



EFFECTS OF CARBON DIOXIDE CONCENTRATION AND TEMPERATURE ON LIPID SYNTHESIS BY YOUNG WHEAT LEAVES

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Key Word Index—Acyl lipid synthesis; carbon dioxide concentration; diacylglycerol; fatty acid formation; phosphatidylcholine; phosphatidylglycerol; temperature; *Triticum aestivum*; wheat.

Abstract—The effects of incubation temperature and CO₂ concentration on lipid synthesis in leaves from 7-day-old wheat plants were studied. Plants were cultivated at 350 $\mu\text{mol mol}^{-1}$ (approximately ambient CO₂) and 20° so that, irrespective of the subsequent incubation conditions, the samples were all derived from plants at the same phenological stage of development. Leaf tissue was incubated with [1-¹⁴C]acetate at 350 $\mu\text{mol mol}^{-1}$ or 700 $\mu\text{mol mol}^{-1}$ CO₂ concentration and at 20° or 24°. Doubling the CO₂ concentration had little or no effect on lipid metabolism. In contrast, a 4° rise in incubation temperature not only increased the rate of radiolabelling but also altered lipid synthesis qualitatively. Most noticeable of these changes was a marked increase in phosphatidylcholine labelling evidently at the expense of that of diacylglycerol. The increase in carbon flux to this extrachloroplastic lipid appeared to be restricted to the distal portions of the leaf tissue, thus indicating that the stage of tissue development was critical. Surprisingly, an increase of polyunsaturated fatty acid labelling was found at the higher incubation temperature. This increase was accompanied by a decrease in labelling of oleate in the main radiolabelled membrane lipids. In phosphatidylcholine the decrease in oleate labelling was compensated by a rise in that of linoleate while in monogalactosyldiacylglycerol, both linoleate and α -linolenate were better labelled at 24°. A molecular basis for these alterations in lipid synthesis and acyl desaturation is suggested. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Wheat is one of the world's major crops and the most important cereal in temperate regions. There has been much speculation as regards the possible effects of elevated atmospheric temperatures, as part of the predicted global warming, may have on such crops. It has been estimated that, by the middle of the twenty-first century, atmospheric temperatures could rise from anywhere between 0.5° and 5.5° [1]. It is well recognised that such climate changes will, in turn, have complex effects on vegetation [2] including important crops [3]. Any changes to crop production will, in turn, have social and economic repercussions. Therefore, an understanding of this possible scenario

will facilitate the appropriate changes in social, ecological and agricultural policies.

Elevated atmospheric temperatures accelerate plant growth and, hence, phenological development [4]. However, enhanced growth does not necessarily mean increased crop yield, because elevated growth rates in determinate crops, such as wheat, limits the time available for grain maturation and, thus, can reduce overall grain yield [5, 6]. Indeed, we have reported previously that grain lipid content is actually reduced in wheat plants grown at elevated temperatures [7, 8].

The effects of alterations in temperature on plant lipids have been reviewed [9]. Considerable emphasis has been placed on determining the effects of low temperature on lipid synthesis because of the importance of understanding cold-tolerance in economically viable crops. As a result a greater understanding of the connections between acyl desaturation and membrane fluidity has been achieved [10, 11]. However, little attention has been paid to the possible effects that elevated temperatures may have on lipid metabolism, and yet, with the perceived threat of global warming, this scenario warrants investigation. Therefore, we

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 Abbreviations: DAG = diacylglycerol; DiPtdGly = diphosphatidylglycerol (cardiolipin); DGDG = digalactosyldiacylglycerol; MGDG = monogalactosyldiacylglycerol; PtdCho = phosphatidylcholine; PtdEtn = phosphatidylethanolamine; PtdGly = phosphatidylglycerol; SQDG = sulphoquinovosyldiacylglycerol.

have been investigating the effects of climate change on lipid synthesis in wheat leaves because, since lipids are vital components of membranes, their metabolism can influence generally growth and development. Wheat was chosen as a model because, as mentioned above, it is a very important crop. As part of the research we have investigated how growth under different conditions can influence the ability of wheat leaves to synthesise membrane lipids. However, in such studies interpretation of results is complicated by the different speed and nature of development in the wheat plants [6]. Therefore, in this study we have chosen to investigate short term effects in order to examine directly alterations to enzyme reactions and metabolism. Therefore, the data are not complicated by overlapping effects of tissue maturation, such as would occur when plants were also grown under different temperatures.

Another well recognised contributing factor to the so-called 'Greenhouse Effect' is that of atmospheric gases such as CO₂. Elevated atmospheric CO₂ is usually associated with increased photosynthesis [12, 13], primarily because of the present sub-optimal levels of this gas as a substrate for ribulose-bisphosphate carboxylase/oxygenase activity in C₃ plants. Although acclimation to raised CO₂ is a well recognised phenomenon in some plants [14, 15], it has not been observed in winter wheat [16]. Elevated temperature and CO₂ levels have been observed to interact as plant growth enhancement factors [17] in soybean [18] rice [19] and wheat [6, 20]. Therefore, we have included elevated CO₂ as an interacting parameter during our incubations. The results show marked effects of even small changes in incubation temperature of lipid synthesis. Unexpectedly, these alterations are qualitative as well as quantitative.

RESULTS AND DISCUSSION

We have found previously that wheat seedlings grown for 7 days in an elevated CO₂ environment were characterised by marked changes in lipid synthesis, particularly in a shift of carbon flux from the 'prokaryotic' to the 'eukaryotic' pathway as well as by a 5.7-fold increase in diphosphatidylglycerol (DiPtdGly) radiolabelling in immature post-meristematic tissue [21]. The increase in formation of this mitochondrial-localised lipid correlated well with significant increases in the amounts of mitochondrial specific proteins (e.g. mitochondrial 2-oxoglutarate dehydrogenase complex and chaperonin protein) and in the number of mitochondria as revealed in electron micrographs. All these observations were indicative of increased mitochondrial biogenesis in young parts of the leaves [22]. In contrast, the preferential channelling of newly-formed oleate into the 'eukaryotic' metabolic pathway [23], was found in all parts of the leaves. Temperature also seemed, in preliminary experiments [24], to cause a similar redistribution of carbon.

In order to study these effects further we chose to use *in vitro* manipulations so that we could make conclusions without having to take alterations in tissue development into consideration. Whereas in our previous studies we found that growing young wheat plants in an elevated CO₂ atmosphere altered subsequent lipid metabolism [21], the results in Table 1 show conclusively that changing the concentration of CO₂ during the incubations had little or no effect on the labelling of lipids. Since we used [1-¹⁴C]acetate as a precursor, there was clearly no change in carbon flow into the acyl-CoA pool caused by elevated CO₂. This result agreed with previous data in showing that ¹⁴C-acetate was an effective precursor for fatty acid biosynthesis in plants [25] and that the different levels of dissolved CO₂ were probably optimal for the incorporation of radiolabelled acetate into lipids [26]. Therefore, for the experiment shown in Table 1, [1-¹⁴C]acetate is able to compete effectively with CO₂ as a precursor of lipids. Thus, the effects of CO₂ which we observed before [21] are most likely due to tissue development.

In contrast to the lack of effect of CO₂, a rise of 4° in the incubations resulted in a significant increase in the rate of lipid radiolabel incorporation (e.g. total lipids $165.4 \pm 4.4 \times 10^3$ dpm/100mg fr wt at 20° as opposed to $193.1 \pm 8.7 \times 10^3$ dpm/100mg fr wt at 24°). Both temperatures are within the physiological range for wheat and, accordingly, an overall increase of about this amount in total incorporation is not unexpected given that chemical reactions often have a Q_{10} of about 2. Phosphatidylcholine (PtdCho) and phosphatidylglycerol (PtdGly) were the most heavily labelled phospholipids. Monogalactosyldiacylglycerol (MGDG) was prominently labelled amongst the glycolipids, but digalactosyldiacylglycerol (DGDG) and sulphoquinovosyldiacylglycerol (SQDG), also major chloroplastic glycolipids [27], were poorly labelled. This was probably due to the incubation time chosen since the latter lipids show generally slow rates of acyl turnover as noted in other studies [28, 29]. Diacylglycerol (DAG) is an important intermediate in acyl lipid synthesis. It is often well labelled and, indeed, in the present studies was, by far, the most labelled non-polar lipid. DiPtdGly is unique to the inner mitochondrial membrane and can, therefore, be considered as a lipid marker for this organelle [30]. It was also labelled, generally at 1–2% of the total incorporation. Phosphatidylethanolamine, the second most abundant non-chloroplastic membrane lipid [27], was labelled at about 5% of the total.

The results in Table 1 show that elevated incubation temperatures caused an increase in the labelling of most lipid classes—a notable exception being DAG. In addition to DAG, the relative rate of labelling of PtdCho (in comparison to that of other lipids) was also changed, as indicated by the % labelling figures in parenthesis. PtdGly and the glycolipids are the major lipid constituents of thylakoid membranes, and their content as well as transmembrane distribution in

Table 1. The effect of temperature on the incorporation of radiolabel from [1-¹⁴C]acetate into lipids by young wheat leaves at two CO₂ concentrations

Lipid	Lipid labelling (dpm × 10 ⁻³)/100mg fr. wt			
	350 μmol mol ⁻¹ CO ₂		700 μmol mol ⁻¹ CO ₂	
	20	24 ^c	20 ^c	24 ^c
Total lipid	165.4 ± 4.4	193.1 ± 8.7	164.5 ± 3.3	187.8 ± 4.7
MGDG	14.8 ± 4.3 (9 ± 3)	16.9 ± 2.9 (9 ± 1)	12.4 ± 2.8 (7 ± 2)	19.4 ± 5.3 (10 ± 3)
DGDG	3.1 ± 1.6 (2 ± 1)	4.1 ± 0.5 (2 ± tr.)	2.6 ± 0.8 (2 ± tr.)	3.4 ± 1.6 (2 ± 1)
SQDG	4.3 ± 1.9 (3 ± 1)	5.8 ± 1.9 (3 ± 1)	3.6 ± 1.3 (2 ± 1)	3.7 ± 1.8 (2 ± 1)
PtdGly	24.9 ± 5.0 (15 ± 3)	32.0 ± 3.1 (17 ± 2)	25.2 ± 5.4 (15 ± 3)	33.7 ± 9.6 (18 ± 5)
PtdCho	46.3 ± 8.0 (28 ± 5)	69.0 ± 2.3 (36 ± 1)	41.3 ± 3.8 (25 ± 2)	57.6 ± 10.7 (31 ± 6)
PtdEtn	8.3 ± 3.3* (5 ± 2)	9.4 ± 1.9 (5 ± 1)	5.4 ± 1.1 (3 ± 1)	8.9 ± 2.7 (5 ± 1)
DiPtdGly	1.6 ± 0.6 (1 ± tr.)	2.9 ± 0.8 (2 ± tr.)	1.6 ± tr. (1 ± tr.)	2.9 ± 1.5 (2 ± 1)
DAG	24.6 ± 1.7 (15 ± 1)	19.1 ± 2.0 (10 ± 1)	25.8 ± 3.2 (15 ± 2)	18.2 ± 1.9 (10 ± 1)

Data represent means ± s.d. ($n = 6$, except * where $n = 3$). Numbers in parentheses represent percentages of total lipid incorporation. tr. = trace (<0.5%).

different plant species has been shown to be relatively conserved [31]. Although the rate of radiolabelling of these lipids increased with elevated temperature, there were no changes in their relative percentage labelling. On the other hand, PtdCho which is the major extra-chloroplastic lipid [27] and which is synthesised in the ER primarily by the CDP-base pathway [32] was better labelled proportionally at higher temperatures. The increase in PtdCho labelling could be accounted for by a corresponding decrease in the relative labelling of DAG, from 15 to 10% of the total lipid labelling. These two lipids are connected directly through the cholinephosphotransferase step of the CDP-base pathway, a reaction which may also be important in the transfer of DAG to the chloroplasts via the 'eukaryotic' pathway for lipid synthesis (see refs [23, 25]).

The use of serial sections of leaves from monocotyledons was first used over 20 years ago [33] and has since proved to be a very useful approach to studying lipid synthesis in differentiating tissue, because each successive section distal from the basal meristematic region is representative of sequential stages of maturity. Thus, we have used this approach in order to identify whether the above mentioned changes in lipid labelling occurs in younger or more mature cells. For younger tissue (segment 'B') and in the post-meristematic region (segment 'A'), there were no marked differences in the labelling rates of the individual lipid classes (or total lipids), caused in response to raised incubation temperature (Table 2). It is surprising that an increased temperature did not raise

labelling rates in the younger (section A) tissue sections and suggests that there may be significant constraints to the incorporation of radioactivity from [1-¹⁴C]acetate, such as the availability of other substrates.

The data in Table 2 is in good agreement with previous published work [33, 34], whereby there is a relative increase in glycolipid labelling in the distal regions which was thought to reflect increased plastid development and differentiation. Indeed, typical 'chloroplast' lipids [27] such as MGDG or PtdGly were considerably better labelled as a percentage of total lipids in the more mature sections. Moreover, because of the involvement of PtdCho in supplying chloroplast fatty acids by the 'eukaryotic' pathway [23] then its increased labelling in sections 'C' and 'D' (Table 2) might also be expected. The basal region was characterised by the least amount of radiolabel incorporation on a fresh weight basis although there was a relatively high proportion of labelling of non-saponifiable lipids including surface wax components (data not shown) which also agreed well with previous work [21]. In contrast, segment 'C', the upper middle leaf segment, was characterised by the highest rate of incorporation in keeping with the substantial membrane biogenesis in this region [33]. The differential effect of temperature in increasing the proportional PtdCho labelling at the expense of that in DAG was clearly seen in the older sections, 'C' and 'D'.

Radioactivity from [1-¹⁴C]acetate is mainly incorporated into the fatty acid moieties of wheat leaf lipids [34]. Palmitate, oleate, linoleate and α -linolenate were the main acids labelled during the incubation period

Table 2. The effect of temperature on the incorporation of radioactivity from [1-¹⁴C]acetate into major lipids of serial sections from young wheat leaves

Lipid	Temperature	Lipid labelling (d.p.m. $\times 10^{-3}$)/100mg fr.wt			
		Section A	Section B	Section C	Section D
Total lipid	20°	158.7 $\pm$ 32.6	422.0 $\pm$ 63.1	523.7 $\pm$ 122.3	388.7 $\pm$ 30.3
	24°	160.8 $\pm$ 69.9	507.1 $\pm$ 85.3	699.6 $\pm$ 133.6	544.7 $\pm$ 36.0
MGDG	20°	8.9 $\pm$ 0.7 (6)	36.4 $\pm$ 2.5 (9)	67.9 $\pm$ 2.1 (13)	55.1 $\pm$ 2.7 (14)
	24°	7.3 $\pm$ tr. (5)	34.5 $\pm$ 4.5 (7)	75.8 $\pm$ 9.5 (11)	71.3 $\pm$ 5.4 (13)
PtdGly	20°	16.6 $\pm$ 4.2 (10)	51.4 $\pm$ 5.1 (12)	85.2 $\pm$ 18.1 (16)	63.9 $\pm$ 12.6 (16)
	24°	16.8 $\pm$ 7.9 (10)	60.1 $\pm$ 8.2 (12)	102.8 $\pm$ 25.1 (15)	86.5 $\pm$ 13.1 (16)
PtdCho	20°	32.2 $\pm$ 6.2 (20)	105.4 $\pm$ 14.8 (25)	143.7 $\pm$ 15.3 (27)	116.9 $\pm$ 9.9 (30)
	24°	36.7 $\pm$ 2.2 (23)	115.2 $\pm$ 29.5 (23)	210.8 $\pm$ 4.9 (30)	189.4 $\pm$ 20.1 (35)
DAG	20°	7.9 $\pm$ 0.6 (5)	91.7 $\pm$ 12.7 (22)	89.9 $\pm$ 18.3 (17)	69.5 $\pm$ 10.3 (18)
	24°	7.8 $\pm$ 1.6 (5)	97.7 $\pm$ 20.3 (19)	90.5 $\pm$ 14.3 (13)	55.1 $\pm$ 6.8 (10)

Data represent means $\pm$ s.d. ($n = 3$). Sections A, B, C, and D are sections of the leaf 0–1, 1–2, 2–3 and > 3 cm from the base, respectively.

(Table 3). This result is in keeping with previously published work [21, 34–36]. There was no effect of CO₂ concentration on fatty acid synthesis during the 4hr incubation (data not shown), but there were indications that elevating the incubation temperature by 4° caused an increase in the relative radiolabelling of linoleate (Table 3).

The effect of temperature on lipid metabolism has been extensively studied [37]. It is well accepted that reduced growth temperatures are often characterised by increased membrane lipid unsaturation [37–39], an observation which is most easily observed in non-photosynthetic tissue [9]. It is thought that increased unsaturation then contributes to the maintenance of membrane fluidity at lower temperatures. Indeed, Murata and coworkers have shown very convincingly that modifications of unsaturation through genetic

manipulation can give rise to cold temperature-resistant organisms [40].

In our experiments we were not concerned with changing temperatures in a range likely to cause any physiological damage to wheat, in contrast to experiments on chilling or heat stress. Nevertheless, it was surprising to note that raising the incubation temperature from 20° to 24° actually produced an *increase* in unsaturation. Thus, the proportion of radiolabel in oleate was reduced and that in linoleate usually raised (Tables 3 and 4). These data pointed to a proportional increase in Δ^{12} -desaturase activity and were unexpected since, in general, desaturation is increased at lower temperatures [37, 41]. The effects were particularly noticeable in the more mature tissues as had been the effects on lipid class labelling (Table 2). Since, PtdCho is a major substrate for the Δ^{12} -desaturase

Table 3. Effect of incubation temperature on the incorporation of radioactivity from [1-¹⁴C]acetate into total fatty acids of young wheat leaves

Incubation temperature	Radiolabelling (% of total fatty acids)					
	16:0	18:0	18:1	18:2	18:3	Others
20°	18.1 $\pm$ 1.1	2.7 $\pm$ 0.9	28.1 $\pm$ 3.0	41.6 $\pm$ 3.1	7.4 $\pm$ 0.8	1.1 $\pm$ tr.
24°	17.4 $\pm$ 0.8	2.3 $\pm$ tr.	24.5 $\pm$ 2.1	48.1 $\pm$ 2.5 ($P < 0.1$)	7.8 $\pm$ 1.0	N.D.

Data represent means $\pm$ s.d. ($n = 3$). Statistical significance (Student's *t*-test). Abbreviations: N.D. = none detected; 16:0 = palmitate; 18:0 = stearate; 18:1 = oleate; 18:2 = linoleate; 18:3 = α -linolenate.

Table 4. The effect of temperature on the incorporation of radioactivity from [1-¹⁴C]acetate into the fatty acids of the total lipids of individual wheat leaf section

Temperature	Leaf segment	Fatty acid radiolabelling (% of total)					
		16:0	18:0	18:1	18:2	18:3	Others
20°	A	25.5 ± 3.3	4.3 ± tr.	34.4 ± 2.4	30.4 ± 4.8	2.8 ± 0.6	2.4 ± 1.0
24°	A	34.2 ± 6.5	3.1 ± 1.2	28.6 ± 2.8 (<i>P</i> < 0.1)	30.3 ± tr.	1.5 ± 0.9	2.3 ± 0.8
20°	B	17.0 ± tr.	1.7 ± 0.6	26.5 ± 3.8	46.4 ± 2.5	7.6 ± 2.1	1.0 ± tr.
24°	B	20.1 ± 1.3 (<i>P</i> < 0.02)	3.2 ± 1.2	21.7 ± 1.6	45.3 ± 2.0	8.3 ± 1.1	1.2 ± tr.
20°	C	15.1 ± 1.2	1.0 ± tr.	27.7 ± 2.6	47.0 ± 2.7	8.7 ± 2.1	tr.
24°	C	16.9 ± tr.	1.9 ± 0.6	20.2 ± 0.9 (<i>P</i> < 0.01)	50.7 ± 1.2	9.9 ± 0.9	tr.
20°	D	15.0 ± 0.9	1.0 ± 0.6	33.9 ± 2.9	43.8 ± 3.4	6.2 ± 1.2	N.D.
24°	D	17.9 ± 1.7	1.0 ± tr.	21.6 ± 2.2 (<i>P</i> < 0.01)	50.3 ± 0.6 (<i>P</i> < 0.05)	9.2 ± 0.8 (<i>P</i> < 0.05)	N.D.

Data represent means ± s.d. (*n* = 3). Statistical significance by Student's *t*-test. Abbreviations see Table 3. For leaf segment descriptions see Table 2.

in most leaves [23, 37, 42] and this phospholipid is increasingly labelled at 24° (Table 2), then we postulate that more substrate oleate is available for desaturation and this leads to a rise in the proportion of [¹⁴C]-linoleate.

Because the major changes in the lipid metabolism were associated with the more mature wheat leaf sections, 'C' and 'D' (see Tables 2 and 4), then we studied the lipids in these segments in more detail. MGDG, the major chloroplastic lipid, is recognised to be a substrate for the Δ^{15} -desaturase [36, 43] and, thus, possesses a very high endogenous amount of α -linolenate [27]. Therefore, it was not surprising to observe that this particular fatty acyl moiety was well labelled in MGDG at both incubation temperatures (Table 5). For MGDG, incubation at 24° caused increased labelling of both linoleate and α -linolenate. The increase in polyunsaturated fatty acid labelling was balanced by a decrease in that of oleate, though the increases were only statistically significant for segment 'C' (Table 5).

PtdCho is the major extrachloroplastic lipid and was characterised by marked labelling of oleate and linoleate. This observation, as well as the relatively poor labelling of α -linolenate, is consistent with the proposal that this lipid is a major substrate for the Δ^{12} -desaturase in photosynthetic tissue [35, 42]. The intracellular distribution of DAG, on the other hand, is not known for the experiments described. It can be formed most obviously by the Kennedy pathway in both the chloroplast and in the endoplasmic reticulum as well as by the reverse of the cholinephosphotransferase reaction referred to earlier. The radiolabelled fatty acid composition of the DAG pool (Table 5), having only 11–13% palmitate, precludes the plastid as being a major site of its production [see ref. 23]. It would also argue against newly formed DAG from the endoplasmic reticulum being a major

contributor although it is possible for significant amounts of polyunsaturated acids and a high C₁₈/C₁₆ composition to be observed in tissues where the acyl-CoA pool is dominated by such acids [44]. However, this is not normally the case in leaves. Thus, we would suggest that a major source of the radiolabelled DAG observed in our experiments is the reversed cholinephosphotransferase reaction. The significantly higher α -linolenate content of DAG compared to PtdCho may be explained either because molecular species of PtdCho containing such moieties participate preferentially in cellular reactions (for example, to supply some α -linolenate to the plastid) or because it originates from other sources. In the latter regard, the galactolipid, galactolipid galactosyltransferase [45], is a possibility, although the poor labelling of DGDG precludes this being a major supplier of radiolabelled DAG. Clearly, detailed experimentation is required to resolve these questions.

Without knowing the relative sources of the radiolabelled DAG pool analysed, it is difficult to comment on its relative decrease in comparison to PtdCho following incubations at 24°. However, the changes in lipid class labelling do provide a possible explanation for the rise in radioactive polyunsaturated fatty acids without requiring any increase in the Δ^{12} -desaturase enzyme *per se*. Thus, the higher entry of radiolabelled oleate into PtdCho provides more substrate for the Δ^{12} -desaturase. In turn, this leads to an increase in the amount of linoleate either as substrate for the extrachloroplastic Δ^{15} (ω 3)-desaturase (coded by the *fad 3* gene [46]) or for the chloroplastic Δ^{15} -desaturase (coded by the *fad 7/fad 8* genes) which use MGDG as substrate [46].

CONCLUSIONS

There have been many reports describing the effects of temperature on plant lipid metabolism. Various

Table 5. Effect of temperature on the incorporation of radioactivity from [1-¹⁴C]acetate into the fatty acids of major labelled acyl lipid classes of wheat leaf sections

Lipid class	Temperature	Leaf segment	Fatty acid radiolabelling (% of total labelling)					
			16:0	18:0	18:1	18:2	18:3	Others
MGDG	20°	C	7.8 ± 0.9	6.3 ± 1.8	20.2 ± 1.2	38.4 ± 1.7	25.3 ± 3.8	1.9 ± 0.8
MGDG	24°	C	6.2 ± tr.	1.6 ± tr. (<i>P</i> < 0.02)	11.4 ± 0.8 (<i>P</i> < 0.01)	42.5 ± tr. (<i>P</i> < 0.05)	37.8 ± 1.6 (<i>P</i> < 0.01)	N.D.
PtdCho	20°	C	7.4 ± 2.2	3.7 ± 1.3	34.9 ± 2.4	50.1 ± 2.9	2.6 ± 0.7	1.0 ± 1.0
PtdCho	24°	C	10.1 ± 1.7	1.9 ± 0.7	28.4 ± 1.2	56.5 ± 2.8	2.7 ± tr.	N.D.
DAG**	20°	C	12.5 ± 1.5	5.5 ± 1.0	25.6 ± 2.5	24.7 ± 1.5	19.1 ± 2.5	12.5 ± 1.5
DAG**	24°	C	10.5 ± 0.5	8.0 ± 2.0	18.5 ± 0.5 (<i>P</i> < 0.1)	25.5 ± 0.5	22.0 ± tr.	15.5 ± 2.5
MGDG	20°	D	5.8 ± 2.4	tr.	16.9 ± 2.3	43.4 ± 1.1	33.3 ± 1.9	N.D.
MGDG	24°	D	3.7 ± 0.7	tr.	11.3 ± tr. (<i>P</i> < 0.02)	47.1 ± 4.7	37.8 ± 3.2	N.D.
PtdCho	20°	D	5.5 ± 0.8	1.5 ± 0.7	37.0 ± 0.6	52.4 ± 2.5	2.4 ± 1.5	1.1 ± 0.8
PtdCho	24°	D	6.7 ± tr.	1.7 ± 0.1	31.6 ± tr. (<i>P</i> < 0.01)	57.3 ± 0.6 (<i>P</i> < 0.02)	2.5 ± tr.	N.D.
DAG**	20°	D	11.9 ± 2.0	8.8 ± 1.5	30.5 ± 1.5	29.0 ± 2.0	15.5 ± 1.0	4.0 ± 0.5
DAG**	24°	D	12.7 ± 0.5	9.4 ± 1.0	18.5 ± 3.5 (<i>P</i> < 0.01)	35.0 ± 0.5 (<i>P</i> < 0.01)	19.7 ± 5.0	4.3 ± 2.0

Data represent means ± S.D. (*n* = 3, except** where *n* = 2). Statistical significance by Student's *t*-test. For abbreviations see Tables 1 and 3. For segment descriptions see Table 2.

theories have been proposed in order to explain the phenomenon of increased desaturation at low growth temperatures. These have been recently reviewed [37]. It is noticeable that the literature is rather devoid, though, of the effects of elevated temperature on plant lipid metabolism. However, with 'Global Warming' threatening the earth's ecosystem the effect of modest elevations of temperature on plant metabolism needs urgent investigation. Indeed, the unexpected results obtained in the current work show the difficulty of predicting what the consequences of elevated environmental temperatures may be. Because the results were obtained by short-term manipulations *in vitro*, the change in fatty acid and lipid patterns are probably mainly reflections of the properties of existing enzymes. They may also reflect the changes in connected/competing enzyme activities which have been invoked before to explain temperature-dependant fatty acid patterns in plants [47]. Certainly, if such alterations in lipid metabolism are mimicked and maintained *in vivo*, then it is hardly surprising (given the vital role of lipids in membranes) that plant growth and differentiation is altered. Since a significant 'Greenhouse Effect' is predicted imminently, then more work on this important topic is clearly needed.

EXPERIMENTAL

Wheat (*Triticum aestivum* L. cv. Hereward) seeds were grown for 7 days in constant environment growth cabinet (Birchover West 2050) at 20° and a CO₂ concentration of 350 µmol mol⁻¹ (approximately ambient levels) with a light/dark cycle of 14 hr/10 hr.

The light intensity was 230 µmol m⁻² s⁻¹. For the incubations with serial leaf sections whole primary leaves were cut transversely under water into four sections. The lower three sections were designated 'A'–'C' and were each 1 cm long. The most distal section, 'D', consisted of the rest of the leaf and was generally between 1 and 2 cm long.

Primary leaves were cut at soil level and incubated with [1-¹⁴C]acetate (1 µCi, sp. activity 60 mCi/mmol⁻¹) in 120 µl of water for 4 hr, which was within the period for linear incorporation of label into lipids (data not shown). A draft of air was used to aid transpiration (0.7 m³ s⁻¹) and, hence, uptake of label. There were two variables applied to the incubation (temp and CO₂). The incubation temp were either 20° or 24°, and the CO₂ concentrations were either 350 or 700 µmol mol⁻¹. The serial sections were incubated with [1-¹⁴C]acetate in a shaking water bath for 4 hr [48].

The incubations were stopped by briefly rinsing the leaf samples with H₂O, followed by heating in hot propan-2-ol for 30 min. The lipids were extracted further using the Garbus high-salt extraction method [49] as modified for plant tissues [50]. Individual lipid classes separated by TLC. Polar lipids separated using pre-coated silica-gel plates (E. Merck, Darmstadt, Germany); solvent system: CHCl₃–MeOH–HOAc–H₂O (170:30:5:7.5). This solvent system is not suitable for the separation of DiPtdGly and phosphatidic acid because these lipids co-chromatograph. Therefore, DiPtdGly was separated from phosphatidic acid in separations of polar lipids using 0.2 M ammonium sulphate-impregnated silica plates; the solvent system:

CHCl_3 -MeOH-6.5 M ammonia soln (17:7:1). Non-polar lipids separated by neutral solvent system: of petrol (60/80° fr.)-Et₂O-HOAc (80:20:1) which separated adequately the main non-polar lipid classes. Identification of individual lipids: co-chromatography with authentic lipid standards and their identities confirmed with specific spray reagents [51]; bands visualised by either spraying lightly with H₂O, prior to scraping and scintillation counting, or by spraying with 8-anilino-1-naphthalenesulphonic acid in dry MeOH.

Incorporation of radiolabel into total lipid and fatty acid methyl esters determined by lipid scintillation counting. Quenching corrected using an external standard ratio method.

Fatty acid methyl esters analysed by GC using either 15% EGSS-X as polar packing or 5% SE-30 as non-polar packing material. The gas chromatogram was connected via an effluent splitter to a LabLogic RAGA gas flow proportional counter. The split ratio between the flame ionisation detector (FID) and the radio detector was 1:6, respectively. The flow rate for H₂ and air were 8 and 300 ml min⁻¹, respectively. Ar used as carrier gas with a flow rate of 8 ml min⁻¹. The injector and detector temps for the polar column were set at 230° and 250°, respectively, with the oven temp isothermally set at 180°. A temp programme was employed for the non-polar column with an initial oven temp of 210° for 10 min, thereafter rising 4 min⁻¹ to a final temp of 250°.

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REFERENCES

- Schneider, S. H., *Science*, 1989, **243**, 771.
- Krupa, S. V. and Kirkert, R. N., *Vegetatio*, 1993, **104/105**, 223.
- Chaves, M. M. and Periera, J. S., *Journal of Experimental Botany*, 1992, **43**, 1131.
- Woolhouse, H. W., in *Plants and Temperature*, vol. 42, ed. S. P. Long and F. I. Woodward. The Company of Biologists, Cambridge, 1988, p. 411.
- Thorne, G. N. and Wood, D. W., *Annals of Botany*, 1987, **59**, 413.
- Mitchell, R. A. C., Mitchell, V. J., Driscoll, S. P., Franklin, J. and Lawlor, D. W., *Plant, Cell Environment*, 1993, **16**, 521.
- Williams, M., Shewry, P. R. and Harwood, J. L., *Journal of Experimental Botany*, 1994, **45**, 1379.
- Williams, M., Shewry, P. R., Lawlor, D. W. and Harwood, J. L., *Plant Cell Environment*, 1995, **18**, 999.
- Harwood, J. L., *Progress in Lipid Research*, 1994, **33**, 193.
- Quinn, P. J., in *Plants and Temperature*, Vol. 42, ed. S. P. Long and F. I. Woodward. The Company of Biologists, Cambridge, 1988, p. 237.
- Mazliak, P., *Journal of Plant Physiology*, 1995, **143**, 399.
- Long, S. P., *Plant Cell Environment*, 1991, **14**, 729.
- Arp, W. J., *Plant Cell Environment*, 1991, **14**, 869.
- Besford, R. T., Ludwig, L. J. and Withers, A. C., *Journal of Experimental Botany*, 1990, **41**, 925.
- Besford, R. T., *Vegetatio*, 1993, **104/105**, 441.
- Delgado, E., Mitchell, R. A. C., Parry, M. A. J., Driscoll, S. P., Mitchell, V. J. and Lawlor, D. W., *Plant Cell Environment*, 1994, **17**, 1205.
- Rozema, J., *Vegetatio*, 1993, **104/105**, 173.
- Baker, J. T., Allen, L. H., Boote, K. J., Jones, P. and Jones, J. W., *Crop Science*, 1989, **29**, 98.
- Baker, J. T., Allen, L. H. and Boote, K. J., *Journal of Experimental Botany*, 1992, **43**, 959.
- McKee, I. F. and Woodward, F. W., *New Phytologist*, 1994, **127**, 447.
- Williams, M., Robertson, E. J., Leech, R. M. and Harwood, J. L., *Journal of Experimental Botany* (submitted).
- Robertson, E. J., Williams, M., Harwood, J. L., Lindsay, J. G., Leaver, C. J. and Leech, R. M., *Plant Physiology*, 1995, **108**, 469.
- Browse, J. and Sommerville, C., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1991, **42**, 467.
- Harwood, J. L., in *Plant Lipid Metabolism*, ed. K. C. Kader and P. Mazliak, Kluwer Academic, Dordrecht, 1995, p. 361.
- Roughan, P. G. and Slack, C. R., *Annual Review of Plant Physiology*, 1982, **33**, 97.
- Murphy, D. J. and Leech, R. M., *FEBS Letters*, 1978, **88**, 192.
- Harwood, J. L., in *The Biochemistry of Plants*, Vol. 4, ed. P. K. Stumpf and E. E. Conn, Academic Press, NY, 1980, p. 1.
- Heinz, E. and Harwood, J. L., *Hoppe-Seyler's Zeitschrift Physiologische Chemie*, 1977, **358**, 897.
- Hawke, J. C., Rumsby, M. G. and Leech, R. M., *Plant Physiology*, 1974, **53**, 555.
- Bligny, R. and Douce, R., *Biochimica et Biophysica Acta*, 1980, **617**, 254.
- Rawlyer, A., Unitt, M. D., Giroud, C., Davies, H., Mayor, J. P., Harwood, J. L. and Siegenthaler, P. A., *Photosynthesis Research*, 1987, **11**, 3.
- Moore, T. S., *Annual Review of Plant Physiology*, 1982, **33**, 235.
- Leech, R. M., Rumsby, M. G. and Thompson, W. W., *Plant Physiology*, 1973, **52**, 240.
- Bolton, P. and Harwood, J. L., *Planta*, 1978, **139**, 267.
- Bolton, P. and Harwood, J. L., *Planta*, 1978, **138**, 223.
- Wharfe, J. and Harwood, J. L., *Biochemical Journal*, 1979, **174**, 163.
- Harwood, J. L., Jones, A. L., Perry, H. J., Rutter, A. J., Smith, K. L. and Williams, M., in *Temperature*

- Adaptation of Biological Membranes*, ed. A. R. Cossins, Portland Press, London, 1994, p. 107.
38. Murata, N., *Nature*, 1992, **356**, 710.
39. Murata, N. and Wada, H., *Biochemical Journal*, 1995, **308**, 1.
40. Murata, N., in *Temperature Adaptation of Biological Membranes*, ed. A. R. Cossins. Portland Press, London, 1994, p. 163.
41. Harwood, J. L., Abulnaja, K. O., Barrett, P. B., Herbert, D., Rutter, A. J. and Williams, M., in *Metabolism, Structure and Utilization of Plant Lipids*, ed. A. Cherif, D. B. Miled-Daoud, B. Marzouk, A. Smaoui and M. Zarrouk. Centre National Pedagogique, Tunis, 1992, p. 319.
42. Harwood, J. L., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1988, **39**, 101.
43. Jones, A. V. M. and Harwood, J. L., *Biochemical Journal*, 1980, **190**, 851.
44. Stymne, S. and Stobart, A. K., in *The Biochemistry of Plants*, Vol. 9, ed. P. K. Stumpf and E. E. Conn. Academic Press, NY 1987, p. 175.
45. Joyard, J., Block, M. A. and Douce, R., *European Journal of Biochemistry*, 1991, **199**, 489.
46. Harwood, J. L., *Biochimica et Biophysica Acta*, 1996, **1301**, 7.
47. Slack, C. R., Roughan, P. G. and Browse, S. A., *Biochemical Journal*, 1979, **235**, 649.
48. Walker, K. A., Ridley, S. M. and Harwood, J. L., *Biochemical Journal*, 1988, **254**, 811.
49. Garbus, J., De Luca, H. F., Loomans, M. E. and Strong, F. M., *Journal of Biological Chemistry*, 1963, **238**, 59.
50. Smith, K. L., Douce, R. and Harwood, J. L., *Phytochemistry*, 1982, **21**, 569.
51. Kates, M., *Techniques of Lipidology*. Elsevier, Amsterdam, 1986.