composition of *X. parietina*, chemical data were correlated to annual variations in temperature and precipitation (rain and snow). Four samplings were done, one for each season, from January to December. The total lipid fraction was separated, for each season considered, into four classes: non-polar and neutral lipids, phospholipids and glycolipids.

In general, the content of the various lipid classes increased from spring to winter (Fig. 1A), ranging from 24.13 to 254.79 $\mu g\, g^{-1}$ dry wt. Figure 1B shows the percentage contribution of each lipid class to the seasonal lipid composition. In particular, free fatty acids (FFA) were proportionally higher during winter and spring, whereas monoacylglycerols (MG), phosphatidylcholine (PC) and digalactosyldiacylglycerols (DGDG) showed higher relative percentages in summer. Phosphatidylglycerol (PG) was proportionally higher in autumn.

Tables 1–4 show the detailed FA composition of the various lipid classes extracted from thalli of *X. parietina* during the four seasons. During spring (Table 1), FFA were composed mainly of saturated FA, while MG had higher amounts of 18:3 and 18:2. The main FA in the diacylglycerols (DG) and PC fractions was represented by 8:0, while 6:0 and 16:0 were the major FA in FFA and triacylglycerols (TG), respectively. Linolenic acid (18:3) was the main FA in phosphatidylethanolamine

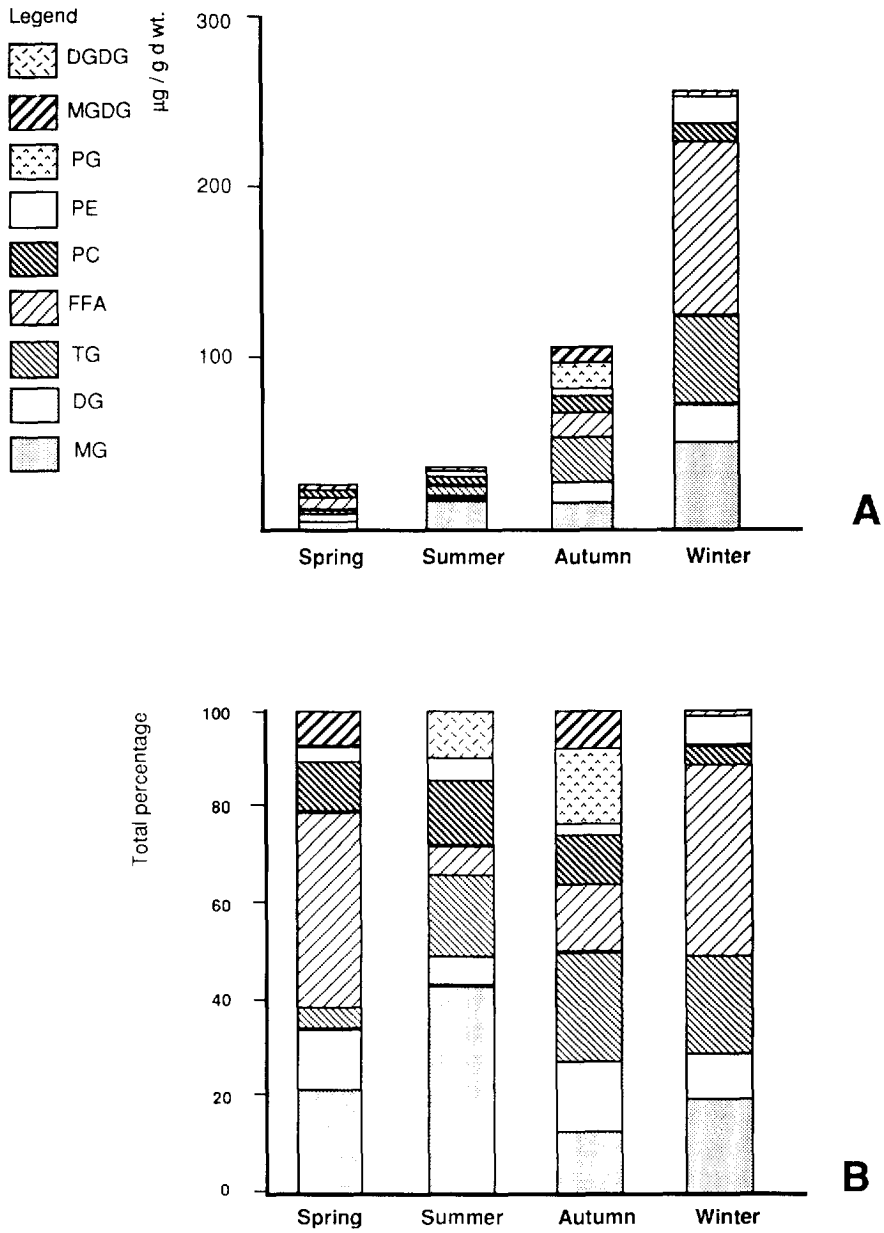

Fig. 1. (A) Seasonal variations in content of various lipid classes in *X. parietina* growing at high elevation; (B) relative percentages of various lipid classes in related season.

(PE) and monogalactosyldiacylglycerols (MGDG). During summer (Table 2), MG had the highest content of saturated FA, 18:1 and 20:2, while the highest content of 16:0 was found in TG. DG were particularly abundant in 20:0 and 20:1, whereas 18:2 and 18:3 were greatest in PC. The FA content of FFA was very low (with the highest value found for 16:0), while 18:3 was the main FA of PE and DGDG. During autumn (Table 3), MG had the highest contents of 16:0 and 18:1, DG had greater contents of 16:1 and 18:0, while 10:0, 12:0, 14:0 and 18:2 were particularly high in TG. PG showed the highest contents of 6:0 and 8:0, whereas 18:3, 20:0 and 20:2 were higher in PE. The main FA of PC was 18:0, whereas 10:0 was the major FA of MGDG. Finally, during winter (Table 4), FFA had the highest contents of almost all FA with the exception of 8:0, 10:0 and 12:0, which were greater in MG; 6:0, which was higher in DG, and 18:1, which was higher in TG. Capric acid (10:0) was the main FA in almost all lipid classes, while 14:0 was the major FA in TG and FFA.

In order to evaluate the changes in the degree of unsaturation in the four seasons we calculated, for each lipid class, the percentage of unsaturation (i.e. percentage of unsaturated FA in total FA) (Fig. 2). A clear decreasing trend in the percentage unsaturation was observed for MG and PC throughout the year, whereas DG, TG, FFA and DGDG showed the highest degree of unsaturation during summer. Unsaturation increased from spring to

Table 1. Fatty acid composition of non-polar, neutral and polar lipids from *X. parietina* collected during spring. Data are expressed as $\mu g\,g^{-1}$ dry wt

	Lipid class						
Fatty acids	MG	DG	TG	FFA	PC	PE	MGDG
6:0	0.29	0.20	0.02	4.11	0.28	0.03	0.06
8:0	0.55	0.64	0.10	1.31	0.84	0.14	0.16
10:0	0.28	0.51	0.10	0.89	0.35	0.09	0.05
12:0	0	0	0.06	0.23	0.05	0	0.05
14:0	0.21	0.61	0.09	0.08	0	0.03	0.05
16:0	0.70	0.61	0.31	1.16	0.18	0.16	0.18
16:1	0.27	0	0.08	1.30	0.15	0	0.06
18:0	0	0	0.08	0.08	0	0	0.80
18:1	0	0	0.08	0.31	0	0	0
18:2	0.55	0	0	0.12	0.53	0	0
18:3	2.27	0.50	0.06	0.28	0.35	0.25	0.27

Table 2. Fatty acid composition of non-polar, neutral and polar lipids from *X. parietina* collected during summer. Data are expressed as $\mu g\,g^{-1}$ dry wt

	Lipid class						
Fatty acids	MG	DG	TG	FFA	PC	PE	DGDG
6:0	0.51	0.04	0.09	0.01	0.15	0.12	0.18
8:0	0.08	0	0.02	0.01	0.02	0.02	0.03
10:0	0.36	0	0.14	0.01	0.05	0.03	0.05
12:0	1.51	0.04	0.14	0.03	0.21	0.16	0.33
14:0	2.74	0.07	0.34	0.06	0.93	0.15	0.21
16:0	1.41	0.35	1.97	0.50	0.48	0.35	0.37
16:1	0.86	0.13	1.34	0.05	0.78	0.20	0.41
18:0	2.02	0.21	0.35	0.25	0.10	0.06	0.20
18:1	3.23	0.35	0.81	0.38	0.34	0.06	0.64
18:2	0.44	0.04	0.40	0.26	0.69	0.03	0.08
18:3	0.79	0.13	0.49	0.21	1.00	0.35	0.66
20:0	0.03	0.48	0	0	0.03	0	0.06
20:1	0.11	0.25	0	0	0.02	0	0.01
20:2	1.12	0	0.10	0.09	0.07	0.11	0.28

autumn in PE, while in MGDG the highest percentages of unsaturation were found during spring and autumn. Figure 3 summarizes the seasonal unsaturation percentages in the four general lipid classes. The highest percentages were found for neutral lipids (Fig. 3, N) during spring and summer; in the latter season, the percentage of unsaturation was almost equal for the remaining lipid classes. Moreover, the percentage of unsaturation of neutral lipids declined from spring to winter. A decreasing unsaturation trend was observed from summer to winter in PL and GL, while NP had the highest percentages of unsaturation in winter.

A direct comparison between general unsaturation and climatic data is represented in Fig. 4. Mean monthly precipitations (rain and snow, expressed in ml of water) indicated a relatively dry period from November to June, with a mid wet spring season and a heavy wet season in summer. The average range was between 20 and 280 ml of water, with minimum and maximum values experienced in January and October, respectively. Mean temperatures were very low in December and January ($-14°$) and gradually increased up to April, to reach the top values in July (27.1°). As the wet season began, temperatures declined rapidly (Fig. 4). Figure 4 also shows the total seasonal percentage of unsaturated FA. A clear increasing trend was present from winter to summer, paralleling the temperature and moisture gradient, then the percentage dropped down along with climatic data (Fig. 4). The statistical analysis performed on the unsaturation percentages indicates a highly significant difference between the four seasons ($F_{3,38} = 5.86$; $P < 0.001$).

The univariate F test calculated on all data indicates highly significant differences for 8:0, 10:0, 12:0, 16:0, 16:1, 18:1, 18:3 and 20:2, while a multivariate statistical

Table 3. Fatty acid composition of non-polar, neutral and polar lipids from *X. parietina* collected during autumn. Data are expressed as $\mu g\,g^{-1}$ dry wt

	Lipid class							
Fatty acids	MG	DG	TG	FFA	PC	PE	PG	MGDG
6:0	0.21	0.55	0.64	0.53	0.44	0.03	1.73	0.82
8:0	3.58	3.10	6.21	3.79	2.42	0.33	7.22	0.47
10:0	5.50	3.45	9.13	5.37	3.18	0.55	5.29	4.68
12:0	0.21	0.20	0.40	0.17	0.04	0.04	0.31	0.26
14:0	0.29	0.41	2.49	1.43	0.05	0.04	0.13	0.17
16:0	1.90	0.66	0.55	0.24	0.16	0.14	0.27	0.22
16:1	0.00	0.55	0.37	0.00	0.04	0.08	0.45	0.10
18:0	0.53	5.17	0	0	3.29	0.09	0	0
18:1	1.00	0.63	0	0.96	0.35	0.38	0.27	0.30
18:2	0	0	4.93	0.58	0.02	0	0	0.10
18:3	0.23	0	0	0.89	0.62	0.97	0.68	0.81
20:0	0	0	0	0	0	0.34	0	0
20:2	0	0	0	0	0.17	0.18	0	0.10

Table 4. Fatty acid composition of non-polar, neutral and polar lipids from *X. parietina* collected during winter. Data are expressed as $\mu g\,g^{-1}$ dry wt

	Lipid class						
Fatty acids	MG	DG	TG	FFA	PC	PE	DGDG
6:0	2.44	2.70	2.21	0.15	1.24	2.10	0.08
8:0	19.65	2.08	9.09	0.32	0.85	1.67	0.83
10:0	22.45	14.41	10.87	0.28	5.65	9.40	1.36
12:0	1.26	0.70	0.54	0.20	0.30	0.41	0.04
14:0	1.05	0.40	17.40	47.56	0.20	0.47	0.04
16:0	1.32	0.54	1.45	8.46	0.33	0.58	0.10
16:1	1.01	0.11	1.91	7.22	0.06	0.01	0
18:0	0	0.16	0	1.37	0.05	0.59	0
18:1	0.78	0.29	2.96	2.50	0.23	0.23	0.29
18:2	0.07	0	1.59	26.11	0	0	0
18:3	0.31	0.32	4.40	6.75	0	0.05	0
20:0	0	0	0	1.38	0	0	0
20:2	0	0	0	0.46	0.13	0.18	0.19

analysis (MANOVA) indicates highly significant Wilk's lambda (0.036; $F_{42,45} = 2.21$; $P = 0.005$), Pillai trace (1.821; $F_{42,51} = 1.875$; $P = 0.016$), Hotelling–Lawley trace (7.481; $F_{42,41} = 2.434$; $P = 0.003$) and Chi-squares (= 72.86; Roots 1 through 2; $df = 42$). In order to evaluate the statistical significance of the differences found for the various lipid classes among the four seasons considered, we used the data matrix of Tables 1–4 to calculate a principal component analysis (PCA), by considering the content of the most significant FA listed above. Figure 5 shows the scatter plot of all lipid classes belonging to the four seasonal samplings on the two main axes of the PCA. The total variance explained by the two PC was 40.6 and 18.7, respectively. PC1 was characterized by positive component loadings for 16:1, 18:1 and 12:0, and negative component loadings for 8:0 and 10:0, thus clearly separating summer's lipid classes from the other seasonal samplings. In contrast, negative PC2 component loadings separated winter and autumn samplings because of higher values for 18:3 and 16:0. Spring lipid classes were located in the PC1 negative and PC2 positive quadrant, which was characterized by high 10:0 component loadings.

Adaptation to low temperatures and drought induce, in lichens, a state of dormancy which is typical of these organisms, enabling them to survive under adverse conditions [13]. During these periods, general lipid metabolism responds to the various environmental factors (i.e. temperatures, moisture, light, etc.) by changing the FA qualitative and quantitative chemical composition [14,

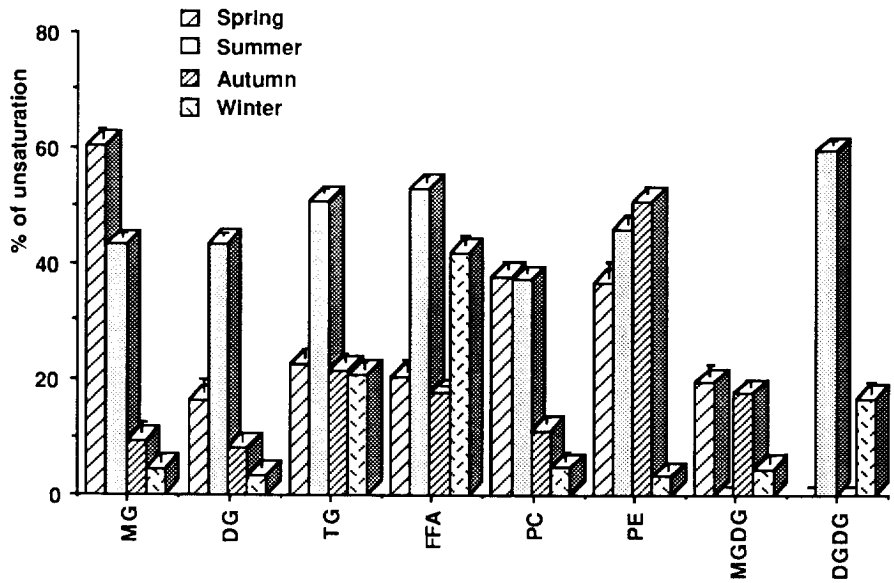

Fig. 2. Seasonal variations in total percentage of unsaturation (total % unsaturated FA in total FA percentage) in the various lipid classes extracted from *X. parietina*. Bars indicate standard deviations.

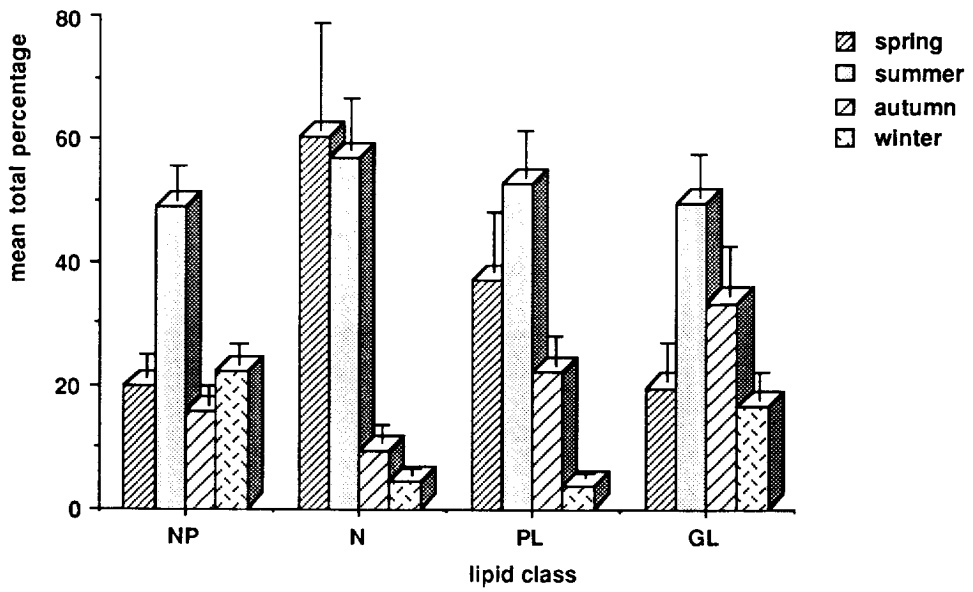

Fig. 3. Seasonal variations of overall FA unsaturation percentage in the main lipid classes extracted from *X. parietina*. Bars indicate standard deviations. NP = non-polar lipids; N = neutral lipids; PL = phospholipids; GL = glycolipids.

and refs cited therein]. In *X. parietina*, in a short-time period, increasing temperatures induces an increase in the NL fraction and a decrease in the glycolipid content, along with a little increase in the total lipid content [14]. In our case, the one-year long-time period of growth shows a dramatic increase of total lipid fraction from spring to winter, with increased FA classes which are typical of storage lipids (i.e. TG). With decreasing temperatures, some endolithic lichen species have been observed to build up lipid reserve into 'oil hyphae', with TG being the predominant lipid component; however, temperatures seem not to alter the degree of unsaturation of the FA analysed in these lichens [15].

Even though the FA percentage of unsaturation has been observed to follow the general pattern typical of higher plants, by drecreasing the degree of unsaturation as temperatures rise [14], the seasonal variations in *X. parietina* growing at high elevations show an opposite

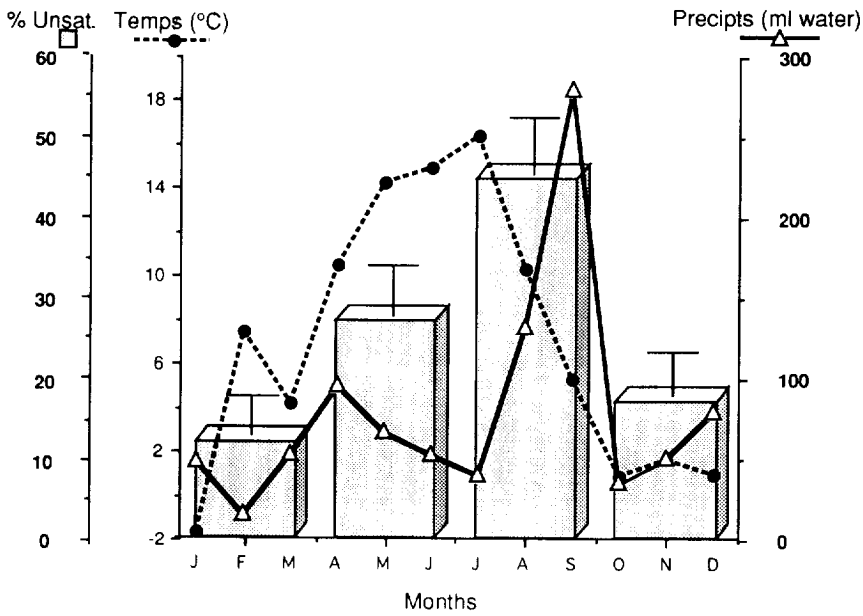

Fig. 4. Direct comparison between climatic data (temperature and precipitation) and total percentage of unsaturated FA of *X. parietina* during four seasons. A general FA unsaturation trend was observed from January to September, then FA unsaturation declined along with temperatures and moisture levels. Bars indicate standard deviations.

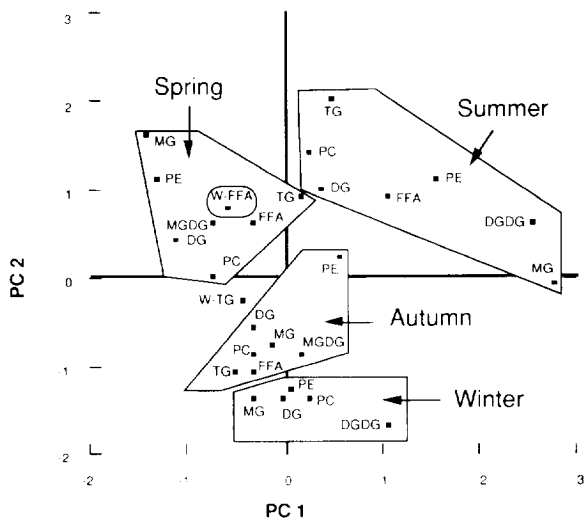

Fig. 5. Scatter plot of various lipid classes extracted from *X. parietina* during four seasons on the two main axes of the PCA. Four general areas are clearly separated, each for a specific seasonal sampling. TG (W-TG) and FFA (W-FFA) from the winter sampling lay outside the winter area.

trend, with higher unsaturation percentages corresponding to the warmest period (summer). These results, in accordance with our previous observations [7], indicate that in *X. parietina* growing at high elevations, low temperatures do not increase the unsaturated FA, which instead seem to parallel increasing temperatures and moisture (Fig. 4). These data do not match with previous studies on the same lichen species [14]. The reason for this incongruence may depend on our wider period of sampling and the effect of elevation. The increasing trend in lipid content from spring to winter reflects a direct relationship between lipid metabolism and the water and thermal status of the symbionts. As the coldest season approaches the lichen accumulates more lipids, though decreasing the unsaturation of FA. Evidently, in *X. parietina* growing at high elevations during the coldest period, unsaturated FA do not play a major role in chilling- and drought-resistance, probably because of the

low metabolic activity of the symbionts. In contrast, unsaturated FA were produced more during the warmest season, when sudden changes in temperature or moisture may injure the plasma and/or organelle membranes of the metabolically active photo- and myco-bionts.

Unfortunately, lichen FA biosynthesis is still little studied and present knowledge does not allow us to formulate considerations on the FA biochemical pathways for enzymes involved in the elongase and/or desaturase reactions [12]. The general difficulty of separating the two lichenic bionts, and the possible different chemical behaviour of the two separated partners cultivated in isolation, makes the study of lichen FA biosynthesis difficult.

In conclusion, the results of this report confirm a clear response of the general lipid metabolism of *X. parietina* to changing seasonal environmental factors and indicates a characteristic behaviour when the lichen is growing at high elevations. This behaviour is an increase in total lipid content from spring to winter and a direct relationship between percentage FA unsaturation and high temperature and precipitation levels.

EXPERIMENTAL

Analyses were carried out on thalli of *X. parietina* (L.) Th. Fr. growing on *Populus nigra* L. bark living at the upper limits of the distribution area for this lichen, at elevations of 1300 m above sea level. Sampling was done every 3 months by collecting thalli on the same trees, 150 cm above the ground level facing north, from plants growing at Bardonecchia. Climatic data (rainfall and temp.) were obtained from the Regione Piemonte.

Thalli (5–7 g dry wt) were thoroughly cleaned and lipids were immediately extracted as previously described [7]. Florisil CC was used to separate lipid extracts into polar, non-polar and neutral lipids using solvents with increased polarity [16].

Lipid extracts were purified by TLC according to the methods described in ref. [16]. TLC plates were sprayed with dichlorofluorescein and observed under UV (353 nm). Spots, corresponding to pure standard compounds, were scraped off and extracted with appropriate solvents. Fatty acid methyl esters (FAME) were obtained, after addition of 30 μg 17:0 as int. standard, according to the method described in ref. [7]. FAME separation was performed by FID-GC using a 50 m × 0.2 mm × 0.3 μm HP-FFAP column as described elsewhere [7]. Peak identifications were based on both R_f comparison with pure standards and GC-MS as described earlier [17]. Statistical analysis included analysis of variance (ANOVA), MANOVA to check for significance differences between lipid compositions for each season and PCA to check for partition among the parameters considered. All analyses were performed with Systat 5.2 software as previously described [18].

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