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THE GLYCERIDES AND ACYL FATTY ACIDS OF GERMINATING HAZEL SEEDS

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Key Word Index—*Corylus avellana*; Betulaceae; glycerides; fatty acids; germinating seeds.

Abstract—An analysis of the neutral lipid fraction of both cotyledon and axis tissue in germinating *Corylus avellana* (L.) seeds has been carried out. In the cotyledons the triglyceride components are utilized non-selectively resulting in little change in the relative acyl-fatty acid content and glyceride groups during germination. Glycerides in the embryo were mainly triglyceride groups containing largely unsaturated acyl fatty acids, and on germination the unsaturated acids decreased and an increase in the saturated (^{14}C) triglycerides was observed.

INTRODUCTION

THE MAJOR storage reserve in hazel seeds is triglyceride which accounts for 30–40% of the fresh weight of the cotyledon tissue. During germination the mobilization of the lipid reserves is essential to provide energy and carbon skeletons for the developing embryonic axis. During the germination of hazel seeds there is increased lipase activity and utilization of ^{14}C- glycerol.[1] However, no differences in the release of $^{14}CO_2$ from carboxyl and universal labelled acyl fatty acids in germinating hazel cotyledon tissue has been observed (Shewry and Stobart, unpublished). The present report describes a qualitative and quantitative analysis of the glycerides in germinating hazel seed cotyledon tissue and embryonic axes.

RESULTS AND DISCUSSION

Figure 1 shows the fresh and dry weight changes in the cotyledon tissue during germination. The fresh weight increase in the cotyledons is due to water uptake since no equivalent increase is observed in the dry weights. The utilization of cotyledon triglyceride (neutral lipid) is not detectable by colorimetric[2] or direct gravimetric measurement until germination and growth are well established (Fig. 1). An increase was observed in the polar lipid (phospholipid) content of the cotyledons after 2 days germination. However, in the germinating embryonic axes there are increases in both the triglyceride and polar lipid fractions which parallel fresh weight increases (Fig. 2).

No changes were observed in the acyl fatty acid content of the cotyledon glyceride fraction during germination. The major fatty acids were $C_{18:1}$ and $C_{18:2}$ with minor quantities of C_{16} and C_{18}. The percentage of acyl fatty acids present (Table 1) is similar to

[1] A. K. STOBART and N. J. PINFIELD, *New Phytol.* **69**, 939 (1970).
[2] N. ZOLLNER and K. KIRSCH, *Z. Ges. Exptl Med.* **135**, 545 (1962).

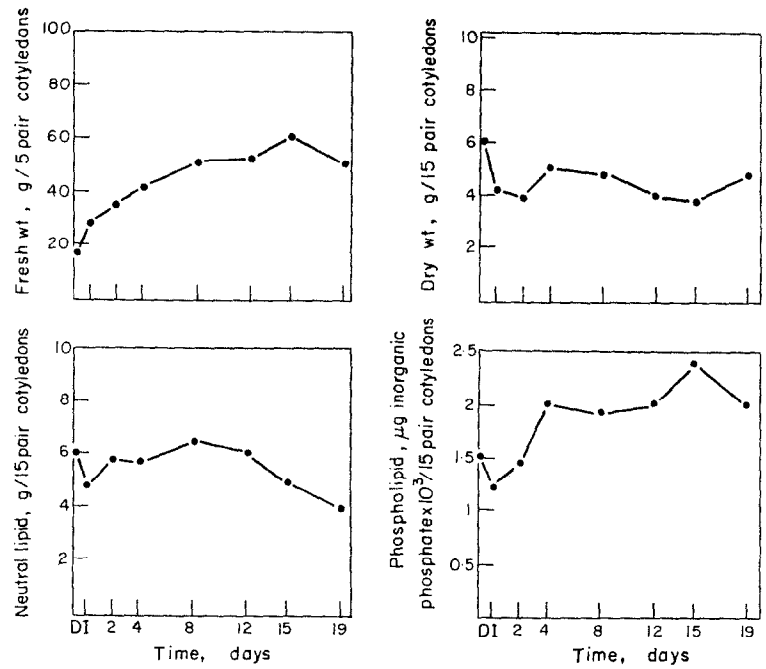

FIG. 1. LIPID AND WEIGHT CHANGES IN THE COTYLEDONS OF GERMINATING HAZEL SEEDS. SEEDS WERE INDUCED TO GERMINATE IN 3×10^{-4} M GA_3.
D—dry seeds; I—imbibed seeds.

values for other varieties of hazel seeds.[3–6] It has been shown similarly that no change occurs in the relative composition of fatty acids synthesized from ^{14}C-acetate in homogenates of germinating Safflower seeds.[7]

In the embryonic axes an increase in saturated short chain acids (C_{14}) took place with a corresponding decrease in C_{18} unsaturated acids (Table 2). An increase in the synthesis of saturated long chain fatty acids has been reported in the neutral lipid fraction of barley[8] and

TABLE 1. ACYL FATTY ACID CONTENT OF COTYLEDON TISSUE IN GERMINATING HAZEL SEEDS

Sample	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry seed	3·8	0·6	74·6	21·0
4 days germination	5·2	1·0	70·0	23·8
15 days germination	3·3	0·6	70·5	25·6

Results are expressed as a percentage of total fatty acid.

[3] H. A. SCHUETTE and C. Y. CHANG, *J. Am. Chem. Soc.* **55,** 3333 (1933).
[4] S. H. BERTRAM, *Ole. Fette. Wachse. Seife. Kosmetik.* **14,** 2 (1936).
[5] C. Y. HOPKINS and M. J. CHISHOLM *Can. J. Chem.* **31,** 1131 (1953).
[6] S. C. FANG and D. E. BULLIS, *J. Am. Oil Chem. Soc.* **26,** 512 (1949).
[7] V. MCMAHON and P. K. STUMPF, *Plant Physiol.* **41,** 148 (1966).
[8] J. C. HAWKE and P. K. STUMPF, *Plant Physiol.* **40,** 1023 (1965).

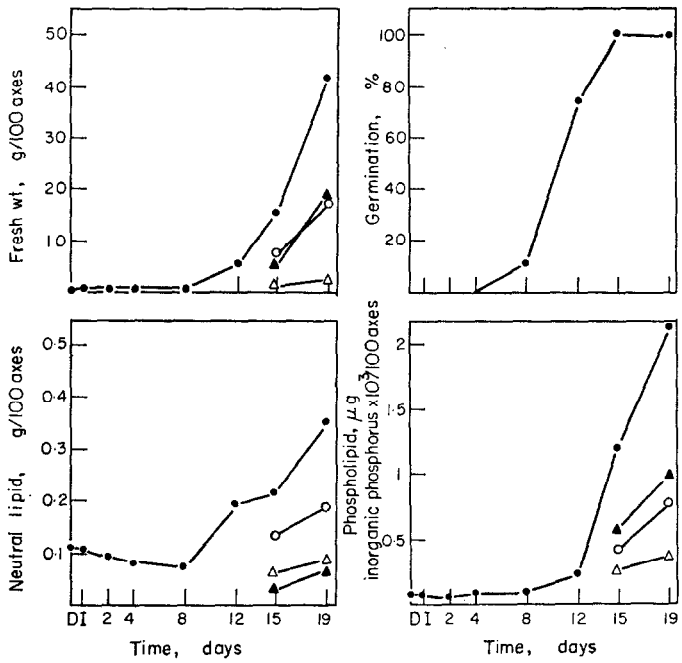

FIG. 2. GERMINATION AND LIPID CHANGES IN EMBRYONIC AXES OF HAZEL SEEDS TREATED WITH 3×10^{-4} M GA_3.
At 15 days germination the axes were separated into component parts: ▲—plumule; ○—radicle + hypocotyl; △—cotyledonary petioles; D—dry seeds; I—imbibed seeds.

the wax and neutral lipid fractions of *Pisum* seeds.[9] No increase in the amounts of saturated long chain fatty acids was found in the cotyledon and embryonic axis neutral lipid fractions of hazel seeds.

TABLE 2. ACYL FATTY ACID CONTENT OF EMBRYONIC AXES FROM GERMINATING HAZEL SEEDS

Sample	C_{14}	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Dry	1·8	5·3	0·5	46·4	45·1	0·9
Imbibed seed	1·4	5·3	0·8	50·9	40·9	0·7
2 days germination	1·9	5·7	0·8	45·1	45·4	1·1
4 days germination	2·3	8·4	1·2	45·8	40·9	1·4
8 days germination	1·9	5·8	0·6	44·0	46·4	1·3
12 days germination	1·7	2·7	0·5	64·2	30·4	0·5
15 days: plumule	27·0	9·5	—	40·9	22·6	—
radicle	10·1	6·1	—	60·8	22·6	0·4
cotyledonary petioles	13·7	6·0	1·3	57·9	21·1	—
Total	13·2	6·5	0·4	57·5	22·4	—
19 days: plumule	37·6	14·2	2·2	32·1	13·9	—
radicle	32·2	11·7	0·6	36·7	18·8	—
cotyledonary petioles	36·9	12·0	—	37·5	13·6	—
Total	34·5	12·3	0·8	35·9	16·5	—

Results are expressed as a percentage of total fatty acid.

[9] J. L. HARWOOD and P. K. STUMPF, *Plant Physiol.* **46**, 500 (1970).

TABLE 3. THE GLYCERIDES OF COTYLEDON AND EMBRYONIC AXIS TISSUE OF HAZEL SEEDS

	Cotyledon glycerides				Embryonic axis glycerides						
Glyceride spot No.	R_f	C_{16}	$C_{18:1}$	$C_{18:2}$	R_f	C_{14}	C_{16}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Co-chromatographed with:
1					0·73	33·0	31·0	35·0	1·0	—	Tripalmitin Tristearin
2					0·59		Not analysed				—
3	0·55	24·7	73·1	2·2	0·53	9·1	27·1	56·0	7·8	—	—
4	0·47	4·2	90·2	5·6	0·47	2·7	10·3	66·5	20·5	—	Triolein
5	0·40	1·6	69·0	29·4	0·42	8·9	7·1	53·7	30·3	—	—
6	0·33	1·6	44·9	53·5	0·38	7·1	6·5	39·2	47·2	—	—
7	0·26	0·7	36·7	62·6	0·34	10·6	8·3	19·4	61·1	0·6	Trilinolein 1,3-Dipalmitin
8					0·29		Not analysed				1,2-Dipalmitin
9					0·26		Not analysed				

The glyceride groups obtained by argentation TLC with CCl_4–$CHCl_3$–HOAc–EtOH (40:60:0·5:0·5) are numbered 1–9 from high to low R_f values. The acyl fatty acid components of the glyceride groups are expressed as a percentage of the total fatty acid. Glyceride groups 3–7 of cotyledon tissue co-chromatographed with groups 3–7 of the embryonic axes.

Argentation TLC showed the presence of five major cotyledon and nine major embryonic axis glyceride groups. Such a separation technique is based on the degree of unsaturation in the molecule.[10] Consequently, each spot does not represent an individual glyceride but rather a number of glycerides having the same degree of unsaturation. The acyl-fatty acid composition of the glyceride groups was established by GLC (Table 3). The dry cotyledon neutral lipid fraction was composed of almost 96% triglycerides the remainder being diglyceride. The five major cotyledon triglyceride groups showed no changes in relative amounts during germination indicating non-selective utilization by lipase (Table 4). Similar non-specific utilization of triglyceride fatty acids has been shown in other germinating seeds.[11–14]

TABLE 4. GLYCERIDES OF COTYLEDON TISSUE IN GERMINATING HAZEL SEEDS

Sample	Glyceride No. 3	4	5	6	7
Dry	12·7	36·7	31·9	14·9	3·8
2 days germination	9·1	40·8	31·9	13·6	4·6
8 days germination	11·6	41·4	32·9	10·6	3·5
15 days germination	8·5	42·3	32·2	12·7	4·3
19 days germination	10·6	42·7	30·7	12·2	3·8

Results are expressed as a percentage of total glyceride. Glyceride numbers refer to the relative position of the glycerides group on $AgNO_3$ impregnated TLC plates (see Table 3).

[10] L. J. MORRIS, in *New Biochemical Separations* (edited by A. T. JAMES and L. J. MORRIS), p. 295, Van Nostrand, London (1964).
[11] D. C. ZIMMERMAN and H. J. KLOSTERMAN, *J. Am. Oil Chem. Soc.* **42**, 58 (1965).
[12] S. G. BOATMAN and W. M. CROMBIE, *J. Exptl Bot.* **9**, 52 (1958).
[13] W. M. CROMBIE and R. COMBER, *J. Exptl Bot.* **7**, 166 (1956).
[14] E. E. HARDMAN and W. M. CROMBIE, *J. Exptl Bot.* **9**, 239 (1958).

The neutral lipid fraction from the embryonic axes showed greater changes in glyceride composition during germination (Table 5). The increase in glyceride '1' represents an increase in the degree of saturation of the component acyl-fatty acids (see Table 3). Increased amounts of diglyceride (Spots 8 and 9) were also observed. From tracer studies (unpublished)

TABLE 5. GLYCERIDES OF EMBRYONIC AXES FROM GERMINATING HAZEL SEEDS

Sample	Glyceride No. 1	2	3	4	5	6	7	8	9
Dry	14·5	—	4·1	19·2	26·3	18·2	15·7	2·0	—
2 days germination	11·9	—	2·6	18·8	24·6	21·2	17·8	3·1	—
4 days germination	17·3	—	7·1	18·4	25·1	15·9	13·2	3·0	—
8 days germination	12·8	—	5·9	21·8	26·6	16·1	14·9	1·9	—
12 days germination	22·4	—	3·8	24·9	23·5	11·4	11·2	2·8	—
15 days: Total embryo axes	57·0	1·7	10·7	8·1	4·2	2·8	1·9	8·9	4·7
plumule	66·8	7·0	5·9	3·5	2·1	1·9	1·7	9·3	1·8
radicle	57·2	—	10·3	6·3	3·5	3·2	2·7	10·0	6·8
Cotyledonary petioles	52·5	3·1	14·4	14·4	6·7	2·3	—	5·4	1·2
19 days: Total embryo axes	54·7	—	—	6·0	6·6	4·1	4·9	16·9	6·8
plumule	58·7	—	—	—	4·8	3·5	7·8	19·8	5·4
radicle	49·1	—	—	9·9	5·9	2·9	2·8	20·1	9·3
Cotyledonary petioles	63·9	—	—	2·1	9·6	7·1	7·3	7·6	2·4

Results are expressed as a percentage of total glyceride. Glyceride numbers refer to the relative position of the glyceride groups on $AgNO_3$ impregnated TLC plates (see Table 3).

it is probable that the diglycerides are involved in phospholipid synthesis in the germinating embryonic axis.

EXPERIMENTAL

Fruits of *Corylus avellana* (L.) (Kent Cob Nuts) were purchased from R. Gould, Mereworth, Kent. After de-shelling and sterilizing, the seeds were imbibed in H_2O and placed in Petri dishes containing 20 ml 3×10^{-4} M GA_3. The hormone is necessary to break the dormancy of the seed.[15] At intervals samples of cotyledon and embryonic axis tissue were harvested, extracted, and fractionated into polar and neutral lipids.[1,16] Embryonic axes from seedlings up to 12-days-old were extracted whole. After 12 days, embryonic axes were sub-divided into plumules, radicle + hypocotyl, and cotyledonary petioles, each part being extracted separately.

Neutral lipid fractions were estimated both colorimetrically[2] and gravimetrically. Phospholipid was determined as inorganic phosphate by a modification of the Bartlett procedure.[17] Aliquots of the neutral lipid fractions were reduced to dryness and transmethylated at 80° for 90 min in sealed glass vials with 2 ml methylating mixture (MeOH–benzene–conc. H_2SO_4, 20:10:1 by vol.). Methyl esters were extracted with 5 ml isopentane and washed with saturated Na_2CO_3 solution. Esters were separated on a 1·5 m × 6 mm glass column of 15% PEGS on 100–120 mesh chromosorb W at 175° in a Pye 104 gas chromatograph. N_2 at 50 ml/min was used as carrier.

Glycerides were separated into saturation groups by argentation TLC. Kieselgel plates (0·25 mm), impregnated with 5% $AgNO_3$ were developed in CCl_4–$CHCl_3$–HOAc–EtOH (40:60:0·5:0·5). Diglycerides were separated from mono-, and triglycerides by TLC on Kieselgel using light petroleum 40–60°–diethyl ether–HOAc (70:20:4). After development TLC plates were sprayed with 50% H_2SO_4 and charred for 20 min at 220°. Quantitative measurements were made by densitometer tracings of the charred areas using a Joyce-Loeble Chromoscan. The fatty acid composition and double bond number of the glyceride groups

[15] P. F. WAREING, in *Physiology of Plant Growth and Development* (edited by M. B. WILKINS), p. 605, McGraw-Hill, London (1969).
[16] D. R. THOMAS and A. K. STOBART, *J. Exptl Bot.* **21**, 274 (1970).
[17] G. V. MARINETTI, in *New Biochemical Separations* (edited by A. T. JAMES and L. J. MORRIS), p. 339 Van Nostrand, London (1964).

were determined by preparative TLC followed by GLC of the fatty acid components. Confirmation of the degree of unsaturation of the glyceride groups was obtained by co-chromatography with authentic glyceride samples.

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