Phytochemistry, Vol. 20, No. 7, pp. 1529–1538, 1981.
Printed in Great Britain.

0031-9522/81/071529-10 $02.00/0

EFFECTS OF TEMPERATURE AND GIBBERELLIC ACID ON PHOSPHOLIPID COMPOSITION OF *AVENA SATIVA* STEM SEGMENTS

MANFRED JUSAITIS, LESLIE G. PALEG and DONALD ASPINALL

Department of Plant Physiology, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, S.A. 5064, Australia

(*Revised received* 3 *November* 1980)

Key Word Index—*Avena sativa*; Gramineae; oat; stem segments; temperature; phospholipids; fatty acids; gibberellic acid.

Abstract—Stem segments taken from *Avena sativa* plants grown at 10°, 20° or 30° varied in their phospholipid composition depending on the growth temperature; as temperature was lowered, there was a shift towards a greater proportion of unsaturated fatty acids. A significant increase was observed in the concentration of linolenic acid (18:3) as growth temperature was lowered. Although prolonged treatment of oat plants with GA_3 produced marked changes in phospholipid composition of stem segments, these changes did not always accompany the GA_3-induced growth response of segments. Treatment of stem segments with GA_3 for only 20 hr produced a significant growth response with little or no effect on phospholipid composition over this time. The data support the hypothesis that GA_3-induced growth in *Avena* stem segments can occur without a concomitant change in phospholipid composition.

INTRODUCTION

The mechanism of action of gibberellic acid (GA_3) in plant tissue is still unclear. Some evidence has suggested that the hormone may act by altering either membrane composition [1, 2] or membrane structure [3, 4]. Compositional alteration may be brought about by changing the ratios of phospholipid: sterol: protein, or by replacing individual phospholipids, sterols and proteins with new or different species (e.g. by changing the fatty acid structure of phospholipids, or by changing sterol composition). Work on the barley aleurone system has indicated a possible effect of GA_3 on enzymes of phospholipid synthesis [5, 6], although more recent studies on wheat aleurone tissue failed to show any control of phospholipid synthesis by GA_3 [7, 8].

The *Avena* stem segment system is an ideal one for studying rapid growth responses to GA_3 [9], and several hypotheses have been suggested to account for the response. The relatively rapid action of GA_3 in causing an increase in growth rate precludes an early effect of the hormone on DNA and RNA synthesis [10]. Koning *et al.* [11] have suggested that the node may be involved in wall-loosening in the internode through GA_3-stimulated acidification [12] leading to enhanced cell wall endohydrolase activity. GA_3 treatment was found to result in an increase in invertase activity in this tissue, but this increase was not the initial response to the hormone as indicated by time-sequence studies [13]. To date, a membrane mechanism of action for GA_3 in this tissue system has not been tested.

In this paper, the hypothesis that GA_3 influences growth by altering phospholipid composition has been investigated. Since the growth response of *Avena* stem segments is strongly influenced by the conditioning of plants prior to excision of segments [9], and particularly by growth temperature of the plants (unpublished results), it was decided to investigate concurrently the effects of both hormone and growth temperature on lipid composition of (p − 1) internode* stem segments. In two sets of experiments, GA_3 was applied either to the plant for a long term, or to the stem segment for a short term, to determine if altered lipid composition was a necessary pre-requisite for GA_3-induced growth.

RESULTS

Application of GA_3 to plants before segment excision (long term)

In this experiment, *Avena* plants grown at 10°, 20° or 30° were treated with GA_3, (p − 1) internode segments were harvested, and phospholipids extracted and analysed. The fatty acid profiles for the five major phospholipids for each treatment are shown in Table 1. Quantitation of phospholipid composition was based on fatty acid content as measured by GC.

Growth temperature had a marked effect on phospholipid composition. Total phospholipid content on a dry wt basis was highest in segments from plants grown at 20° (Table 1). This was also true for the major phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC). There was a significant effect

*(p − 1) internode = internode immediately below the peduncular node.

Table 1. Fatty acid profiles of phospholipid classes from *Avena* stem segments from plants grown at 10, 20 or 30° and treated with GA_3. Values are expressed as μg fatty acid/g dry wt (mean of 3 replicates). (A = 10° plants, B = 20° plants, C = 30° plants)

Treatment	Fatty acid	Phospholipid					Total fatty acid
		PE	PG	PC	PI	PS	
A		(μg/g dry wt.)					
Control	14:0	22	14	7	26	9	80
	16:0	873	169	999	51	25	2119
	16:1	0	0	0	0	0	1
	18:0	8	5	13	4	4	35
	18:1	136	15	347	9	10	519
	18:2	1142	104	1656	41	46	2990
	18:3	1752	212	1811	51	55	3883
	Total phospholipid	3936	523	4835	184	151	9631
$+GA_3$	14:0	7	8	11	7	7	42
	16:0	726	113	650	36	20	1547
	16:1	0	0	0	0	0	1
	18:0	8	4	8	2	4	28
	18:1	112	9	221	7	7	358
	18:2	894	54	1048	26	29	2053
	18:3	1475	120	1270	34	37	2939
	Total phospholipid	3224	311	3210	115	108	6970
B		(μg/g dry wt.)					
Control	14:0	2	2	5	0	1	12
	16:0	1248	456	1712	84	31	3531
	16:1	12	3	13	5	6	42
	18:0	21	10	26	13	11	81
	18:1	247	47	680	19	12	1008
	18:2	2400	307	3485	83	31	6307
	18:3	1704	317	1667	58	18	3766
	Total phospholipid	5638	1144	7591	264	112	14751
$+GA_3$	14:0	2	2	2	0	0	8
	16:0	638	294	698	48	28	1709
	16:1	5	1	7	2	2	20
	18:0	21	10	11	4	6	53
	18:1	144	81	371	14	9	620
	18:2	1110	318	1419	39	20	2908
	18:3	901	244	742	31	12	1932
	Total phospholipid	2824	954	3253	141	80	7254

Table 1. (*Continued*)

Treatment	Fatty acid	Phospholipid PE	PG	PC	PI	PS	Total fatty acid
C				(μg/g dry wt.)			
Control	14:0	23	50	90	44	49	257
	16:0	833	394	1168	93	216	2705
	16:1	13	5	19	14	11	64
	18:0	126	100	62	24	52	366
	18:1	194	63	444	25	32	760
	18:2	1508	270	2081	78	136	4074
	18:3	1229	216	892	38	73	2450
	Total phospholipid	3929	1101	4759	318	572	10680
$+GA_3$	14:0	24	14	41	69	76	225
	16:0	455	178	492	55	115	1296
	16:1	9	4	8	6	10	39
	18:0	70	42	29	17	66	225
	18:1	94	31	162	16	15	320
	18:2	690	111	655	36	63	1557
	18:3	651	93	287	19	29	1081
	Total phospholipid	1995	476	1676	220	376	4744

Table 2. *F*-values for the effects of growth temperature and *in vivo* GA_3 treatment on fatty acid and phospholipid composition of stem segments

Fatty acid	Source of variation	Phospholipid PE	PG	PC	PI	PS	Total fatty acid
14:0	Temp.	7.5*	3.5	22.3**	9.1*	35.4**	35.2**
	GA_3	0.6	1.8	3.6	0.2	0.4	1.4
	$T \times GA_3$	0.8	1.1	4.0	7.0*	0.5	0.3
16:0	Temp.	3.2	6.9	5.6	4.1	21.3**	6.5
	GA_3	42.9***	9.2*	37.5***	14.3**	3.7	78.9***
	$T \times GA_3$	5.4*	1.0	3.0	0.9	3.0	6.6*
16:1	Temp.	14.7*	9.3*	5.4	15.1*	6.8	19.1**
	GA_3	7.2*	2.4	3.0	3.4	0.5	6.4*
	$T \times GA_3$	2.2	0.9	0.8	1.5	0.3	1.7
18:0	Temp.	2.6	5.8	2.6	8.8*	6.2	8.9*
	GA_3	2.5	0.9	7.4*	2.7	0.0	1.9
	$T \times GA_3$	2.5	0.8	1.7	0.4	0.1	0.9
18:1	Temp.	9.8*	3.0	50.8**	9.2*	9.5*	42.8**
	GA_3	125.0***	0.0	45.7***	30.0**	6.3*	165.2***
	$T \times GA_3$	14.2**	1.0	2.6	4.3	2.5	11.1**
18:2	Temp.	31.4**	3.5	32.3**	10.2*	41.1**	33.2**
	GA_3	223.8***	1.0	55.3***	46.9***	6.6*	131.1***
	$T \times GA_3$	32.9***	0.6	5.3*	3.4	2.3	13.0**
18:3	Temp.	17.6*	3.8	47.1**	5.3	10.5*	34.9**
	GA_3	93.6***	6.0*	39.1***	18.0**	10.9*	131.5***
	$T \times GA_3$	7.1*	0.1	1.2	0.4	2.8	4.5
Total phospholipid	Temp.	6.4	4.2	14.9*	5.8	30.0**	10.5*
	GA_3	133.1***	3.9	51.7***	30.6**	4.6	142.5***
	$T \times GA_3$	14.9**	0.7	3.5	0.8	1.6	10.0*

Significance levels: * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$.

of temperature on phosphatidylserine (PS) (Tables 1 and 2) for which a greater than five-fold increase was observed in segments at 30° over and above those at 10° and 20°. The amounts of all fatty acids were affected to a greater or lesser extent by temperature (Tables 1 and 2), the most significant effects being on myristic acid (14:0), palmitoleic acid (16:1), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). Although palmitic acid (16:0), 18:1 and 18:2 were all maximal at 20°, the concentration of 18:3 decreased as temperature was increased. The quantities of 14:0 and 16:1, although low, were higher at 30° than at 10° or 20°.

Total lipid, total phospholipid, and most of the individual phospholipids and fatty acids on a per segment basis decreased markedly as growth temperature was raised (Figs. 1B, 2A and 2B). These decreases accompanied the decrease in dry wt of segments as temperature was increased (Fig. 1D). A corresponding decrease in fresh wt of segments was observed (Fig. 1C).

Growth temperature had a very significant effect on the 18:2/18:3 ratio (Fig. 3A). As temperature was decreased, particularly from 20° to 10°, the 18:2/18:3 ratio was lowered as a result of an increase in 18:3 and a concomitant decrease in 18:2. An effect of temperature on the overall degree of unsaturation was also noted, in that as growth temperature was lowered, the unsaturated/saturated fatty acid ratio generally increased (Fig. 3B). Except in the case of PS, the larger increase occurred between 30° and 20°, while a smaller change was observed between 20° and 10°.

GA_3 treatment caused a significant decrease in total phospholipid on a dry wt basis at all three growth temperatures (Table 2). This decrease was largely due to decreases in PC and PE. The level of phosphatidylinositol (PI) was also significantly reduced by GA_3 (Table 1), though phosphatidylglycerol (PG) and PS demonstrated no significant change in response to GA_3 treatment. The decreases in PE, PC and PI were associated primarily with amounts of 18:2, 18:3, 16:0 and 18:1, which also constituted the major fatty acid components of these phospholipids (Tables 1 and 2). Overall total fatty acid concentrations reflected similar changes, indicating that prolonged GA_3 treatment of the plant brings about a reduction in these components of the phospholipids. It seems likely that GA_3 would bring about a reduction in the other fatty acids as well if the response involves merely a general degradation of phospholipids rather than a specific effect on particular fatty acids. A decrease in 16:1, stearic acid (18:0) and 14:0 may have in fact occurred, but its significance could not be established due to the very small amounts of fatty acid and high degree of variability between replicates.

Linolenic acid (18:3) was the major component of the total phospholipids and of most of the individual phospholipids in segments from plants grown at 10°. However, at growth temperatures of 20° and 30°, 18:2 became the predominant fatty acid in the total phospholipid fraction. 16:0 was the largest component of PG, PI and PS in segments from these plants, while PE and PC were composed primarily of 18:2. PC and PE were the major phospholipid components regardless of growth temperature or GA_3 treatment.

Results plotted on a per segment basis once again indicated a decrease in phospholipid and fatty acid content with GA_3 treatment, particularly in segments from plants grown at 10° and 30° (Fig. 2A and 2B).

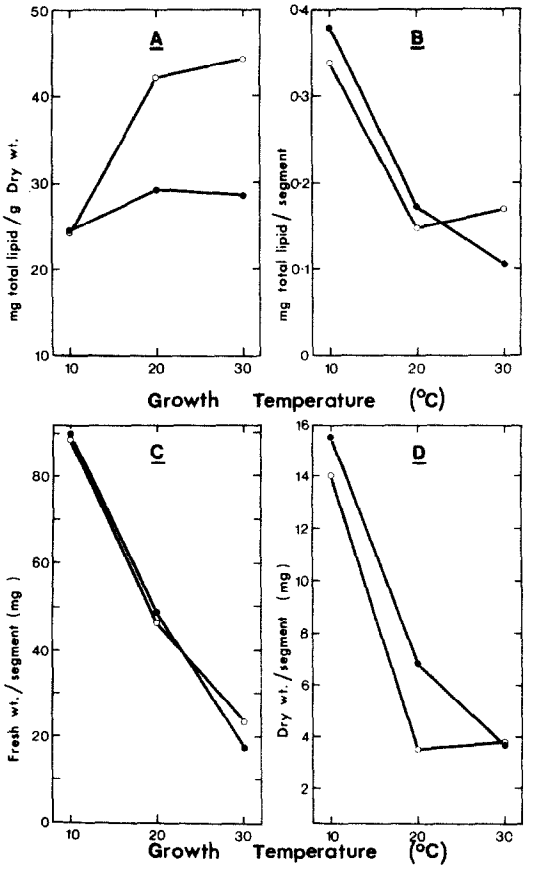

Fig. 1. Effects of growth temperature and *in vivo* GA_3 treatment on lipid and stem segment weights. (A) Total lipid (dry wt basis), (B) total lipid (per segment basis), (C) mean fresh wt of segment, (D) mean dry wt of segment. ○ = Control, ● = 100 μg GA_3/plant.

Segments from 30° plants demonstrated a greater than 50% reduction in total phospholipid in response to GA_3 treatment, and this was reflected by similar decreases in all of the component phospholipids (Fig. 2A). Changes of similar magnitude occurred with each of the fatty acid classes from 30° grown plants (Fig. 2B). A similar decrease was evident in the total lipid extracted per segment from these plants (Fig. 1B), although this difference was not statistically significant.

GA_3 treatment of plants grown at 10° and 20° resulted in increased segment dry wt, while treatment of 30° plants had no effect on dry wt, although fresh wt was significantly reduced (Fig. 1C, 1D). Total lipid extracted was significantly lower on a dry wt basis in segments from 20° and 30° plants treated with GA_3 (Fig. 1A). Since GA_3 treatment results in a general decrease in segment fatty acid and phospholipid content, and the increased dry wt of segments cannot be completely accounted for by an increase in total lipid per segment (Fig. 1B), some other cellular constituent must be contributing to the increased dry wt. The effect of GA_3 on dry wt of segments from 10° and 20° plants suggests that the effects on the lipids may be a *result* of growth differences, rather than a direct effect of the hormone on synthesis or lipid breakdown. The 18:2/18:3 and the unsaturated/saturated fatty acid ratios were not changed significantly by GA_3 treatment (Fig. 3B).

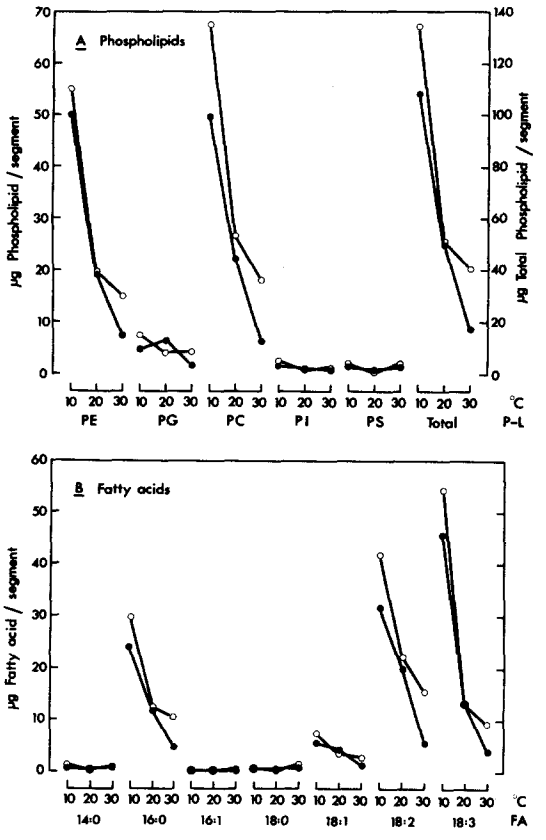

Fig. 2. Effect of growth temperature and *in vivo* GA_3 treatment on (A) phospholipid (P-L) and (B) fatty acid (FA) composition of stem segments (per segment basis). ○ = Control, ● = 100 μg GA_3/plant.

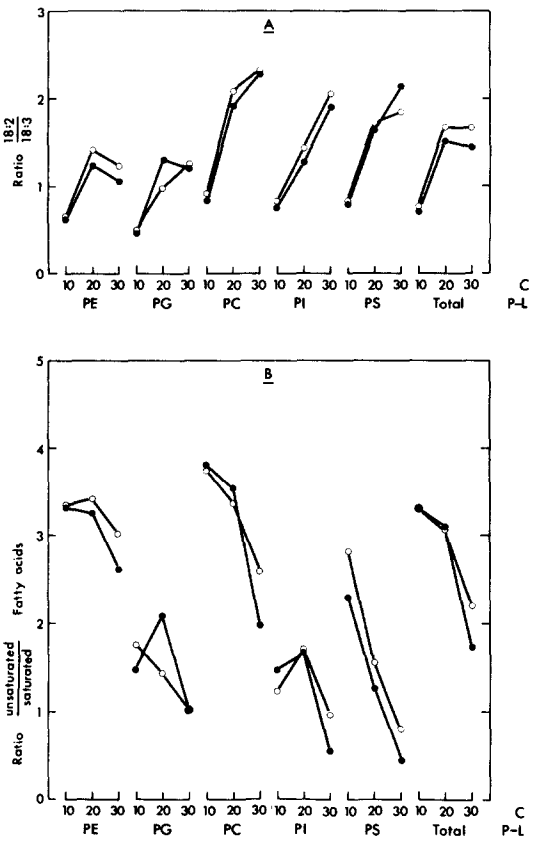

Fig. 3. Effect of growth temperature and *in vivo* GA_3 treatment on (A) the 18:2/18:3, and (B) the unsaturated/saturated fatty acid ratios. ○ = Control, ● = 100 μg GA_3/plant.

Application of GA_3 to segments after excision (short term)

Stem segments from plants grown at 10°, 20° or 30° were incubated in 10^{-6} M GA_3 in 0.1 M sucrose for 20 hr prior to extraction and analysis of phospholipids from these segments. Significant GA_3-induced growth occurred over this period.

Table 3 gives data for the distribution of the major fatty acids of the phospholipid classes separated from these stem segments after 20 hr. The fact that segments had been incubated for 20 hr before lipid extraction did not markedly affect their fatty acid distribution with respect to growth temperature. Total phospholipid and total lipid on a dry wt basis were somewhat lower when compared with segments which were extracted immediately after excision from the plant. This suggests that a certain amount of lipid breakdown or metabolism occurred during the incubation period, a phenomenon commonly observed in senescing tissues [14, 15].

The most significant effect of growth temperature was to increase drastically the proportion of 18:3, particularly in PE, as the temperature was lowered. This resulted in a very significant decrease in the 18:2/18:3 ratio with decreasing growth temperature (Fig. 4A). The ratio of unsaturated/saturated fatty acids was also significantly affected by growth temperature, although not in a consistent fashion.

Total lipid, total phospholipid, and individual phospholipids and fatty acids on a per segment basis, decreased significantly as growth temperature increased (Figs. 5 and 6). The mean fresh wt per segment was much higher (*ca* 10–15 mg/segment) in segments from all growth temperatures after incubation in growth medium for 20 hr due to the uptake of water during this period by both control and GA_3-treated segments (Fig. 1C and 6C).

Total phospholipid content was not significantly affected by GA_3 treatment of the segments (Table 4). No significant effects were observed on individual phospholipids, except for a slight decrease in PS from 20°and 30° plants. Similarly, little effect was seen on the individual fatty acids. The significant differences observed with 16:1 and 18:1 were slight, and seemed to be predominantly due to increases of these fatty acids in PC and PG, respectively, in segments from 30°-grown plants. The significance of these effects can be seen from Table 4; in no case was an effect due to GA_3 significant at $P < 0.01$.

GA_3 treatment did not affect the fatty acid or phospholipid content on a per segment basis (Fig. 5), even though the dry wt of segments from 10° and 20° plants were slightly affected (Fig. 6D). Although 20°-grown segments were the only ones to show a GA_3-induced increase in dry wt over the 20 hr incubation period, significant increases in fresh wt of GA_3-treated segments were found for segments from all three growth temperatures (Fig. 6C), suggesting that the increased growth of treated segments was largely due to a greater uptake of water rather than a higher rate of biosynthesis

Table 3. Fatty acid profiles of phospholipid classes from *Avena* stem segments cultured in the presence and absence of GA_3 after excision. Values are expressed as μg fatty acid/g dry wt (mean of 3 replicates). (A = 10° plants, B = 20° plants, C = 30° plants)

Treatment	Fatty acid	Phospholipid					Total fatty acid
		PE	PG	PC	PI	PS	
A		(μg/g dry wt)					
Control	14:0	5	4	11	2	2	26
	16:0	595	212	983	71	25	1888
	16:1	1	1	5	1	1	10
	18:0	13	8	17	3	3	45
	18:1	101	15	267	9	6	399
	18:2	851	95	1387	39	38	2412
	18:3	1348	203	1691	59	49	3353
	Total phospholipid	2916	540	4365	186	126	8135
$+GA_3$	14:0	7	3	8	3	2	25
	16:0	685	226	879	118	24	1933
	16:1	5	1	6	1	0	15
	18:0	13	4	10	5	3	37
	18:1	112	15	291	17	6	443
	18:2	978	106	1309	71	30	2494
	18:3	1426	213	1426	89	48	3203
	Total phospholipid	3227	570	3931	307	115	8152
B		(μg/g dry wt)					
Control	14:0	6	3	9	2	24	2
	16:0	690	273	942	42	67	2016
	16:1	6	2	5	2	2	18
	18:0	16	7	18	4	4	51
	18:1	158	27	325	11	8	530
	18:2	1511	168	1856	36	39	3611
	18:3	1461	187	1184	21	26	2881
	Total phospholipid	3851	669	4341	120	150	9134
$+GA_3$	14:0	4	5	7	2	1	20
	16:0	556	296	790	84	39	1767
	16:1	4	0	8	2	1	17
	18:0	13	6	13	6	5	45
	18:1	156	43	376	30	8	615
	18:2	1426	254	1677	102	33	3493
	18:3	1351	216	1004	63	20	2657
	Total phospholipid	3512	823	3879	292	110	8617

Table 3. (*Continued*)

Treatment	Fatty acid	Phospholipid PE	PG	PC	PI	PS	Total fatty acid
		(μg/g dry wt.)					
C Control	14:0	7	5	9	3	3	29
	16:0	403	259	886	99	39	1688
	16:1	9	2	11	2	3	29
	18:0	13	10	17	11	9	62
	18:1	75	26	341	16	15	475
	18:2	883	176	1719	78	54	2912
	18:3	289	130	699	42	27	1190
	Total phospholipid	1681	610	3686	254	154	6387
$+GA_3$	14:0	7	5	16	3	3	36
	16:0	443	259	948	104	24	1780
	16:1	7	2	18	3	3	35
	18:0	19	15	50	10	9	106
	18:1	104	44	395	19	9	572
	18:2	989	187	1832	77	27	3114
	18:3	337	119	741	43	15	1257
	Total phospholipid	1910	634	4003	262	93	6904

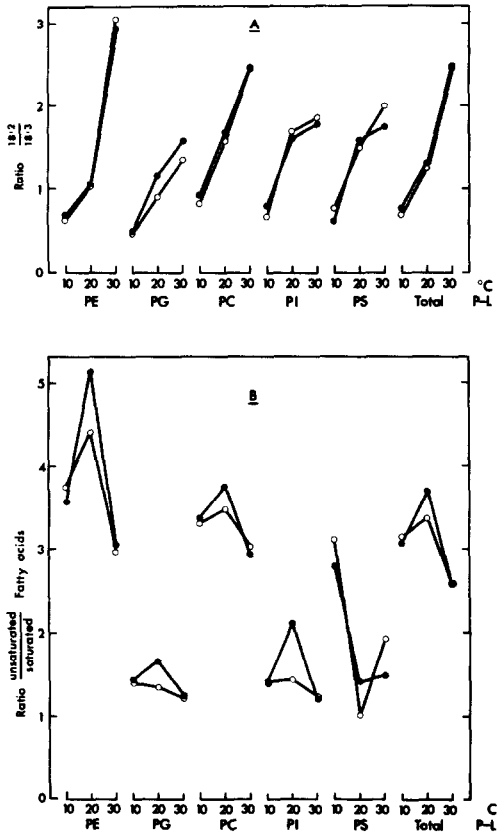

Fig. 4. Effect of growth temperature and GA_3 treatment of excised stem segments on (A) the 18:2/18:3, and (B) the unsaturated/saturated fatty acid ratios. ○ = Segments incubated for 20 hr with 0.1 M sucrose, ● = segments incubated for 20 hr with 0.1 M sucrose $+10^{-6}$ M GA_3.

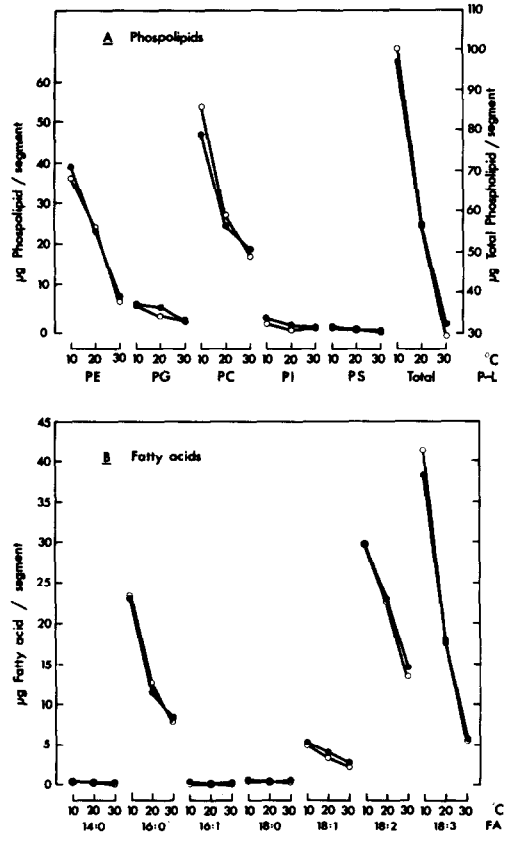

Fig. 5. Effect of growth temperature and GA_3 treatment of excised stem segments on (A) phospholipid (P-L) and (B) fatty acid (FA) composition (per segment basis). ○ = Segments incubated for 20 hr with 0.1 M sucrose, ● = segments incubated for 20 hr with 0.1 M sucrose + 10^{-6} M GA_3.

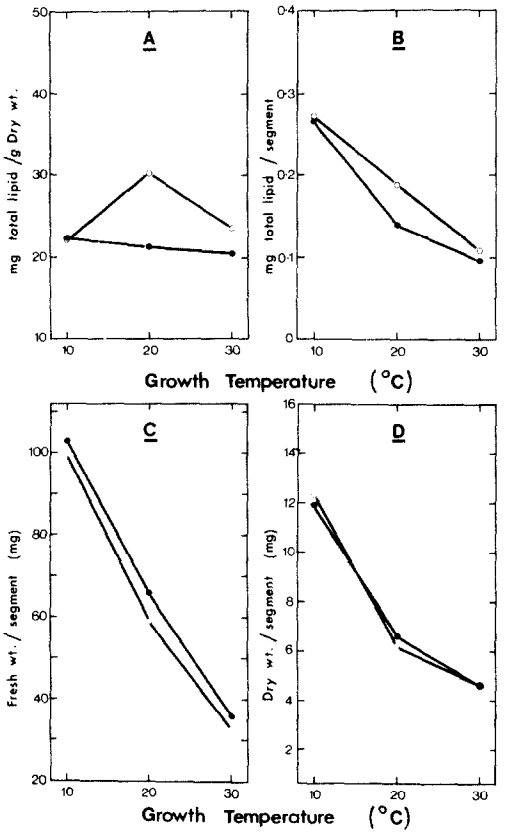

Fig. 6. Effect of growth temperature and GA_3 treatment of excised stem segments on lipid and stem segment weights. (A) Total lipid (dry wt basis), (B) total lipid (per segment basis), (C) mean fresh wt of segment, (D) mean dry wt of segment. ○ = Segments incubated for 20 hr with 0.1 M sucrose, ● = segments incubated for 20 hr with 0.1 M sucrose + 10^{-6} M GA_3.

or cell division. Total lipid on a dry wt or per segment basis from 10° or 30° plants did not change significantly, but GA_3 treatment reduced the total lipid in segments from 20° plants (Fig. 6A and 6B).

The 18:2/18:3 ratios were unaffected by GA_3 treatment (Fig. 4A). The unsaturated/saturated fatty acid ratios remained similarly unaffected, except in the case of PI where GA_3 treatment significantly increased the ratio in segments from 20°-grown plants (Fig. 4B).

DISCUSSION

Although the relative proportions of individual fatty acids remained fairly constant, long term GA_3 treatment brought about a general decrease in the concentrations of each of the phospholipids, particularly PE, PC and PI (Table 1). Hale *et al.* [1] found a decrease in total lipids in stems of peanut (*Arachis hypogaea*) treated with GA_3. Several studies on the breakdown of lipids reserves during seed germination indicate that GA_3 markedly stimulated the rate of lipid breakdown [16–18]. However, an important factor with all these studies is the time-sequence involved. Invariably GA_3 had had ample time to elicit a response before the lipids were analysed. For *Avena* stem segments, it seems that GA_3 treatment will inevitably affect lipid composition after prolonged treatment of the plant, but this probably happens as a secondary response to the hormone. Prolonged treatment with GA_3 does not always result in an effect on membrane lipid composition; for example, GA_3 applied to soybean suspension cultures for 24 weeks produced no effect on fatty acid composition unless applied together with another hormone [2].

Because this *in vivo* GA_3 treatment extended over such a long period (3 weeks), the experiment gave no indication as to whether altered lipid composition was a pre-requisite for GA_3-induced growth, or merely a result of it. The second experiment, using short-term application of the hormone to *Avena* stem segments, clarified this situation. Although significant growth occurred in response to GA_3 treatment, total phospholipid content was not affected (Tables 3 and 4). A small but significant effect of GA_3 on 16:0, 16:1 and 18:1 of PS prevents us from concluding unreservedly that lipid (membrane) composition was unaffected by GA_3 (Table 4). A small change in membrane composition could well produce a large physiological effect [19]. However, an incubation period of 20 hr with the hormone may still be too long in terms of observing the initial response to the hormone, particularly since this system is capable of an essentially immediate growth response to GA_3 (Kaufman, personal communication).

There are several reasons for looking at the 18:2/18:3 ratio. Several workers have found that the 18:2/18:3 ratio is considerably influenced by growth temperature [20–22]. Also, 18:2 and 18:3 are major components of phospholipids in *Avena* stem segments. There is some evidence that plant hormones may affect the 18:2/18:3 ratio [2]. However, in our tissue system, GA_3 had no significant effect on this ratio when applied to the segments for 20 hr.

Low temperature, on the other hand, markedly decreased the ratio as a result of increased concentration of 18:3, and relative decrease in concentration of 18:2. *Avena* stem segment lipids contained more unsaturated fatty acids than saturated ones, as shown by the unsaturated/saturated fatty acid ratios (Figs. 3 and 4). This ratio generally increased as temperature was lowered, but remained unaffected by GA_3 treatment. Increased unsaturation of membranes at low temperatures has often been observed [23–26], and has been associated with low temperature tolerance [27–29]. The increased unsaturation may be due to changes in fatty acids, or to preferential synthesis of particular phospholipids enriched in unsaturated fatty acids. Since plants grown at 10° had phospholipid compositions similar to those grown at 30° (Table 1), the increase in unsaturation of lipids (membranes) synthesized at 10° is likely to be a result of altered fatty acid desaturase activity [30] rather than preferential biosynthesis of individual phosphilipids. Membranes containing higher proportions of unsaturated fatty acids tend to have lower transition temperatures and hence are more fluid at lower temperatures [31], thus maintaining a more favourable lipid milieu for membrane-bound enzymes, [32, 33].

It is evident that GA_3 applied to *Avena* plants over a long period produced significant changes to both phospholipid and 4-desmethylsterol [34] composition of stem segments. It was difficult to assess whether these changes were involved in producing the growth response of the tissue to GA_3, or whether they occurred as a result

Table 4. *F*-values for the effects of growth temperature and GA_3 (short-term treatment) on fatty acid and phospholipid composition of excised stem segments

Fatty acid	Source of variation	Phospholipid					Total fatty acid
		PE	PG	PC	PI	PS	
14:0	Temp.	2.7	1.3	7.4*	4.8	8.3*	20.1**
	GA_3	0.2	2.1	0.2	1.1	5.9	0.1
	$T \times GA_3$	2.6	2.2	7.3*	1.4	4.9	5.3*
16:0	Temp.	16.6*	0.6	0.3	5.7	10.3*	2.8
	GA_3	0.0	0.6	0.7	4.0	28.5**	0.0
	$T \times GA_3$	8.8*	0.2	0.7	0.7	8.0*	2.1
16:1	Temp.	280.0***	4.6	29.7**	24.3**	22.2**	54.7**
	GA_3	0.0	1.0	14.7**	0.5	9.9*	9.1*
	$T \times GA_3$	1.3	1.6	3.5	1.6	0.8	4.2
18:0	Temp.	0.6	4.7	9.1*	10.9*	6.3	17.9*
	GA_3	0.0	0.0	2.7	0.5	1.4	3.2
	$T \times GA_3$	4.7	1.8	7.7*	0.1	0.1	8.7*
18:1	Temp	48.2**	3.4	3.3	1.4	4.0	16.2*
	GA_3	2.5	23.9**	3.1	7.0*	10.2*	10.2*
	$T \times GA_3$	2.0	5.7*	0.2	1.6	11.5**	0.5
18:2	Temp.	98.3**	3.0	3.8	1.5	0.3	38.9**
	GA_3	0.7	5.5	0.2	4.5	5.2	0.4
	$T \times GA_3$	2.5	2.6	0.6	1.6	1.2	0.6
18:3	Temp.	248.6***	2.9	15.9*	6.7	10.0*	144.6***
	GA_3	0.7	0.6	2.4	5.9	2.6	0.0
	$T \times GA_3$	2.7	0.9	1.1	1.5	0.6	1.2
Total phospho-lipid	Temp.	321.0***	0.8	0.3	1.0	0.1	21.9**
	GA_3	0.7	2.7	0.4	5.1	10.2*	0.2
	$T \times GA_3$	5.5*	1.0	0.8	1.2	1.6	1.1

Significance levels: * $P = 0.05$, ** $P = 0.01$, *** $P = 0.001$.

of growth induced by GA_3. The latter theory seems the more plausible since short-term treatment of segments with GA_3 resulted in no marked effects on phospholipid and sterol components, although significant growth response to GA_3 was achieved. Thus neither the mechanism nor the mode (at least in the short term) of GA_3 action in this system involves an alteration of lipid (membrane) composition. The finding that GA_3 treatment of *Avena* stem segments resulted in little or no change in membrane phospholipid or sterol composition was not unexpected. It is unlikely that the rapid timing of many responses to GA_3 would allow a mechanism or mode of action involving biosynthesis or even incorporation of new membrane components, both of which are time-consuming processes. Similarly, studies of the time requirements for protein compositional changes and for GA_3-induced responses suggest that altered membrane protein composition is not involved in the earliest responses to the hormone [35]. Thus, it is reasonable to assume that the three major components of membranes (phospholipids, sterols and proteins) remain largely unaffected (at least in the short term) by GA_3 treatment, and that the primary response of GA_3 does not involve compositional alteration of the membrane. Changes in phospholipid and sterol composition occurred after considerably longer periods, and these changes probably maintained rather than initiated the increased growth observed with GA_3 treatment.

EXPERIMENTAL

Avena sativa cv. Avon was grown at 10°, 20° or 30° and treated with GA_3 (100 μg/plant) on a 6-day treatment schedule, beginning *ca* 3 weeks before harvest of stem segments. All conditions and techniques of harvest and *in vitro* culture of (p − 1) internode stem segments were as previously described [34].

Lipid extraction and fatty acid analysis were performed as outlined previously [34]. Fatty acid data were subjected to analysis of variance to test the significance of the temperature, GA_3 and interaction effects on indidividual fatty acids of each phospholipid on the overall amounts of each phospholipid (based on fatty acid content) and each fatty acid, and on the total fatty acid content. Expts were replicated 3 times, and the data presented are mean values.

REFERENCES

1. Hale, M. H., Orcutt, D. M. and Moore, L. D. (1977) *Plant Physiol. (Suppl.)* **59,** 30.
2. Stearns, E. M., Jr., and Morton, W. T. (1975) *Phytochemistry* **14,** 619.
3. Wood, A. and Paleg, L. G. (1974) *Aust. J. Plant Physiol.* **1,** 31.
4. Nelles, A. (1977) *Planta* **137,** 293.
5. Evins, W. H. and Varner, J. E. (1971) *Proc. Natl. Acad. Sci.* **68,** 1631.
6. Koehler, D. E. and Varner, J. E. (1973) *Plant Physiol.* **52,** 208.

7. Laidman, D. L., Colbourne, A. J., Doig, R. I. and Varty, K. (1974) in *Plant Growth Substances* 1973, p. 626. Hirokawa, Tokyo.
8. Varty, K. and Laidman, D. L. (1976) *J. Exp. Botany* **27,** 748.
9. Adams, P. A., Kaufman, P. B. and Ikuma, H. (1973) *Plant Physiol.* **51,** 1102.
10. Kaufman, P. B. (1975) in *Gibberellins and Plant Growth* (Krishnamoorthy, H. N., ed.) p. 225. Halsted Press, John Wiley, London.
11. Koning, R., Tkaczyk, A., Kaufman, P. B., Pharis, R. P. and Morf, W. (1977) *Physiol. Plant* **40,** 119.
12. Hebard, F. V., Amatangelo, S. J., Dayanandan, P. and Kaufman, P. B. (1976) *Plant Physiol.* **58,** 670.
13. Kaufman, P. B., Ghosheh, N. S., LaCroix, J. D., Soni, S. L. and Ikuma, H. (1973) *Plant Physiol.* **52,** 221.
14. Bentelmann, P. and Kende, H. (1977) *Plant Physiol.* **59,** 888.
15. Fong, F. and Heath, R. L. (1977) *Phytochemistry* **16,** 205.
16. Bhatia, I. S., Singh, I. P. and Sukhija, P. S. (1974) *Physiol. Plant.* **30,** 288.
17. Marriott, K. M. and Northcote, D. H. (1975) *Biochem. J.* **148,** 139.
18. Younis, M. E., Foda, H. A. and El-Ghobashy, A. S. (1971) *Physiol. Plant.* **24,** 411.
19. Haze, J. R. (1973) in *Effects of Temperature on Ectothermic Organisms* (Weiser, W., ed.) p. 55. Springer, Berlin.
20. Farkas, T., Deri-Hadlaczky, E. and Belea, A. (1975) *Lipids* **10,** 331.
21. Galliard, T., Berkeley, H. D. and Matthew, J. A. (1975) *J. Sci. Food Agric.* **26,** 1163.
22. Mannella, C. A. and Bonner, W. D., Jr., (1975) *Biochim. Biophys. Acta* **413,** 213.
23. De La Roche, I. A., Andrews, C. J., Pomeroy, M. K., Weinberger, P. and Kates, M. (1972) *Can. J. Botany* **50,** 2401.
24. De La Roche, I. A., Pomeroy, M. K. and Andrews, C. J. (1975) *Cryobiology* **12,** 506.
25. Rivera, C. M. and Penner, D. (1977) *Plant Physiol. (Suppl.)* **59,** 31.
26. Wilson, J. M. and Crawford, R. M. M. (1974) *New Phytol.* **73,** 805.
27. Moore, P. D. (1975) *Nature* **253,** 11.
28. Smolenska, G. and Kuiper, P. J. C. (1977) *Physiol. Plant* **41,** 29.
29. St. John, J. B. and Christiansen, M. N. (1976) *Plant Physiol.* **57,** 257.
30. Harris, P. and James, A. T. (1969) *Biochem. J.* **112,** 325.
31. Hasiam, J. M. and Fellows, N. F. (1977) *Biochem. J.* **166,** 565.
32. Lyons, J. M. (1972) *Cryobiology* **9,** 341.
33. Lyons, J. M. (1973) *Annu. Rev. Plant Physiol.* **24,** 445.
34. Jusaitis, M., Paleg, L. G. and Aspinall, D. (1981) *Phytochemistry* **20,** 937.
35. McComb, A. J. and Broughton, W. J. (1972) in *Plant Growth Substances* 1970 (Carr, D. J., ed.) p. 407. Springer-Verlag, Berlin.