FATTY ACIDS IN GERMINATING SEEDS OF SUNFLOWER (*HELIANTHUS ANNUUS*) AND COTTON (*GOSSYPIUM BARBADENSE*)

By

Ahmed El-Sayed Mohamed Abdalla

Thesis submitted to the University of Glasgow
for the degree of Doctor of Philosophy

Faculty of Science
University of Glasgow
February 1986

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

To my parents,

my sisters, Liala and Fatima,

and my brother, Gaber.

CONTENTS

ACKNOWLEDGEMENTS

I am particularly grateful to my supervisor, Professor John R. Hillman for his continuous assistance, advice and encouragement during the course of this study and the preparation of the manuscript. I also wish to express my deep gratitude to Dr. A.M.M. Berrie for his guidance, assistance with gas chromatography and helpful suggestions during the preparation of the manuscript. Thanks are also due to Dr. V.B. Math for assistance with mass spectrometry. It is a pleasure to acknowledge the helpful discussions with Drs. C. Brett, E. Baydoun (Yarmouk University, Jordan), J. Milner and K. Waldron, and Professor M.B. Wilkins, as well as the advice of my fellow research students, especially Mark Evans and Christopher Mackenzie. I would like to thank Mr. E. Robertson and Mr. N. Tait for their photographical assistance. Thanks are also due to Mrs. M. Gourlay for typing the manuscript.

This study was carried out at the University of Glasgow while the author was in receipt of grants from the Egyptian Government and C.V.C.P. of the Universities of the U.K.

Finally, I would like to convey my thanks to Professors Entisar El-Difrawy, F. Rehab and K. El-Etr, Faculty of Agriculture, Helwan University, Egypt for their encouragement.

ABBREVIATIONS

ADP	Adenosine diphosphate
AFID	Alkali-flame-ionization detector
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
b.p.	boiling point
BHT	Butylated hydroxy toluene
BSA	Bovine serum albumin
DG	Diglyceride(Diacylglycerol)
DTT	Dithiothreitol
D.wt.	Dry weight
ECD	Electron-capture detector
EDTA	Ethylenediaminetetraacetate
ER	Endoplasmic reticulum
et al.	*et alia*
FA	Fatty acid
FFA	Free fatty acid
FID	Flame-ionization detector
Fig.	Figure
FPD	Flame-photometric detector
f.s.d.	Full scale deflection
F.wt.	Fresh weight
GA	Golgi apparatus
GC-MS	Combined gas chromatography - mass spectrometry
GL	Glycolipid
GLC	Gas-liquid chromatography
HETP	Height equivalent to a theoretic plate (efficiency
HPLC	High-performance liquid chromatography

IDP	Inosine diphosphate
IDPase	Inosine diphosphatase
IR	Infra-red
LCFA	Long-chain fatty acid
LCFAME	Long-chain fatty acid methyl ester
M	Mitochondria
Me	Methyl
m/z	mass-to-charge ratio
MG	Monoglyceride(Monoacylglycerol)
MS	Mass spectrometry
MW	Molecular weight
NADH	Nicotinamide adenine dinucleotide, reduced form
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
ND	Not detected
nd	not determined
NL	Neutral lipid
NMR	Nuclear magnetic resonance
NSM	Non-saponifiable material
PA	Phosphatidic acid
PC	Phosphatidyl choline
PE	Phosphatidyl ethanolamine
PG	Phosphatidyl glycerol
PI	Phosphatidyl inositol
PL	Phospholipid
PM	Plasma membrane
PS	Phosphatidyl serine

R	Resolution
R_f	Retention factor
R_t	Retention time
SCFA	Short-chain fatty acid
SCFAME	Short-chain fatty acid methyl ester
SCOT	Support-coated open tubular
SD	Standard division
S.E.	Standard error
SM	Saponifiable material
TCA	Tricarboxylic acid
TCD	Thermal-conductivity detector
TG	Triglyceride(Triacylglycerol)
TLC	Thin-layer chromatography
Tris	Tris (hydroxymethyl) aminomethane
UV	Ultra-violet
v/v	volume to volume
WCOT	Wall-coated open tubular
w/v	weight to volume
w/w	weight to weight

Wherever possible, the S.I. convention has been adopted, with the exception of time units, and minutes (min), hours (h), days (d), weeks and months have been used according to generally accepted practice.

ABSTRACT

Analyses of long-chain and short-chain fatty acids were carried out on sunflower (Helianthus annuus L.) and cotton (Gossypium barbadense L.) seeds and seedlings under optimum conditions of temperature and water availability.

The total lipid content and the levels of the three lipid subfractions (neutral, phospholipid and glycolipid) were determined in the cotyledons, radicles and seed coats of both species. A major proportion of the total lipid in the cotyledons was neutral lipid which decreased gradually during the germination period, but the levels of the two other subfractions increased. No significant changes in the amounts of the three lipid subfractions were noted in extracts of the pericarp and testa, although slight changes were noted in extracts of radicles.

Long-chain fatty acid composition of total lipid and the lipid subfractions was determined using gas-liquid chromatography with flame-ionization detection (GLC-FID) for quantification and combined gas-chromatography - mass spectrometry (GC-MS) for characterisation. Linoleic ($C_{18:2}$) was the most abundant fatty acid and palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$) were the other major fatty acids. Of the fatty acids from the neutral lipid subfraction, C_{16} decreased slightly in Gossypium cotyledons, $C_{18:1}$ decreased in the cotyledons of both species, and $C_{18:2}$ decreased gradually in Gossypium cotyledons. Only slight changes were detected in the levels of fatty acids from the phospholipid and

glycolipid subfractions during the three-day germination period.

Short-chain fatty acids in both species were determined using GC-FID and GC-MS. Lauric (C_{12}) was the most abundant fatty acid and caprylic (C_8), pelargonic (C_9), capric (C_{10}) were the other major fatty acids. C_8 and C_9 levels decreased in the whole seeds as well as in cotyledons but C_{10} and C_{12} increased in the whole seeds, and C_{12} increased sharply in the cotyledons of both species. The quantities of these short-chain fatty acids changed in radicles and seed coats during the 72 h germination period.

Investigations of the spherosomal preparation (lipid-storage organelles) from the cotyledons of both species during germination included morphological studies using electron microscopy and lipid-chemistry studies using GC-FID and GC-MS. The ultrastructural appearance of the spherosomal preparations were in agreement with previous publications. Spherosome diameter varied between 0.5 - 1.0 µm in Helianthus and 0.3 - 2.0 µm in Gossypium, and spherosomes were bounded by a half-unit membrane. The total lipid content and lipid subfractions, and their fatty acid composition were studied in the spherosomal preparations from the cotyledons of both species during germination. Neutral lipid was the most abundant lipid in the spherosomal preparation, and its levels decreased during germination. The major fatty acids of spherosomal preparations were the same for those of intact seeds. C_{16} and C_{18} from spherosomal neutral lipid increased

about three-fold but $C_{18:1}$ and $C_{18:2}$ decreased to less than half the original level in both species during the three-day germination period.

Mitochondria from the cotyledons of both species were isolated, and the constituent lipid and fatty acid composition studied during germination. The mitochondria showed enrichment in the enzyme marker succinate dehydrogenase. Phospholipid was the most abundant lipid in the mitochondria-rich fraction, and its levels increased during germination. C_{16} and C_{18} from the mitochondrial phospholipid subfraction increased about three-fold in Helianthus but C_{18} decreased to about half content in Gossypium; $C_{18:1}$ decreased to about one-third in Helianthus, but increased in Gossypium during the germination period.

INTRODUCTION

This thesis is concerned with fatty acids in seeds and seedlings of two economically important oilseeds, viz. Helianthus annuus L. (sunflower) and Gossypium barbadense L. (cotton).

The Introduction to this thesis is divided into six sections. The first deals with the economic significance and production of oilseeds and vegetable oils. The second section deals with the physical and chemical characteristics and biochemistry of vegetable oils. In the third section, an account is given of Helianthus and Gossypium as oilseeds. The fourth section briefly gives general considerations for studying germination processes in seeds, and this is followed in section five by a selective review of the subcellular localization of lipids and fatty acids. In the final section the aims and rationale of the project are outlined.

1. The economic significance and production of oilseeds and vegetable oils

Oilseeds are an important component of agricultural products, for they provide easily available and highly nutritious food for humans and livestock, and are the main source of vegetable oils. The term "oilseed" in commerce is applied to the true seed, and also in some cases to fruits such as those of sunflower, safflower and niger,or even a part of a fruit such as copra (Weiss, 1983). Plant oilseeds provide both edible oils for human and animal consumption directly(or after hydrogenation to produce margarine and

shortening), and drying oils for industrial purposes mainly in the production of paint and soap. Those seeds or fruits which contain economically important reserves of lipid include not only the group traditionally referred to as the oilseeds, but also certain selections of crops which do not store triglycerides as food reserves, e.g. cereals such as maize and rice. Most of the main types of oilseeds are given in Table 1.

In the last twenty years, the production of oilseeds increased almost two-fold from 100 million tonnes to 181.5 million tonnes and during the last five years this has stabilized in the amount of 180 million tonnes (Table 2). The share of the world oilseed production attributable to soybeans increased during 1980-85 and now comprises over one-half of the world total oilseed production.

In determining the economics of oilseed production, three aspects show potential for improvement: first, improvement of the yield of seed by using better cultural practices (principally irrigation and nutrition), more adaptable varieties, and advances in resistance to diseases, insects and other pests. The development of hybrid varieties can greatly increase the yield of oilseeds such as sunflower, palm and coconut. Second, the yield of oil can be raised through breeding higher oil-content characteristics into the seed and by employing improved extraction methods. Third, the quality of oil can be raised through breeding by ridding the oil of undesirable components and by changing the fatty acid composition (Doty, 1983).

Vegetable oils are derived from the seed of plants which grow in many parts of the world. About 40 different species

Table 1. Selected oilseed crops. Information derived from Daussant et al., 1983; Duffus and Slaughter, 1980; Gill and Vear, 1980; Goldsworthy and Fisher, 1984; Gurr, 1980; Hebblethwaite, 1980; Hilditch and Williams, 1964; Khan and Hanna, 1983; Murray, 1984; Noggle and Fritz, 1983; Osagie and Kates, 1984; Swain, 1963; Thomson, 1979; Vaughan, 1970; Weiss, 1983 and Williams, 1966.

Common name	Species	Plant Family	Oil content (% d.wt.)
Aceituno	Simarouba glauca	Simaroubaceae	kernel 62
Afzelia	Afzelia spp.	Caesalpiniaceae	seed 23-29
Almond	Prunus amygdalus	Rosaceae	kernel 50-60
Apricot	Prunus armeniaca	Rosaceae	kernel 40-45
Avocado	Persea americana	Lauraceae	seed 1 pulp 15-20
Babassu	Orbignya speciosa	Arecaceae	kernel 63-70
Bakayan (China berry)	Melia azederach	Meliaceae	seed 40
Bambarra-groundnut	Voandzeia subterranea	Fabaceae	seed kernel 46-52
Bayberry wax (Myrtle wax)	Myrica spp.	Myricaceae	25
Beech	Fagus sylvatica	Fagaceae	kernel 40
Ben (Horse radish tree)	Moringa oleifera	Moringaceae	seed 25-50
Borneo illipe	Shorea spp.	Dipterocarpaceae	kernel 45-70
Brazil nut	Bertholletia excelsa	Lecythidaceae	kernel 65-70
Camelina	Camelina sativa	Brassicaceae	seed 34-42
Cashew	Anacardium occidentale	Anacardiaceae	kernel 47
Castor	Ricinus communis	Euphorbiaceae	seed 50
Cayete	Omphalea megacarpa	Euphorbiaceae	seed 65
Chaulmoogra	Hydnocarpus spp.	Flacourtiaceae	kernel 16-56
Cherry	Prunus cerasus	Rosaceae	kernel 35-39

Table 1 (Cont'd.)

Common name	Species	Plant Family	Oil content (% d.wt.)
Cheyi (numbuni)	Polygala butyraceae	Polygalaceae	seed 30
Chilean molasses palm	Juboea spectabilis	Arecaceae	kernel 40
Chinese jute	Abutilon avicennae	Malvaceae	seed 16-18
Chuddapah	Buchanania latifolia	Anacardiaceae	seed 61
Citrus seeds	Citrus spp.	Rutaceae	seeds 22-34
Cocoa	Theobroma cacao	Sterculiaceae	nibs 45-56 (crushed beans)
Coconut	Cocos nucifera	Arecaceae	copra 64.5
Coffee	Coffea arabica	Rubiaceae	seed 5-10
Cohune	Attalea cohune	Arecaceae	seed 65-72
Cokerite (Anaja)	Maximiliana regia	Arecaceae	kernel 60
Cotton	Gossypium spp.	Malvaceae	seed 15-24
Crabwood (Andiroba)	Carapa spp.	Meliaceae	kernel 60 or seed 43
Crambe	Crambe hispanica	Brassicaceae	seed 50
Cucumber	Cucumis sativus	Cucurbitaceae	cotyledons 22-40
Curua	Attalea spectabilis	Arecaceae	kernel 6.5
Dhupa (Malabar)	Vateria indica	Dipterocarpaceae	kernel 20-22 or seed 22-27
Dika nut	Irvingia gabonesis	Simaroubaceae	kernel 54-68
Douglas fir	Pseudotsuga menziesii	Pinaceae	megagametophyte 36
Dukudu	Celastrus paniculatus	Celastraceae	seed 50-70
Elsholtzia	Elsholtzia cristata	Lamiaceae	fruit 34-42
Elozy	Ximenia americana	Olacaceae	kernel 63-66
English Walnut	Juglans regia	Juglandaceae	kernel 60-64
Firseed	Pinus cembra	Pinaceae	seed 23

Table 1 (Cont'd.)

Common name	Species	Plant Family	Oil content (% d.wt.)
Gemsbok	*Bauhinia esculenta*	Caesalpiniaceae	kernel 42
Grape	*Vitis vinifera*	Vitaceae	seed 6-20
Groundnut (peanut)	*Arachis hypogaea*	Fabaceae	seed kernel 46-52
Habai (tangallak)	*Litsea sebifera*	Lauraceae	seed 48
Hazelnut	*Corylus* spp.	Corylaceae	kernel 68
Hemp	*Cannabis sativa*	Cannabinaceae	seed 30-35
Horse chestnut	*Aesculus hippocastanum*	Hippocastanaceae	seed 8
Indian kapok	*Bombax malabaricum*	Bombacaceae	seed 18-26
Inoi nut	*Poga oleosa*	Rhizophoraceae	seed 50-60
Jamba (rocket or taramira)	*Eruca sativa*	Brassicaceae	seed 30-35
Japan tallow	*Rhus* spp.	Anacardiaceae	kernel 25-40
Java almond	*Canarium* spp.	Burseraceae	kernel 70-80
Java olive (stinking bean)	*Sterculia foetida*	Sterculiaceae	seed 34
Jojaba	*Simmondsia californica*	Buxaceae	kernel 50
Kapok	*Ceiba pentandra*	Bombacaceae	seed 25
Kenaf	*Hibiscus cannabinus*	Malvaceae	seed 20
Koeme	*Telfairia pedata*	Cucurbitaceae	seed 36
Kokum	*Garcinia indica*	Clusiaceae	kernel 20
Kombo	*Pycnanthus kombo*	Myristicaceae	seed kernel 54-73
Lallemantia	*Lallemantia iberica*	Lamiaceae	fruit 27-33
Laurel	*Laurus nobilis*	Lauraceae	kernel 13-30
Linseed	*Linum usitatissimum*	Linaceae	seed 35-44
Lettuce	*Lactuca sativa*	Asteraceae	cotyledon 33-37
Macamba	*Heisteria* spp.	Olacaceae	kernel 31-46
Madia	*Madia sativa*	Asteraceae	fruit 33

Table 1 (Cont'd.)

Common name	Species	Plant Family	Oil content (% d.wt.)
Mafura nut (cap mahogany)	Trichilia emetica	Meliaceae	kernel 60
Maize	Zea maize	Gramineae	embryo 50-56
Meni (Niam)	Lophira alata	Ochnaceae	kernel 30-40
Mexican poppy	Argemone mexicana	Papaveraceae	seed 22-40
Millet	Pennisetum americanum	Gramineae	seed 7.2
Mowrah	Bassia latifolia	Sapotaceae	kernel 52-60
Myrobalan	Terminalia catappa	Combretaceae	kernel 55
Murumuru	Astrocaryum spp.	Arecaceae	kernel 37-41
Mustard	Sinapis spp.	Brassicaceae	seed 40
Nahor	Mesua ferrea	Clusiaceae	kernel 70
Neem (Margosa)	Melia azadirachta	Meliaceae	kernel 40-45 or fruit 15
Niger	Guizotia abyssinica	Asteraceae	fruit 40-45
Nutmeg	Myristica fragrans	Myristicaceae	kernel 24-30
Oil palm	Elaeis guineensis	Arecaceae	kernel 46-48 and pulp 56
Oiticica	Licania rigida	Rosaceae	kernel 62
Okra	Hibiscus esculentus	Malvaceae	seed 14-20
Olive	Olea europaea	Oleaceae	kernel 12-28
Oyster nut	Telfairia pedata	Cucurbitaceae	fruit pulp 75 -
Paraguay cocopalm	Acrocomia totai	Arecaceae	kernel 60 fruit pulp 25-30
Peach	Prunus persica	Rosaceae	kernel 42
Pecan	Carya illinoensis	Juglandaceae	kernel 65
Pentaclethra	Pentaclethra spp.	Mimosaceae	kernel 49-51
Perilla	Perilla frutescens	Lamiaceae	seed 35-40
Phulwara	Bassia butyracea	Sapotaceae	kernel 52-60
Pine	Pinus spp.	Pinaceae	kernel 50-60
Pistachio	Pistacia vera	Anacardiaceae	kernel 47
Plum	Prunus domestica	Rosaceae	kernel 39-52
Pongam (Honge)	Pongamia pinnata	Fabaceae	seed 27-39
Poppy	Papaver somniferum	Papaveraceae	seed 40-55
Pumpkin	Cucurbita pepo	Cucurbitaceae	seed 47-48

Table 1 (Cont'd.)

Common name	Species	Plant Family	Oil content (% d.wt.)
Queensland nut	*Macadamia ternifolia*	Proteaceae	kernel 75-79
Ravison	*Brassica campestris*	Brassicaceae	seed 35
Rape	*Brassica napus*	Brassicaceae	cotyledon 35-48
Rice	*Oryza sativa*	Gramineae	germ 15-20
Rohituka	*Amoora rohituka*	Meliaceae	kernel 47
Safflower	*Carthamus tinctorius*	Asteraceae	fruit 24-40
Sesame (Beniseed)	*Sesamum indicum*	Pedaliaceae	seed 50
Sequa	*Fevillea cordifollia*	Cucurbitaceae	seed 42
Shea	*Butyrospermum parkii*	Sapotaceae	kernel 40-55
Soybean	*Glycine max*	Fabaceae	seed 21
Sunflower	*Helianthus annuus*	Asteraceae	seed 25-32
Teaseed	*Camellia sasanqua*	Theaceae	kernel 51-60
Tigernut	*Cyperus esculenta*	Gramineae	tuber 25
Tobacco	*Nicotiana tabacum*	Solanaceae	seed 33-34
Tomato	*Lycopersicon esculentum*	Solanaceae	seed 20
Tucum	*Astrocaryum tucuma*	Arecaceae	kernel 44-52
Tung	*Aleurites* spp.	Euphorbiaceae	kernel 33
Ucuhuba	*Virola* spp.	Myristicaceae	kernel 65
Undi	*Calophyllum inophyllum*	Clusiaceae	kernel 60
Watermelon	*Citrullus vulgaris*	Cucurbitaceae	
Wild olive (Tallow nut)	*Ximenia americana*	Oleaceae	kernel 60
Yucca	*Yucca whipplei*	Liliaceae	perisperm 23-27
Zachun (Hegli)	*Balanites* spp.	Zygophyllaceae	kernel 41-58

Table 2. World production of major oilseeds in million tonnes

Sources: Britannica Year Books (1980-1985); Doty, 1983 and Weiss, 1983. ND = not determined

Major oilseeds	1960	1970	1980	1981/82	1982/83	1983/84	1984/85
Castor	0.7	0.9	1.0	0.9	ND	ND	ND
Copra	4.0	4.0	5.0	4.8	4.5	4.1	4.6
Cotton	20.0	21.0	24.0	28.0	27.3	26.8	32.7
Flax	4.0	4.0	3.0	2.1	2.6	2.2	2.4
Groundnut	12.0	12.0	14.0	19.9	17.1	18.7	19.2
Olive	6.0	7.0	8.0	ND	ND	ND	ND
Palm kernels	1.0	1.0	2.0	1.9	1.8	2.0	2.1
Rape	4.0	7.0	12.0	12.4	14.8	14.3	15.9
Safflower	0.5	0.6	1.0	0.8	ND	ND	ND
Sesame	2.0	2.0	3.0	1.9	ND	ND	ND
Soybean	27.0	45.0	93.0	86.3	93.3	81.9	90.2
Sunflower	7.0	10.0	15.5	14.7	16.5	15.5	17.2
World total of all oilseeds	100.0	114.5	181.5	170.3	178.8	166.4	184.9

Table 3. Estimated world production of major vegetable oils in million tonnes

Sources: Britannica Year Books (1970-1980) and Weiss, 1983

Major vegetable oils	1970	1975	1980
Castor	0.4	0.4	0.5
Coconut	2.4	2.9	3.2
Cottonseed	2.5	3.0	3.2
Flaxseed	1.0	1.0	1.0
Groundnut	3.0	3.0	3.2
Olive	2.0	2.0	2.0
Palm	2.0	3.0	4.5
Rapeseed	2.0	3.0	4.0
Safflower	0.2	0.2	0.3
Sesame	1.0	1.0	1.0
Soybean	6.0	9.0	15.0
Sunflower	2.0	3.0	4.0
Tung	0.1	0.1	0.1
World total of all vegetable oils	35.0	46.0	60.0

provide seeds that can be harvested for commercial oil production (Murray, 1984). Nine species account for about 90% of total production. These are soybean (Glycine max), sunflower (Helianthus annuus), groundnut (Arachis hypogaea), cotton (Gossypium spp.), rape (Brassica spp.), coconut (Cocos nucifera), palm (Elaeis guineensis), linseed (Linum usitatissimum) and castor bean (Ricinus communis) (Table 3).

World production of vegetable oils increased steadily up to 1970, but since then production has almost doubled from 35 million tonnes to over 60 million tonnes in 1980 (Table 3). Soybean oil production increased more than two-fold from 1970 to 1980. Sunflower, rapeseed and palm oils nearly doubled in production in the same period. The relative importance of these oils has changed during the last five years, a period in which the vegetable oils in the world decreased to 55 million tonnes in 1981-82 and then increased to about 59.4 million tonnes in 1984-85.

2. Characteristics and biochemistry of vegetable oils

Vegetable oils and fats are obtained from oilseeds and fruits by two methods - mechanical pressure (expeller process), or extraction by solvents, either separately or in combination. The isolated oils and fats possess relatively well-defined physical and chemical characteristics. Their biochemistry has been the subject of prolonged study with the advent of modern analytical techniques.

2.1 Physical and chemical characteristics of vegetable oils

The so-called 'physical and chemical tests' are employed to determine the nature of, and sometimes for the identification

of oils and fats. These physical and chemical tests have been standardised by the British Standard Institution (BSI) and the American Oil Chemists' Society (AOCS).

The important physical properties of vegetable oils include:

1. Density, which depends upon the composition of the fat/oil of the sample and typically ranges between 0.880 to 0.970 at 60°C in most vegetable oils (Achaya, 1975).

2. Melting-point; this depends on the saturated fatty acid content in the oil; natural fats are mixtures of various glycerides and do not have rapid melting transition points but melt over a range of temperatures (Weiss, 1983).

3. Titre test; this defines the solidifying point of the separated fatty acids of an oil or fat and is a useful characteristic property of fat. This solidification is most apparent with oil containing appreciable quantities of linoleic and linolenic acid residues (Doby, 1965).

4. Smoke, fire and flash points; these are almost wholly dependent upon the free fatty acid (FFA) content of the oils though mono- and di-glycerides contribute. A low smoke point indicates the presence of FFA and also mono- or di-glycerides (AOCS Methods, 1973).

5. Refractive index; this is the ratio of the sine of the angle of incidence to the sine of the angle of refraction, when a ray of light of defined wave-length passes from air into the oil kept at a constant temperature (BSI Methods, 1976).

6. Colour; this is primarily due to the presence of fat-soluble plant pigments and is of considerable importance commercially. Spectrophotometers or Wesson-type colorimeters

are used for designating the colour of fats and oils (AOCS Methods, 1973).

Some physical properties and chemical characteristics of the major vegetable oils are given in Table 4.

The important chemical characteristics of vegetable oils include:

1. Iodine value, which is the number of grams of iodine absorbed by 100g of oil, and a well-known measure of unsaturation, or content of double bonds in the oil (AOCS Methods, 1973).

2. Saponification value, which is the number of mg of potassium hydroxide or sodium hydroxide required to saponify one gram of oil and therefore a measure of the average molecular weight of the glycerides in oil (Paech and Tracey, 1955).

3. Unsaponifiable matter, which includes sterols, hydrocarbons, tocopherols, aliphatic alcohols, terpenic alcohols, which after treatment with alcoholic potassium hydroxide are insoluble in water and soluble in diethyl ether or petroleum ether (Christie, 1982).

4. Acetyl and hydroxyl values, which are a measure of the free hydroxyl groups present in oils. The acetyl value is the number of milligrams of potassium hydroxide required to neutralise the acetic acid obtained by the sponification of one gram of acetylated oil. Castor oil is the common commercial oil with a high content of hydroxyl groups, owing to the presence of about 90% of a hydroxy acid (ricinoleic acid) with a hydroxyl value of about 165 and an acetyl value of 145 (Achaya, 1975).

Table 4 Some physical properties and chemical characteristics of common vegetable oils

Sources: Achaya, 1975; AOCS Methods, 1973; BSI Methods, 1976; Christie, 1982; Doby, 1965; Paech *et al.*, 1955; Weiss, 1983; and Williams, 1966. ND = not determined

Oil	Physical properties				Chemical characteristics		
	Density at 60°C	Melting point (°C)	Titre (°C)	Refractive index at 60°C	Iodine value	Saponification value	Unsaponifiable matter %
Coconut	0.890 - 0.895	22 - 26	20 - 24	ND	9	255	0.1 - 0.3
Cottonseed	0.889 - 0.894	5 - 10	32 - 37	1.4572	108	195	0.5 - 2.0
Flaxseed	0.902 - 0.907	0	14 - 16	1.4655	180	192	0.5 - 1.5
Groundnut	0.885 - 0.890	5 - 10	28 - 32	1.4550	90	189	0.4 - 1.0
Maize	0.892 - 0.897	0	18 - 20	1.4596	125	191	0.5 - 3.0
Olive	0.885 - 0.890	0 - 10	18 - 25	1.4542	83	192	0.5 - 2.0
Palm	0.882 - 0.887	25 - 30	40 - 45	1.4508	53	198	0.2 - 0.6
Rapeseed	0.880 - 0.885	0	11 - 15	1.4582	102	175	0.5 - 1.5
Safflower	0.898 - 0.903	0	15 - 17	1.4620	145	191	0.5 - 1.5
Sesame	0.889 - 0.894	0	22 - 26	1.4582	111	192	0.5 - 1.5
Soybean	0.895 - 0.900	0	20 - 32	1.4600	130	192	0.5 - 1.5
Sunflower	0.894 - 0.899	0	17 - 20	1.4597	128	191	0.3 - 0.5
Tung	ND	ND	36 - 37	1.4665	165	192	0.5 - 1.2

2.2 Biochemistry of vegetable oils

Lipids constitute one of the four major classes of organic compounds which are found in living tissues, the others being carbohydrates, proteins, and nucleic acids. Lipids occur in considerable amounts in the seed or fruits of a number of plants and provide such plants with utilizable food reserves to be used during germination and to be mobilised for early seedling development.

The term "lipid" includes a structurally diverse range of compounds which have three features in common: their presence in living tissues; their insolubility in water; and their general solubility in organic solvents such as diethyl ether, hexane, benzene, chloroform and methanol.

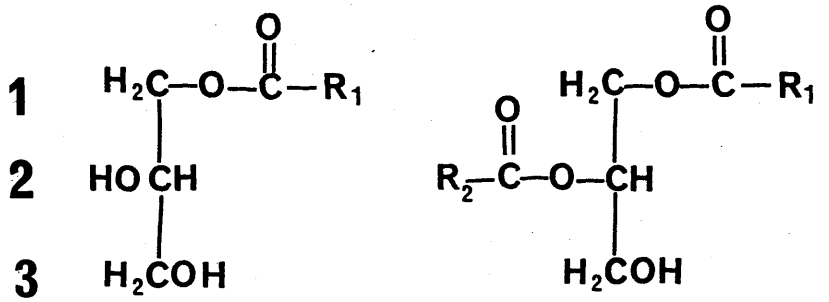
Lipids are conveniently classified into two classes:

1. Simple (neutral) lipids, which yield only fatty acids and glycerol on complete hydrolysis. These include mono-, di-, triglycerides (fats and oils), waxes, sterol esters and esters of vitamin alcohols.
2. Complex (polar) lipids which also contain fatty acids may in addition yield phosphate, carbohydrates, and nitrogenous compounds on hydrolysis. These lipids include glycerophosphatides (phospholipids), glycolipids and sphingolipids.

The structures of simple and complex lipids in plants are shown in Figure 1.

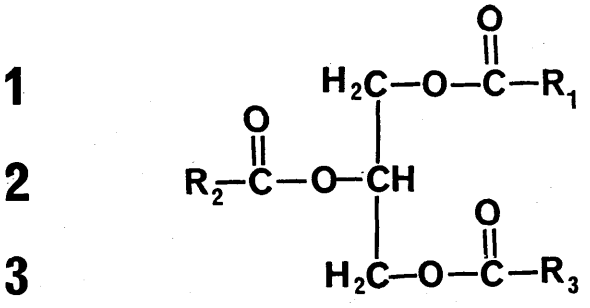
In higher plants the triglycerides are the major constituents of this class. They are found in exceptionally high concentrations in oil-containing seeds, but glycolipids are the major lipid constituents in leaf tissue. No triglycerides apparently occur in chloroplasts, and only a

Position



1-monoacylglycerol (MG)

1,2-diacylglycerol (DG)



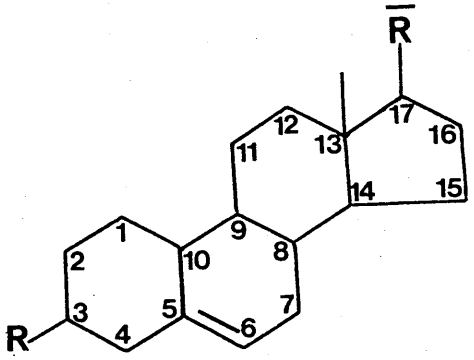
triacylglycerol (TG)

a- neutral glycerides

$CH_3(CH_2)_nCH_3$	$R{\cdot}CH_2OH$	$R{\cdot}COOH$
n-alkanes	primary alcohols	acids
usually C_{29} or C_{31}	usually C_{26} and C_{28}	usually C_{24} - C_{28}

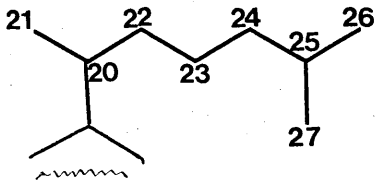
b- major components of plant waxes

c - common plant sterols

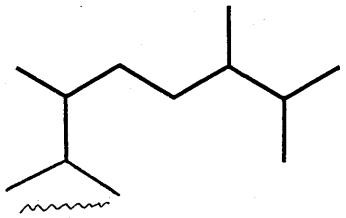


sterol (**R=OH**) $\overline{\text{R}}$ substituent

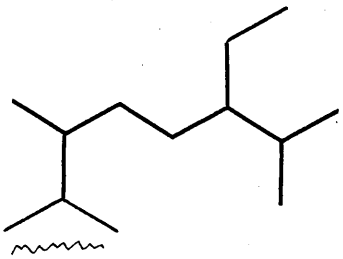
1. cholesterol



2. campesterol



3. sitosterol



4. stigmasterol

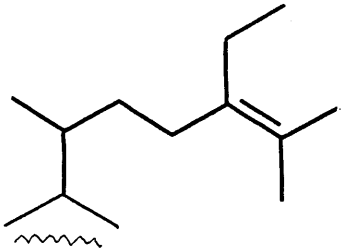
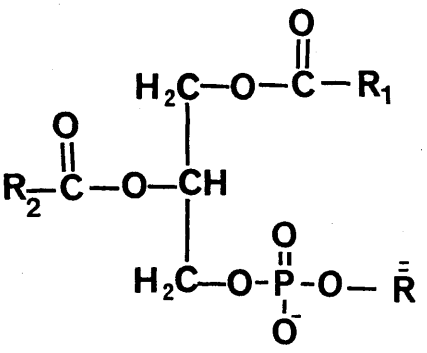


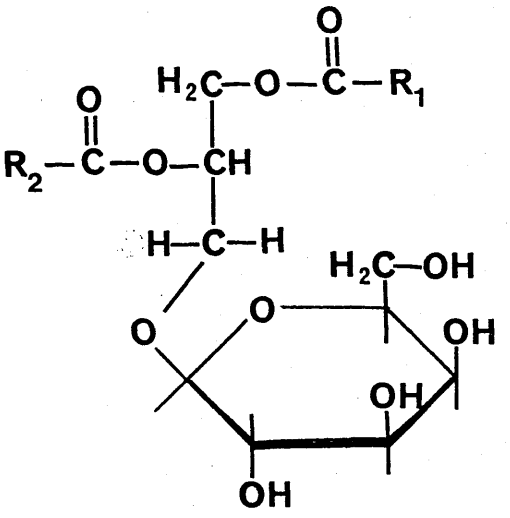
Figure 1.1 Structures of simple (neutral) lipids in plants (Contd.)



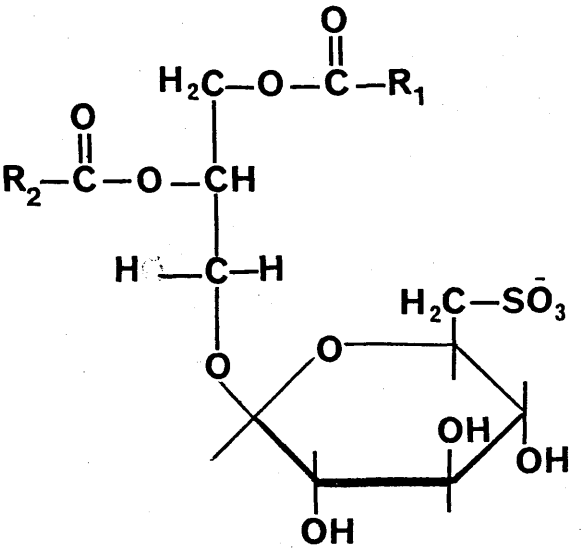
general formula

Substituent ($\bar{\bar{R}}$)	Phospholipid
H	phosphatidic acid
serine ($-CH_2CH(NH_2)COOH$)	phosphatidylserine
ethanolamine ($-CH_2CH_2NH_2$)	phosphatidylethanolamine
choline ($-CH_2CH_2\overset{+}{N}(CH_3)_3$)	phosphatidylcholine
glycerol ($-CH_2OH-CHOH-CH_2OH$)	phosphatidylglycerol
inositol ($-C_6H_6(OH)_5$)	phosphatidylinositol

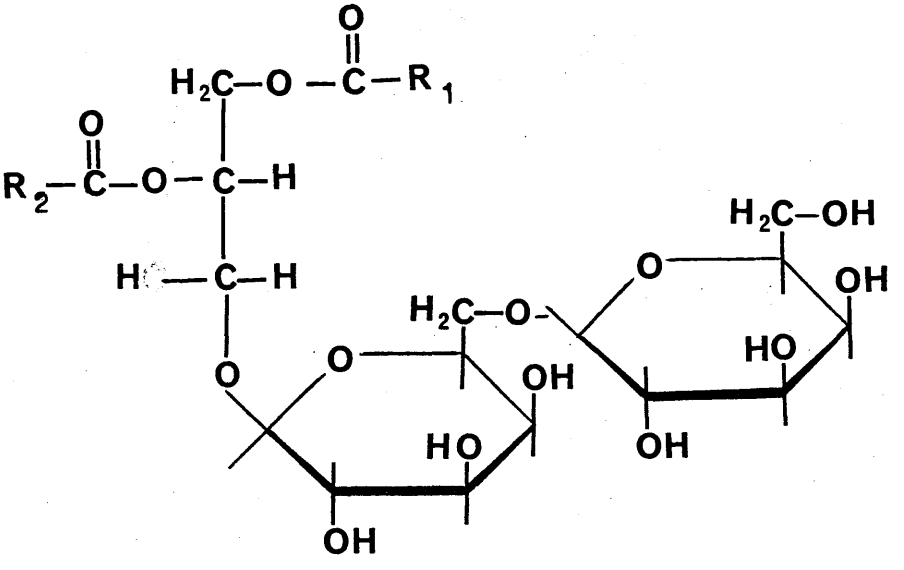
a - important phosphoglycerides



Diacylgalactosylglycerol

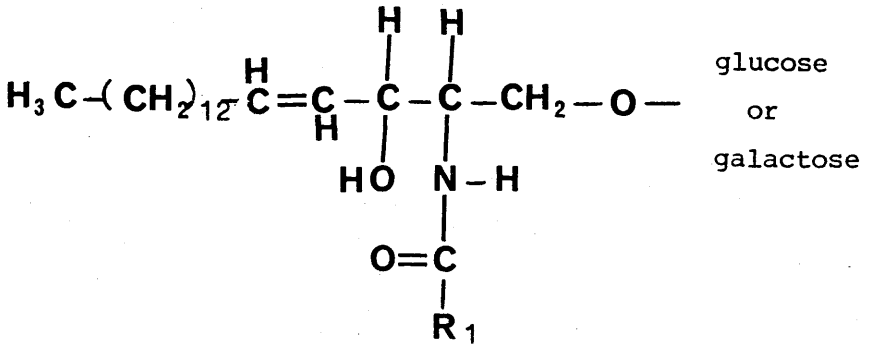


Diacylsulphoquinovosylglycerol

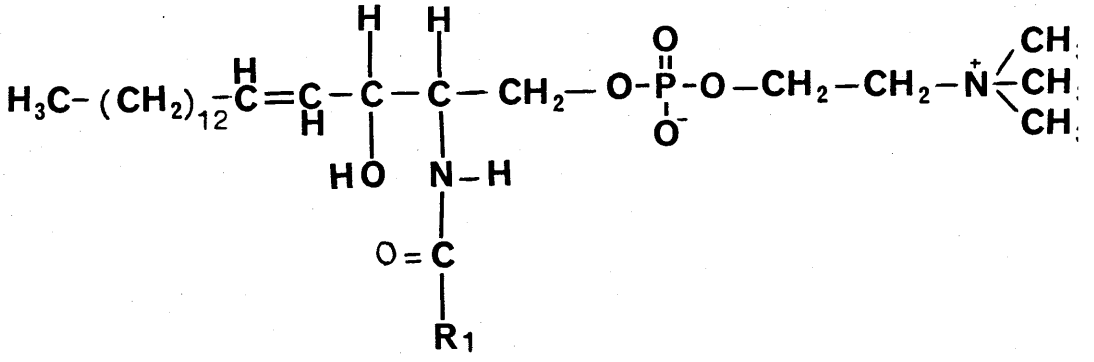


Diacyldigalactosylglycerol

b - some glycolipids in plants



Cerebroside (sphingosine + sugar + fatty acid)



Sphingomyelin (sphingosine + phosphoryl choline unit + fatty acid)

C - some sphingolipids in plants

(R_1, R_2 and R_3 are fatty acids)

Figure 1.2 Structure of complex (polar) lipids in plants

minor amount of phospholipids are present (Bonner and Varner, 1965). In the majority of mature seeds the quantitatively major class of lipid is glyceride, which may constitute between 10 and 70% of the tissue dry weight; saturated fatty acids occupy position 1 on the molecule (Fig. 1.1), with unsaturated acids concentrated at position 2. Analytical procedures for determining the identities of fatty acids at each of the three positions are described and discussed by Gurr (1980). Saturated fatty acids usually occupy positions 1 and 3, whereas unsaturated fatty acids (notably oleic, linoleic and linolenic) are found predominantly in position 2. In most oilseeds, unsaturated fatty acids constitute more than 33% of the total fatty acids, thus unsaturated acids must "spill over" to occupy one or both of the other positions.

In a study of the amount of triglycerides in seed tissues Murray (1984) found that triglycerides varied between greater than 50% by weight and as little as 1 - 2% by weight in the cotyledons of most legumes, and in cereal grains the embryo axis and scutellum can contain up to 50% by weight of triglycerides. Osagie and Kates (1984) found that the lipid content of millet seed was 7.2% of the seed dry weight and this lipid consisted of 85% of neutral lipid, 12% phospholipid and 3% glycolipids.

The term 'waxes' are usually applied to lipids which are esters of fatty acids with higher aliphatic alcohols. These alcohols and acids vary from C_{26} to C_{30}, but some are in the C_{34} to C_{36} range. Occasionally waxes may act as the main storage or reserve lipid of a plant such as jojoba, which has exclusively 50% liquid wax esters (Spencer et al., 1977 and Opute, 1978).

Phospholipids and glycolipids normally represent less than 2% of the total seed lipids (Mattson and Volpenheim, 1963; Hitchcock and Nichols, 1971, and Weber et al. 1971). Galliard and Mercer (1975) showed that seed oils include a small proportion of phospholipid which is associated with membranes such as those enclosing the oil bodies. Carver and Wilson (1984a) found that phospholipids are involved with triglycerides in developing soybean seed. They suggested that phospholipids may be metabolised to diglyceride (DG), a substrate for the enzyme DG-acyltransferase that catalyzes triglyceride formation.

The major sterols found in plants are sitosterol (about 70%), stigmasterol (20%), campasterol (about 5%) and cholesterol (about 5% of total sterols in lipid). In addition to free sterols, plants also contain sterol esters (where a fatty acid is esterified to the 3-hydroxyl sterol ester), sterol glycosides (where the 3-hydroxyl of the sterol forms a glycoside linkage with the 1-position of a hexose, usually glucose) and acylated sterol glycosides (where the 6-position of the hexose is esterified with a fatty acid) (Harwood and Russell, 1984).

Fatty acids (FAs) are usually among the products obtained from the hydrolysis of lipids. The common fatty acids of plants contain even numbers of carbon atoms (4 - 24) in straight chains with a terminal carboxyl group and may be fully saturated or contain one, two or more double bonds, which generally but not always have a cis-configuration (Christie, 1982).
Plant fatty acids may be classed as "major" and "unusual" fatty acids (Goodwin and Mercer, 1983). Seven types account for approximately 95% of the total fatty acids of most commercial

oilseeds; these are saturated (lauric, C_{12}; myristic, C_{14}; palmitic, C_{16} and stearic, C_{18}) and unsaturated (oleic, $C_{18:1}$; linoleic, $C_{18:2}$ and α-linolenic, $C_{18:3}$) acids (Harwood and Russell, 1984). Table 5 shows the structures of fatty acids commonly found in vegetable oils.

Vijayalkshmi and Rao (1972) showed that when unusual fatty acids accumulate in seeds they are present as triglycerides and not as phospholipids. The fatty acid composition of triacylglycerol extracted from selected seeds of important species are listed in Table 6. In most of these lipids, saturated fatty acids constitute only a small percentage of the total fatty acid content with the exceptions of coconut and oil palm which both contain more than 90% saturated fatty acids. Other exceptions are species such as castor bean, crambe, rapeseed, tung and mustard seed oils with a high proportion of unusual fatty acids.

Sreenivasan (1968) examined 19 different samples of oils and fats for their component acids and composition by Gas-Liquid Chromatography (GLC). He found for the first time in traditional oils C_{15} in cottonseed (0.6% of total FA), $C_{20:1}$ in sesame oil (0.1% of total FA), C_{22} in soybean oil (0.1% of total FA) and $C_{24:2}$ in mustard seed oil (0.1% of total FA), but the results require confirmation. The composition of the exocarp oil is usually significantly different from the corresponding seed within the fruit (Galliard and Mercer, 1975). For example, palm fruit coat oil (palm oil) contains main C_{16}, $C_{18:1}$ and $C_{18:2}$ acids, while the oil from the nut of the same fruit (palm kernel oil) is rich in C_{12}, C_{14}, C_{16} and $C_{18:1}$ acids.

Table 5. Structure of some fatty acids (FA) in oilseeds

Sources: Bonner and Varner, 1965; Christie, 1982; Doby, 1965; Goodwin and Mercer, 1983 and Harborne, 1984

ND: not determined

Common name	Symbol	Structure	Melting point °C
Saturated FA			
Butyric	4:0	$CH_3(CH_2)_2COOH$	-5.5
Caproic	6:0	$CH_3(CH_2)_4COOH$	-1.5
Caprylic	8:0	$CH_3(CH_2)_6COOH$	16.5
Capric	10:0	$CH_3(CH_2)_8COOH$	31.4
Lauric	12:0	$CH_3(CH_2)_{10}COOH$	48.0
Myristic	14:0	$CH_3(CH_2)_{12}COOH$	57
Palmitic	16:0	$CH_3(CH_2)_{14}COOH$	63.1
Stearic	18:0	$CH_3(CH_2)_{16}COOH$	70.1
Arachidic	20:0	$CH_3(CH_2)_{18}COOH$	77.0
Behenic	22:0	$CH_3(CH_2)_{20}COOH$	80.0
Lignoceric	24:0	$CH_3(CH_2)_{22}COOH$	85
Unsaturated FA			
Palmitoleic	16:1(9c)	$CH_3(CH_2)_5HC{=}CH(CH_2)_7COOH$	ND
Oleic	18:1(9c)	$CH_3(CH_2)_7HC{=}CH(CH_2)_7COOH$	16.4
Linoleic	18:2(9c, 12c)	$CH_3(CH_2)_4HC{=}CH{-}CH_2{-}HC{=}CH(CH_2)_7COOH$	9.5
α-Linolenic	18:3(9c, 12c,15c)	$CH_3CH_2HC{=}CH\ CH_2HC{=}CH\ CH_2\ HC{=}CH(CH_2)_7COOH$	ND
Arachidonic	20:4(5c, 8c,11c, 14c)	$CH_3(CH_2)_4HC{=}CH\ CH_2\ HC{=}CH\ CH_2\ HC{=}CH\ CH_2\ HC{=}CH{-}(CH_2)_3COOH$	ND

Table 5 (Cont'd.)

Common name	Symbol	Structure	Melting point °C
Unusual FA			
Petroselinic	16:1(7c)	$CH_3(CH_2)_7HC{=}CH(CH_2)_5COOH$	30
Tariric		$CH_3(CH_2)_{10}C{\equiv}C(CH_2)_4COOH$	ND
Sterculic		$CH_3(CH_2)_7C(CH_2)_7COOH$ (C=C bridged by CH_2)	ND
Erucic	22:1(13c)	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	33
Ricinoleic		$CH_3(CH_2)_5CHOH\ CH_2HC{=}CH(CH_2)_7COOH$	4
Chaulmoogric		$CH{=}CH{-}CH(CH_2)_{12}COOH$ (CH_2–CH_2)	ND
Malvalic		$CH_3(CH_2)_7C(CH_2)_6COOH$ (C=C bridged by CH_2)	ND

Table 6. Fatty acid composition of triacylglycerols in selected oilseeds

Sources: Doby, 1965; Gurr, 1980; Hilditch and Williams, 1964; Murray, 1984 and Sreenivasan, 1968.

Plant	Fatty acid composition (% of total FA)													
	8:0	10:0	12:0	14:0	16:0	18:0	20:0	22:0	16:1	18:1	18:2	18:3	20:1	other
Almond	-	-	-	1	5	-	-	-	-	77	17	-	-	-
Brazilnut	-	-	-	2	14	3	-	-	-	58	23	-	-	-
Castor	-	-	-	-	-	-	-	-	-	7	5	-	-	ricinoleic 88
Coconut	10	5	51	18	8	3	-	-	-	4	1	-	-	-
Cotton	-	-	-	1	21	2	-	-	-	31	45	-	-	-
Crambe	-	-	-	-	2	1	1	2	-	18	8	6	2	erucic 60
Cucumber	-	-	-	-	4	17	-	-	-	39	40	-	-	-
Dikanut	-	4	62	31	2	-	-	-	-	1	-	-	-	-
Dhupa	-	-	-	-	20	41	1	-	-	36	-	-	-	-
Groundnut	-	-	-	-	9	4	6		-	54	27	-	-	-
Hazelnut	-	-	-	-	3	2	-	-	-	91	4	-	-	-
Hemp	-	-	-	-	6	2	1	-	-	6	70	15	-	-

Table 6 (Cont'd.)

	Fatty acid composition (% of total FA)													
	8:0	10:0	12:0	14:0	16:0	18:0	20:0	22:0	16:1	18:1	18:2	18:3	20:1	other
Linseed	-	-	-	-	7	9	-	-	-	13	17	54	-	-
Maize	-	-	-	1	10	3	-	-	2	50	34	-	-	-
Neem	-	-	-	-	16	15	1	-	-	60	8	-	-	-
Niger	-	-	-	3	8	5	1	-	-	30	53	-	-	-
Oil palm	3	3	51	15	8	3	-	-	-	16	1	-	-	-
Okra	-	-	-	-	26	6	-	-	-	27	41	-	-	-
Olive	-	-	-	-	6	4	-	-	-	83	7	-	-	-
Pecan	-	-	-	-	3	2	-	-	-	79	16	-	-	-
Pine	-	-	-	-	-	-	-	-	3	53	37	-	-	-
Poppy	-	-	-	-	11	4	1	-	-	14	70	-	-	-
Pumpkin	-	-	-	-	6	7	-	-	-	41	46	-	-	-
Queensland nut	-	-	-	2	9	3	2	-	21	59	2	-	2	-
* Rapeseed	-	-	-	-	3	1	1	1	-	11	15	16	9	erucic 43
Safflower	-	-	-	-	2	2	1	-	-	38	57	-	-	-
Sesame	-	-	-	-	8	5	1	-	-	48	38	-	-	-

Table 6 (Cont'd.)

	Fatty acid composition (% of total FA)													
	8:0	10:0	12:0	14:0	16:0	18:0	20:0	22:0	16:1	18:1	18:2	18:3	20:1	other
Soybean	-	-	-	-	7	6	-	-	-	24	60	3	-	-
Sunflower	-	-	-	-	14		-	-	-	17	69	-	-	-
Tobacco	-	-	-	-	7	3	1	-	-	17	71	1	-	-
Tomato	-	-	-	-	18		-	-	-	24	56	2	-	-
Tung	-	-	-	-	7		-	-	-	19	-	-	-	eleostearic 74
Water melon	-	1	1	-	8	6	-	-	-	36	48	-	-	-
Yellow mustard	-	-	-	-	3	-	1	-	-	24	15	6	-	erucic 51

* in commercial edible rapeseed oil, erucic acid should not be present and in the currently grown cultivars there has been selection for low erucic acid content.

2.3 Techniques for lipid and fatty acid analysis

The first step in most modern analyses is the isolation of lipid from tissues by extraction with organic solvents, and the removal of non-lipid contaminants from these extracts. If the fatty acid composition of the total lipid is required, the lipids are saponified and the fatty acids converted to a volatile derivative by an appropriate procedure for gas chromatographic analysis, with care to minimise autoxidation of polyunsaturated fatty acids.

Single classes of lipid can be isolated from mixtures by combination of chromatographic techniques which differentiate lipids according to the degree of unsaturation, polarity and acidity or basicity of their constituent parts. They can often be provisionally identified by their chromatographic behaviour relative to that of authentic standards, and by spectroscopic techniques and specific spray reagents.

2.3.1 Extraction of lipids

This process should be carried out as soon as possible in order to minimise changes in levels of endogenous lipids during storage (Hitchcock and Nichols, 1971, and Harwood, 1980). Furthermore, many hydrolytic enzymes (especially in leaves) are not destroyed by organic solvents such as chloroform and diethyl ether. Accordingly, an initial extraction with boiling propan-2-ol in order to inactive lipases usually precedes extraction with chloroform-methanol (2:1, by volume) mixtures (Christie, 1982). Butylated hydroxytoluene (BHT) is usually included at 0.005% to prevent oxidation of polyenoic fatty acids (Holman, 1966). Most polar organic solvents used to extract lipids from tissues also

extract significant amounts of non-lipid contaminants such as sugar, urea, amino acids and salts (Lucas and Ridout, 1967). Most of these compounds can be removed from the chloroform-methanol (2:1, by volume) extract simply by shaking the combined solvents with one quarter their total volume of 0.88% potassium chloride solution (Folch et al., 1957). The lower phase, which comprises about 60% of the total volume, contains the purified lipid and the upper phase contains the non-lipid contaminants.

Holman (1966) has recommended that lipid extracts be stored under chloroform in all-glass containers or in bottles with Teflon-lined caps at -20°C and Harborne (1984) advised that direct lipid extracts should be stored at -5°C in the presence of antioxidant (0.005% BHT) if they are not to be processed immediately.

2.3.2 Separation methods

Before further analysis, it is frequently desirable at this stage to separate the lipid into neutral and polar fractions and to remove steroids and any remaining contaminants until pure lipid classes are obtained. Chromatographic technique is one of the most convenient methods for separation and purification of compounds. All chromatographic systems consist of two phases; the stationary phase which may be solid, gel, liquid or a solid/liquid mixture which is immobilised, and the mobile phase may be liquid or gaseous and flows over or through the stationary phase. Partition chromatography is a partition equilibrium between a stationary liquid (or semi-liquid) and a mobile liquid phase. Adsorption chromatography is an adsorption equilibrium between a stationary solid and a mobile liquid phase, and these methods are

generally used to prepare each of the lipid classes. These may also be combined with ion-exchange chromatography until the necessary separations are obtained.

2.3.2.1 Column Chromatography

The components of the applied sample are separated by the continuous passage of a suitable eluant (mobile phase) through the column. The glass column used should have a means of supporting the adsorbent as near to the base of the column as possible in order to minimise the "dead space" below the column support in which post-column mixing of separated compounds could occur (Williams and Wilson, 1981).

The most widely used adsorbent is silicic acid (commonly 200-mesh), a partially hydrated silicon dioxide. The amount of lipid that can be applied to a column is variable and depends on the magnitude of the differences in polarity between the various components to be separated. In general, 30 mg of lipid per g of adsorbent is a reasonable load but this can be varied with circumstances. The sample should be applied to the column in as small a volume as possible of the least polar eluting solvent, washed carefully on to the bed of adsorbent until no lipid remains above the surface, and then the main solvent reservoir can be attached. The quality of the separation is also dependent on the flow rate of the eluant (Christie, 1982).

Ion-exchange (DEAE) cellulose column chromatography is a useful technique for the separation of complex lipids in comparatively large amounts (Rouser et al., 1967). The choice of the ion-exchanger depends upon the stability of the sample

components, their molecular weights and the specific requirements of the separation. The principal of the separation process is partly ion-exchange chromatography of the ionic moieties of the polar lipids, and partly adsorption of highly polar non-ionic parts of complex lipids, for example the hydroxyl groups of inositol or carbohydrates (Christie, 1982).

2.3.2.2 Thin-Layer Chromatography (TLC)

This is a commonly employed technique in the analyses of lipids. The usual adsorbent is silica gel supported on a glass plate and used at 2 μm thickness for analytical purpose and up to 2 mm thickness for preparative work. The plates are air-dried briefly then activated by heating in an oven at 110-120°C for 2 h, and stored in an airtight box or in a desiccator. The sample is applied to the plate by means of a micropipette or syringe. After spotting, the plate is then placed in a tank containing the eluting solvent system after ensuring that the atmosphere within the tank becomes saturated with solvent vapours. Unless this is done, irregular running of the solvent will occur as it ascends the plate by capillary action, resulting in poor separation. The detection spray may be a chemical reagent which is specific for certain types of lipid or for certain functional groups, or it may be a non-specific reagent that renders all lipids visible. Lipids separated by TLC can be recovered after they have been detected, by scraping the adsorbent band into a small chromatographic column or sintered disc funnel and eluting with solvents of appropriate polarity. In order to

improve the resolution of particular separations, the technique of two-dimensional chromatography has been used (Trelease, 1969 and Williams and Wilson 1981).

Table 7 shows selected TLC separation systems for neutral and polar lipids.

2.3.2.3 Gas-Liquid Chromatography (GLC)

The stationary phase of GLC is a liquid coated onto a solid support material. The separating principle depends upon the differences in the partition coefficient between the liquid and gas phases of the constituents of a mixture. Those components with a high affinity for the stationary phase will travel through the column more slowly than those with little or no affinity for the stationary phase. The sample is introduced into the carrier gas (hydrogen, helium or nitrogen) by means of a microlitre syringe via a septum in an injection port at one end of the column. A detector, placed at the end of the column, responds to the compounds as they are eluted and produces an electrical signal which is then amplified and displayed on a recorder in the form of a trace called a chromatogram.

The detectors commonly used are flame-ionization detector (FID), electron-capture (ECD), thermal-conductivity (TCD), alkali-flame-ionization (AFID) and flame-photometric detector (FPD). The most widely used detector is the FID. It responds to almost all organic compounds and has a wide linear response range. When organic compounds are burned in a hydrogen and air flame, ions are produced. The ions are collected by a pair of polarised electrodes inside the

Table 7. Selected TLC separation systems for neutral and polar lipids

	Compounds	Adsorbent	Solvent system	Detection	Reference
1.	Neutral lipid mono-,di-, and tri-glycerides	Silica gel	C_6H_{14}:$(CH_2CH_3)_2O$:HCOOH (80:20:2 by volume)	Acidic ferric chloride solution	Christie, 1982
		Silica gel	C_6H_6:$(CH_2CH_3)_2O$:$CH_3COOC_2H_5$:CH_3COOH (80:10:10:2 by volume)		Christie, 1982
		Silica gel	Petroleum ether:$(CH_2CH_3)_2O$:CH_3COCH_3 (90:10:1 by volume)	-	Williams and Wilson, 1981
		Silica gel	CH_3COCH_3:C_6H_6:H_2O (91:30:8 by volume)	-	Schlotzhauer et al., 1977
		Silica gel	C_6H_{14}:$(CH_2CH_3)_2O$:CH_3COOH (90:10:1 by volume)	-	Mangold and Malins, 1960
		Silica gel $AgNO_3$	$CH_3CHOHCH_3$:$CHCl_3$ (3:197,v/v)	Rhodamine B	Harborne, 1984
		Silica gel	Petroleum ether:$(CH_2CH_3)_2O$:CH_3COOH (80:20:1 by volume)	Iodine vapour	Trelease, 1969

Table 7 (Cont'd.)

	Compounds	Adsorbent	Solvent system	Detection	Reference
2.	Polar lipid phospholipids	Silica gel	$CHCl_3$:CH_3OH:HOAC:H_2O (170:30:30:7 by volume)	Rhodamine B	Nichols et al., 1965
		Silica gel	CH_3COCH_3:CH_3COOH:H_2O (100:2:1 by volume)	-	Gardner, 1968
		Silica gel	$CHCl_3$:CH_3OH:H_2O (65:25:4 by volume)	-	Williams and Wilson, 1981
		Silica gel	CH_3COCH_3:C_6H_6:H_2O (91:30:8 by volume)	-	Pohl et al., 1970
		Silica gel	$CHCl_3$:CH_3OH:NH_4OH (70:20:1.5 by volume)	-	Carver and Wilson, 1984b
		Silica gel	$CHCl_3$:CH_3OH:H_2O (95:35:4 by volume)	Iodine vapour	Trelease, 1969

detector and the current produced is amplified before being passed to a recorder.

In the early days of GLC, the coated supports were packed in glass, teflon, aluminium or even copper columns with diameters of up to 10 mm. The efficiency of separation is determined by a number of factors particularly the efficiency of coating, the support with stationary phase, gas flow rate, temperature programme, and the temperature and dimensions of the column (length and width). As GLC developed, the support has been discarded and the stationary phase is now generally attached to the wall of a thin capillary tube, the so-called 'capillary columns'.

GLC may be performed using capillary columns which are made of glass or metal with diameters of between 0.03 - 1.0 mm and may be up to 100 m in length. There are two types of column systems known as wall-coated open tubular (WCOT) columns and support-coated open tubular (SCOT) columns. Such columns are highly efficient overall giving much greater resolution than that of packed columns, and these systems are useful for the analysis of complex mixtures. The efficiency of a column is usually expressed in terms of the concept of numbers of theoretical plates, originally devised for fractional distillation columns, which can be calculated using the formula:

$$n = 16\left(\frac{t}{w}\right)^2$$

where n is the number of theoretical plates, w the width of the base (distance between two tangents drawn from the side of the peak to the base line) and t the retention time (or distance) of the components. The efficiency of a column

which can be expressed by equation $H = HETP = \frac{l}{n}$ which shows the height equivalent to a theoretical plate, HETP, being equal to the total length of the column divided by the number of theoretical plates. The total number of theoretical plates available is important in determining whether a separation can be made. The efficiency, or HETP, is a measure of how good the column is in comparison to other columns of the same length (Supina, 1974). Capillary columns may have efficiencies of 20,000 - 100,000 theoretical plates. The term resolution takes into account both the selectivity of the column which determines the distance between the two peak maxima, and also the efficiency of a column which determines the peak width. The resolution can be calculated:

$$R = 2\left(\frac{t_2 - t_1}{w_1 + w_2}\right)$$

The recent method of High-Pressure Liquid Chromtography (HPLC) is now being applied to lipid analysis. The excellent resolution of this technique means that it can be easily applied to molecular species (Ando et al., 1976) or cis-trans separations (Warthen, 1975). HPLC may employ the principles of adsorption, partition, ion-exchange, exclusion and affinity chromatography. The technique has the advantages of high efficiency, fast speed of resolution and it is suitable for preparative and analytical qualitative and quantitative analysis (Williams and Wilson, 1981).

2.3.2.4 Spectroscopy

Infrared (IR) and Ultraviolet (UV) spectroscopy were the first of the spectroscopic methods to be applied to the

analysis of lipid and fatty acids. Nowadays, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) have been widely used for determining lipid-structure.

The IR spectra are obtained when the energy of radiation in the IR region at a given frequency is absorbed by a molecule, thereby increasing the amplitude of the vibration of specific bonds between atoms in the molecule (Christie, 1982). IR spectroscopy has been used to recognise functional groups in the seed oils (Wolff and Miwa, 1965).

The UV spectrum of a compound is generally measured over the range 220-400 nm and is used principally in the analysis of natural fatty acids containing conjugated double bond systems or for the estimation of conjugated dienes formed by the action of lipoxygenase or as a result of autoxidation (Chapman, 1965).

Nuclear Magnetic Resonance (NMR) spectroscopy essentially provides a means of determining the structure of an organic compound by measuring the magnetic moments of its hydrogen atoms. In most compounds, hydrogen atoms are attached to different groups such as $-CH_2$, $-CH_3$, -CHO, $-NH_2$ and -CHOH. The proton NMR spectrum provides a record of the number of hydrogen atoms in these different situations (Harborne, 1984). The application of NMR spectroscopy in the analysis of lipids has been the subject of several detailed reviews (Chapman, 1965; Gunstone and Inglis, 1971; Klein and Kemp, 1977; Khan and Scheinmann, 1978; and Macdonald et al., 1984). The technique has been widely used for lipid

structure determinations, particularly for the identification and location of double bonds in fatty acids, but also to detect and locate other functional groups such as cyclopropane rings, hydroxyl groups and triple bonds.

Mass spectrometry (MS) is an excellent tool for the structure_determination of fatty acids. The use of mass spectrometry for determining the structure of fatty acids has been reviewed by McCloskey (1970); Waller (1972); Zeman and Scharmann (1973); Galliard and Mercer (1975) and Christie (1982). This is a very effective procedure and when combined with the separating efficiency of gas chromatography it provides one of the most powerful methods of structure determination and requires much less than one mg of material. Gas chromatography-mass spectrometry (GC/MS) becomes more efficient when linked to a computer. It is possible to determine not only the molecular weight but in most cases also the nature and positions of branches and other substituents on the carbon chain. Most of the mass spectrometric structure work on fatty acids has been performed on the corresponding methyl esters. The methyl ester is more volatile than the free acid and is relatively stable with regard to thermal decomposition. In the mass spectrometer, organic compounds in the vapour phase form positively charged ions. These ions are separated according to their mass to charge (m/z) ratio, and are displayed as peaks on a chart. The largest peak or base peak in the spectrum is given an arbitrary intensity value of 100 and the intensities of all the other ions are normalised against this, so that data can be presented

in a uniform manner. The ion with the highest m/z value is generally that of the original ionised molecule and is termed the parent or molecular ion (M^+). Double bond positions can be determined if the unsaturated esters are first converted to a suitable derivative, preferably one which is sufficiently volatile to be subjected to gas chromatography. Methyl esters of branched-chain fatty acids have mass spectra superficially similar to those of the corresponding straight-chain compounds with the same number of carbon atoms, but changes in the intensities of some ions can be used to indicate the presence and fix the position of the branch (Ryhage and Stenhagen, 1960, and Apon and Nicolaides, 1975).

3. Two important oilseeds - Helianthus and Gossypium

3.1 Helianthus annuus

The sunflower, Helianthus annuus L., is a member of the Compositae, a large and successful family of flowering plants occurring throughout the world. The genus Helianthus is named from the Greek Helios, meaning sun, and anthos, meaning flower. Sunflower probably originated in the south-west United States-Mexico area and from early times its seed was used for food (Vranceanu, 1974 , Heiser, 1976 and Carter, 1978). The sunflower "seed" is, strictly speaking, a type of fruit termed 'achene' composed of two major subunits: the "hull", botanically identified as the pericarp, and the "nut meat", botanically identified as embryonic tissue. The embryo is composed of oil and non-oil fractions (Zimmer and Zimmerman, 1972). There are two general types of sunflower seed - the thick-hulled, often grey-striped "confectionary"

type, and the black, smaller "oil" type (Lusas, 1983). The smooth shell (pericarp) which encloses the seed constitutes by weight some 50-55% of the whole seed, the remaining 45-50% being made up of the oleoginous seed. Commercially grown varieties range in colour from black through to white, but brown, striped or mottled seed can also occur. Although there is often a relationship between a dark hull and high average seed-oil content, an increasing number of hybrids have light coloured seed with a high oil content. The seed varies greatly in size and weight, but is generally a compressed, flattish oblong, with the top truncated and base pointed, roughly diamond-shaped in section, and usually within the range 10 - 25 mm long, 7.5 - 15 mm wide and 3 - 7.5 mm thick (Williams, 1966 , Purseglove, 1968 , and Weiss, 1983).

3.1.1 Production of sunflower

World sunflower production was dominated by the USSR which accounted for some 50% of commercial crops, and in 1970 some 6 million tonnes were harvested in that country (Table 8). In 1979-80, sunflower seed harvested in the USA reached 3.5 million tonnes, half that of the USSR, and the world production that year was 15.5 million tonnes. The amount of sunflower oil produced in relation to seed production would appear to indicate the majority was crushed for oil, and a high proportion entered world trade (Weiss, 1983). The world production of sunflower oil for the year 1979-80 is estimated at 4 million tonnes and 5.13 million tonnes for 1981-82, and 5.75 million tonnes for 1982-83, making sunflower oil production the third

Table 8. Estimated production of sunflower (Helianthus annuus) in selected countries (in thousand tonnes)

Sources: Britannica Year Books (1970-80), Campbell, 1983, and Weiss, 1983. ND: not determined

Selected countries	1970	1975	1978	1979	1980
Argentina	750	850	1600	1300	1400
Australia	50	105	155	186	142
Bulgaria	400	420	420	390	350
Canada	25	30	85	114	200
Chile	25	28	30	32	35
China	50	80	150	275	375
Ethiopia	30	26	15	ND	ND
France	56	75	92	90	155
Hungary	140	150	295	315	450
India	10	10	45	105	125
Iran	30	43	20	13	ND
Italy	25	33	51	42	60
Rumania	760	725	800	790	890
South Africa	100	210	484	500	318
Spain	124	323	470	490	300
Tanzania	15	6	7	5	7
Turkey	395	420	455	520	560
Uruguay	50	51	73	50	35
USA	90	550	1300	1800	3500
USSR	6000	5000	5500	5400	5500
Yugoslavia	325	275	425	540	430
Zambia	ND	15	11	13	9
World	10000	9800	13000	13500	15500

largest of any edible vegetable oil throughout the world. Only in the last few years has sunflower oil production fallen behind palm oil. Sunflower oil is currently the fourth largest edible oil commodity traded on the world market, following soybean, palm oil and coconut oil (Campbell, 1983).

3.1.2 Characteristics and uses of sunflower seed oil

Crude sunflower oil ranges from yellow to dark amber in colour and contains some phosphatides and mucilaginous matter (but less than cottonseed or corn oils) and when refined is light yellow in colour. The oil has a relatively low level of natural antioxidants, and wax esters are present which have their origin in the hull fraction (Robertson, 1972, and Campbell, 1983). The taste and smell of the oil is pleasant and it has a distinctive odour, though not very pronounced (Williams, 1966). Characteristics of the crude sunflower oil are shown in Table 9.

Sunflower seed are produced for three markets - bird feed, human food, and oil. The principal uses of sunflower oil are as a salad and cooking oil, where its high concentration of $C_{18:2}$ acid and pleasing flavour and odour contribute to make the oil highly acceptable for food uses. In Europe, it has been used extensively in shortening and margarine. Margarine includes 50-75% liquid sunflower oil along with a suitable hardstock, generally a soybean-cottonseed blend. Sunflower oil gives margarine excellent flavour characteristics and high polyunsaturated fat levels. Commercially, sunflower oil is used for frying snack foods such as potato chips and for other

culinary processes where a liquid oil with a high smoke point is desired. In addition, sunflower oil with a high $C_{18:2}$ acid content can be used for a number of industrial purposes such as the manufacture of non-yellowing alkyd resins for the paint industry, and it is classed as a semi-drying oil. Being a semi-drying oil, it is used in blends with linseed and other drying oils in paint and varnishes. Sunflower meal remaining after oil extraction is a high-quality protein source which may be used as fillers in animal feed cakes and meals (Purseglove, 1968; Vaughan, 1970; Robertson, 1972; Gill and Vear, 1980; and Campbell, 1983).

Table 9. Characteristics of crude sunflower seed oil

Source: Williams (1966) and Campbell (1983)

Density at 60°C	0.897
Titre °C	16 - 20
Specific gravity 20/20°C	0.918 - 0.923
Refractive index 40°C	1.467 - 1.469
Iodine value	110 - 143
Saponification value	188 - 194
Unsaponifiable matter %	1.5

3.1.3 Lipid and fatty acid composition of sunflower seed oil

The composition of sunflower seed oil is shown in Table 10. Robertson and Morrison (1979) reported that the fatty acid composition of sunflower oil makes it desirable for use as edible oil. It is relatively low in the saturated fatty acids and it contains only small amounts of $C_{16:1}$, $C_{18:3}$, C_{20}, C_{22} and C_{24}. The low content of $C_{18:3}$ acid is primarily responsible for its excellent storage qualities. The variation in fatty acid composition of the oil from seeds located in different positions within an individual sunflower head was determined by Zimmerman and Vick (1973). The $C_{18:2}$ and C_{16} contents of the oil increased (from 60.4 - 71.1% and from 6 - 6.5% respectively) and the $C_{18:1}$ decreased (from 26.0 - 17.1%) from the perimeter toward the centre of the head. Other workers have investigated the effect of inflorescence head size and shape on the oil content and fatty acid composition in sunflower (Afzalpurkar and Lakshminarayana, 1980). Medium and small sized sunflower heads of convex shap yielded seeds with 44% total oil by weight, and 48% of oil content being $C_{18:2}$; big-sized flower heads of convex shape yielded seeds with 42% and 27.3% $C_{18:2}$. The total oil contents of three sunflower varieties; high (Peredovik), medium (INRA 6501) and low oil content (Dahlgren 694) were determined using two methods (Robertson, 1974). In one method, the seeds were ground with an equivalent weight of diatomaceous earth, and in the second method the seeds were ground in a high-speed grinder without diatomaceous earth using petroleum ether in both methods. The oil content of Peredovik was 50.73 $\pm$ 0.7% and 49.81 $\pm$ 0.35%, INRA was 44.85 $\pm$ 0.48% and 44.17 $\pm$ 0.58%

Table 10. Composition of sunflower oil

Material & Analytical method	Total oil content % whole seed	kernel	Lipid class % neutral	polar	SCFA total %	LCFA saturated % C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	total	unsaturated % $C_{16:1}$	$C_{18:1}$	$C_{20:1}$	$C_{22:1}$	$C_{18:2}$	$C_{18:3}$	total	Other FA	References
Different sources																					
six varieties	40.6	60.0					5.6	3.0						41.3			49.2				Kinman and Earle, 1964
20 samples	17-32.7						4.6-6.8	1.7-3.9		0.3-1.0				29.3-56.2			29.9-61.8	0.2-0.4			Gummins et al., 1967
California							4.4-12.9	1.0-9.0						10.8-51.6			39.2-77.6				Knowles et al., 1970
six varieties							5.5-7.0	3.7-6.5						16.4-36.7			51.5-73.7	0.1		1.7	Robertson, 1972
							3.5-4.1	2-2.5						14.8-15.5			78-79.8				Dorrell and Whelan, 19
different varieties	38.2-39.4						7.4-8.1	4.5-5.3						18.8-20.3			58.4-67.8				Beard and Geng, 1982
USA, Canada, Argentina and USSR							5.1-6.3	3.0-5.6						17.8-26.0			64.4-70.4	0.2-0.5			Campbell, 1983
Different methods																					
NMR	42.9																				Robertson and Morrison
	44.8																				Robertson and Windham,
AOCS	44																				Robertson and Windham,
Crude Oil												12-14							58-67		Hilditch and Williams,
Commercial Oil							6	5						20			63				Knowles et al., 1970
							5	2	1					35			57				Weiss, 1983
Triglycerides			57-58				6-6.9	3.1-3.5						15.7-19.7			70.0-75.3				Smidovnik et al., 1981
												14		17			69				Murray, 1984
Phospholipids				2.5			14.8	3.0						13.3			68.7				Greval et al., 1978

and Dahlgren was 31.17 ± 0.46% and 30.81 ± 0.30% respectively. Campbell (1983) subdivided sunflower into 'high-oil seed sunflower' which contains 40% oil and 'low-oil seed sunflower' which contains 30% oil.

3.2 Gossypium spp.

Cotton, Gossypium, belongs to the family Malvaceae. The genus Gossypium is named from Gossypion, the Latin name of the cotton plant. Cotton is grown primarily for its lint, the most important of the vegetable fibres. Cottonseed oil is therefore regarded as a by-product (of the cotton industry). The plant is cultivated widely in many tropical and subtropical regions.

It would appear that about 75% of the world production of seed is processed for oil and meal (Vaughan, 1970). The cultivated races of cotton are normally grouped under four species: (i) G. hirsutum L. (American Upland), one of the most important of cultivated cottons, which originated in the New World; (ii) G. barbadense L. (Sea Island cotton), which also originated in the New World; (iii) G. herbaceum L. (Levant cotton), an Old World species which is grown in India and Africa but it is not so important economically as the two previous species; (iv) G. arboreum L. (Tree cotton), an Old World species but only cultivated on a limited scale (Vaughan, 1970, Berrie, 1977, and Goldsworthy and Fisher, 1984). The seed is up to 12 mm in length, pear-shaped, and after removal of the seed hairs (short fibres - fuzz) is seen to be brown or dark in colour. The weight of 100 seeds of Upland cotton is about 10-13 g (Purseglove, 1968).

3.2.1 Production of cotton

World cotton production increased from 60 million bales to over 70 million bales (218 kg per bale) during 1978 to 1981, dropped to 65 million bales in 1983, but increased to about 80 million bales in 1984-85 (Table 11). The production of cotton in the USA peaked in 1980-81 (15.6 million bales), decreasing to 13.3 million bales in 1984-85. In the USSR it was 14.3 million bales in 1980, decreasing to 12.5 in 1984-85. The production of cotton in China increased continuously from 1978 (10 million bales) to 25.3 million bales in 1984-85.

3.2.2 Characteristics and uses of cottonseed oil

Crude cottonseed oil is very dark in colour due to the presence of pigments, and pale yellow after refining (Williams, 1966). Commercial cottonseed contains approximately 16-20% protein, 18-24% oil, 30% carbohydrate and 22% crude fiber and from 0.4-2.0% gossypol (Purseglove, 1968). The characteristics of crude cottonseed oil are shown in Table 12.

Cottonseed oil is used in lard substitutes, as salad and cooking oil, and in margarine manufacture and low-grade oil is used in the manufacture of soap, lubricants, sulphonated oils and protective coatings. It is often used for deep-fat frying of food such as potato chips and fish (El-Kateeb and El-Zeany, 1983). Cottonseed meal contains the phenolic (toxic) compound gossypo that has undesirable effects in nonruminant animals, so its use is mainly confined to cattle feeds (Pryde, 1983).

Table 11. World cotton production [in million (218 Kg) bales]

Sources: Britannica Year Books (1978-84)

ND: not determined

Region	1978	1979	1980	1980/ 81	1981/ 82	1982/ 83	1983/ 84	1984/ 85
Africa	5.0	5.3	5.3	5.3	5.5	5.5	5.5	5.5
Asia and Oceania	23.2	24.3	26.3	28.4	31.6	32.0	34.8	41.0
Brazil	2.3	2.6	2.8	2.9	3.0	2.8	2.5	3.0
China	10.0	10.1	12.4	13.6	16.5	17.0	21.3	25.3
Egypt	2.0	2.2	2.4	2.3	2.1	2.0	1.9	1.8
Europe	0.9	0.8	0.8	0.9	0.7	0.8	0.8	1.1
India	6.3	6.1	6.1	6.3	6.3	6.6	5.9	6.2
Mexico	1.6	1.5	1.4	1.4	0.8	0.9	1.0	1.3
Pakistan	2.1	3.4	3.3	3.5	3.8	3.1	2.2	3.7
South America	4.7	4.9	4.8	5.0	4.6	ND	ND	ND
Sudan	ND	ND	ND	0.7	0.9	1.0	1.0	1.0
Turkey	2.2	2.2	2.2	2.2	2.2	2.3	2.4	2.6
USA	10.9	14.6	11.1	15.6	12.0	7.5	7.8	13.3
USSR	12.3	13.1	14.3	13.5	11.9	13.0	12.3	12.5
TOTAL	60.0	65.6	65.4	71.0	67.7	65.3	67.3	80.7

Table 12 Characteristics of crude cottonseed oil

Sources: Williams, 1966 and Achaya, 1975

Density at 60°C	0.890 - 0.895
Titre °C	31 - 37
Specific gravity 15/15°C	0.922 - 0.930
Refractive index 40°C	1.474 - 1.694
Iodine value	99 - 115
Saponification value	189 - 198
Unsaponifiable matter %	0.5 - 1.5

3.2.3 Lipid and fatty acid composition of cottonseed oil

Cottonseed oil consists predominantly of triglycerides, a certain proportion of fatty acids in the free form (FFA), and minor amounts of a variety of other substances, such as phospholipids, sterols and hydrocarbons (Achaya,1975). The composition of cottonseed oil is shown in Table 13. In the study of glyceride components of cottonseed oil, there is a fair measure of agreement regarding the component glyceride classes. Trisaturated glycerides comprise about 1%, disaturated mono-unsaturated glycerides make up about 45-50% and triunsaturated glycerides range from 30-40% of total cottonseed glycerides (Bailey, 1948 and Achaya, 1975). Evans et al. (1969) suggested that all saturated acids are present exclusively in the 1,3-positions, randomly distributed, and that $C_{18:1}$ and $C_{18:2}$ acids are then distributed randomly

Table 13. Composition of cottonseed oil

Material & Analytical method	Total oil content %		Lipid class %		SCFA Total %	LCFA												Total	Other FA	Referen
						saturated %						Total	unsaturated %							
	whole seed	kernel	neutral	polar		C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}		$C_{16:1}$	$C_{18:1}$	$C_{20:1}$	$C_{22:1}$	$C_{18:2}$	$C_{18:3}$			
Different sources and varieties																				
IV;105;SE;286.4						1.4	23.4	1.3					2	22.9			47.8				Baiely,
	20	37					20-25	2-7						18-30			40-45				Williams
					0.9	0.8	19.9	3.1					0.4	25.7			48.5	0.1			Sreeniva
Indian (IV;106)						0.5	25.8	2.3					1.2	19.1			51.1				Achaya,
American (G.hirsutum)	18-25											12-25		20-44			33-54				Duffus a Slaught 19
Pakistan (IV; 104,SN;186,NSM; 2.3%)	12-24					1-2	28-43	3-9					0.3-3	16-32			31-41				Raie et 19
Commercial oil							22	2						21			50				Knowles 19
Triglycerides																				
LLL;29.1,PLL+POL; 42.4,SLL;6.5,PPL; 18.0,PSL;3.0 and PPP;1.0							29.8	2.4					0.8	16.6			49.4				Youngs a Subbara
IV;97(PLL;28,POL; 20,PLL;17,LLL;11 and OLL;9)	20		86			1.1	27.3	3.1					1.4	16.7			50.4				Gunston Qureshi
β-position (G.hirsutum)						0.2	3.2	0.1					0.6	21.1			74.8				Mazuelos
			89			1	22	2						31			45				Murray,1
Phospholipids																				
							24.5							33.2			42.3				Vijayala et al.,
[illegible]					1.2-2.0	1.0-2.7	26-29	2.4				1.4-3.2		18-20			38-47				Karshie

and equally in all three positions. For cottonseed oil, the 2-position consists of 33.3% of $C_{18:1}$ and 50.1% of $C_{18:2}$. Phospholipids are present in the oil, the seed and the cake, and they may be obtained either by the hydration of oils (when the products are rendered insoluble and can be separated) or by the extraction of oilseeds with solvents such as methanol. Since all phospholipids contain phosphorus, it is a common practice to estimate the content of phosphorus and multiply the value by 25 as a fair approximation of the phospholipid content. The content of phospholipids in the kernel of cottonseed is about 1.3 - 1.5% and about 1% in the whole seed. Between 0.93 - 2.74% of the total lipid was phospholipid in Indian cottonseed oil (Achaya, 1975). Karshiev et al., (1981) extracted phospholipids from cottonseed (variety F-108) and showed that after the usual extraction of kernels with chloroform-methanol (2:1,v/v), about 8% of phospholipids remains in the meal, and after extraction with nitric acid (0.33%) about 2.3% of phospholipid remained, which was easily extracted with a mixture of solvents acidified with a mineral acid.

4. Germination of seeds (general consideration)

The seed of angiosperms develops from a fertilised ovule. It consists of an embryo surrounded by the testa or seed coat and the endosperm may persist as a storage organ or it may partly or fully degenerate, or may be become fused to the seed or fruit coat (Mayer and Polijakoff-Mayber, 1982). The embryo consists of the embryonic axis bearing one or two 'seed leaves' - the cotyledons - and is composed of the

hypocotyl to which the cotyledons are attached, the radicle, and the plumule (the shoot apex with the first true leaf or leaves) (Bewley and Black, 1978).

Seeds are described as endospermic or non-endospermic depending on the presence or absence of a well-formed endosperm. Endospermous seeds retain the endosperm to maturity while in non-endospermous seeds the endosperm is utilised completely or almost completely during maturation. Non-endospermous seeds store their food reserves in the cotyledons (Berrie, 1984). The size and shape of seeds depends on the species, the size of embryo, the amount of endosperm and the genotype.

The process of germination leads eventually to the development of the embryo into a seedling within two phases; the first phase refers to the period up until the radicle emergence and the second relates to seedling growth, dependent on seed reserves. Seedlings are classified as "epigeal" in which the cotyledons come above ground and are usually photosynthetic, as in Gossypium and Helianthus, and "hypogeal" in which the cotyledons remain below ground.

4.1 Germination behaviour

Seed germination, an important aspect in the life of the plant, is an interesting and complex physiological phenomenon. The term "germination" is commonly used to encompass the imbibition of water, the formation of enzyme systems, commencement of growth and radicle emergence, and finally the growth of the seedling with characteristic features. In order that a non-dormant seed can germinate,

it must be placed in environmental conditions favourable to this process, but others do not respond in such circumstances and are considered to be dormant. Among the conditions required are an adequate supply of water, suitable temperature and composition of the gases in the atmosphere, as well as light for certain seeds (Leopold and Kriedemann, 1975; Duffus and Slaughter, 1980 and Mayer and Polijakoff-Mayber, 1982).

The first process which occurs during germination is the uptake of water by the seed, an uptake due to the process of imbibition. When dry seeds are exposed to liquid water there is an initial rapid increase in moisture content which is followed by a slower uptake until the water content reaches about 60% of the initial dry weight (Berrie and Drennan, 1971). Imbibition is determined by three factors: (i) the composition of the seed, (ii) the permeability of the seed coat to water, and (iii) the availability of water in the environment (Vicente et al., 1969). Imbibition is a physical process related to the properties of colloids and is of great importance in the process of germination as it may lead to the breaking of the seed coat and also to some extent makes room in the soil for the developing seedling. McArthur et al. (1975) showed that radicle emergence of cottonseed can be noted after 24 h after a small amount of water reaches the interior of the cottonseed coat. The process of imbibition is completed within 36-48 h in cottonseed (Wanjura and Buxton, 1972). Many seeds have a specific temperature range within which they germinate. At very low temperatures (c. 5°C) and very high

temperatures (c. 50°C) the germination of all seeds is usually prevented. The optimal temperature may be taken to be that at which the highest percentage of germination is attained in the shortest time. Bhatti (1974) found that the relatively high temperature of 27°C increased the percentage of germination of cottonseeds. Abdel Magid and Osman (1977) showed that the maximum germination percentage of cottonseed occursat 30°C with decreased germination and failure at 15 and 45°C, but Goldsworthy and Fisher (1984) mentioned that the optimum temperature for cottonseed germination is 34.4°C.

There is a little evidence for light as a major factor influencing germination in this species. Seeds may be divided into these which: (i) germinate only in the dark, (ii) germinate only in continuous light, (iii) germinate after given a brief illumination, and (iv) are unaffected by the presence or absence of light during germination. Berrie (1984) classified seeds into photoblastic which respond to light and non-photoblastic or non-responding seeds. He showed that light may induce or promote germination in positively photoblastic seeds, or light may prevent or retard germination in negatively photoblastic seeds.

Many seeds do not germinate when placed under conditions favourable for germination. This dormancy may be due to the immaturity of the embryo, impermeability of the seed coat to water or to gases, prevention of embryo development due to mechanical causes, special requirements for temperature or light, or the presence of substances inhibiting germination. Many methods have been used to eliminate the hard coat effect

and thus promote germination; among the best known ones are mechanical abrasion or impact, high and low temperature treatments, and chemical treatment to remove or dissolve the impermeable portions of the coat (Barton, 1965). The percentage germination of cottonseed is increased by removal of seed coat (Halloin, 1976).

The effect of delinting on the germination of cottonseed was studied by Marani and Amirav (1970). They found that acid delinting increased the permeability of the seed coat and acid-delinted seeds showed a high percentage germination.

4.2 Lipid content and fatty acid composition during germination

Seeds characteristically contain relatively large amounts of food reserves which support growth and development of the seedling. These reserves include lipid, protein, carbohydrate, organic sulphate and various inorganic compounds. The fat and fatty acid contents of seeds change during germination, and they are utilised during the process of germination. Total lipid content decreased during germination of Ricinus (Yamada, 1957), Citrullus vulgaris and Elaeis guineensis (Hardman and Crombie, 1958 and Boatman and Crombie, 1958) and Douglas fir (Ching, 1963). Zimmerman and Klosterman (1965) found that during the first 18 h of flax seed germination there was only a slight decrease in the level of triglycerides but thereafter this fraction was metabolised more rapidly, so that after 90 h germination the oil content decreased by 53%. The constant increase in FFA levels in the germinating tissues from 18 h, suggested that lipase activity

was greater than that of the enzymes involved in the oxidative breakdown of the fatty acids. McMahon and Stumpf (1966) studied post-germination safflower seedlings for their capacity to incorporate ^{14}C-acetate into fatty acids. They found that most of the fatty-acid-synthesising capacity was confined to the developing cotyledons and progressively increased with time, whereas the activity in the hypocotyl tissue was much lower and decreased with time. Katayama and Funahashi (1969) traced the incorporation of label from ^{32}P orthophosphate into the lipids of the seed cotyledons and into the hypocotyls and radicles of mung bean (Vigna radiata) seedlings. The greater part of the incorporated activity was observed in the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fractions in the cotyledons, and in the PE fractions of the hypocotyls and radicles. Joshi et al. (1974) found that C_{16} and $C_{18:1}$ decreased in the cotyledons of soybean seeds from 6 - 12 d, while $C_{18:2}$ increased during the same period. Germinating cotton seeds incorporated significant amounts of ^{14}C-acetate into the very long chain fatty acids in spite of a low tissue concentration of these substances (Harwood and Stumpf, 1970; Harwood, 1975): during germination at 30°C, radioactivity was present in the fatty acid fractions viz.: C_{16} (48%), C_{18} (33%), C_{20} (2%), C_{22} (9%), C_{24} (3%) and others (5%).

Seeds from two Egyptian cotton varieties attained similar maximum germination percentages at 30°C (Abdel Magid and Osman, 1977). The disappearance of oil from the cotyledons of germinating seed was gradual over the period of 5 d (from 35%

to 5%) while starch breakdown was very rapid during the first day. During the first 11 d after imbibition the cotyledons of peanut decreased in dry weight from 345 mg to 143 mg and fat content decreased to 55% of fat content from dry seeds; this represents hydrolysis of 9.4 μmoles of triglyceride per cotyledon per day (Bewley and Black, 1978). Bhatia et al. (1978) found that the total and non-polar lipid contents in germinating sunflower seeds decreased between days 1 - 5 of germination and lipase activity steadily increased. Gopalakrishman et al. (1982) isolated lipids from root, stem, leaves, buds, flowers and seeds at different stages of growth of Althaea rosea. They found that C_{18} and $C_{18:1}$ acids decreased during growth. Moreover, Doman et al. (1982) studied the metabolism of carbohydrate and lipid reserves during germination (0 - 12 h) and postgerminative growth (12 - 48 h) of cottonseeds, noting that the neutral lipids in the cotyledons decreased from 20.4 ± 1.4 to 13.6 ± 0.4 mg per cotyledon pair during germination (from initial soaking to 48 h). Such values indicated that the lipid could be used exclusively for root growth and the labelling data with 2-^{14}C acetate showed that its metabolites were partitioned between the axis and cotyledons.

High temperatures during sunflower seed germination reduced total oil content from 41% to 24% (Harris et al., 1978). With decreasing temperature during development of sunflower there was an increase in $C_{18:2}$ in the range 49 - 72%. The marked reduction in $C_{18:2}$ at high temperatures may be attributable to an inhibition of desaturase, responsible for

the conversion of $C_{18:1}$ to $C_{18:2}$ acid (Weiss, 1983). Indeed, Silver et al. (1984) detected the decrease of $C_{18:2}$ in developing sunflower seed embryos in response to high temperature and suggested that alterations in the fatty acid composition of seed oil in response to temperature are produced by an effect on desaturation of newly synthesised oleate rather than through turnover of existing lipid. Seven-day-old seedlings of Echinochloa crus-galli variety Oryzicola (Vasing) had a higher total lipid content when germinated under N_2 (262 µg seed^{-1}) than in air (191 µg seed^{-1}), although dry seeds contained 319 µg seed^{-1} (Knowles and Kennedy, 1984). Triglycerides and polar lipids were present at 170 and 38 µg seedling^{-1} when germinated under N_2 and 78 and 50 µg seedling^{-1} in air, and 171 and 40 µg seedling^{-1} in dry seeds. There were no significant changes in the fatty acid composition of triglycerides but there were significant changes in C_{16} and $C_{18:1}$ acids of polar lipids. Changes in haustorial lipids were followed until the eighth week after germination of oil palm oil (Khor and Cheang, 1984). SCFA C_{12} increased from 19.5% after one week to 47.9% after three weeks germination, C_{14} decreased during first three weeks and increased thereafter. LCFA C_{16} decreased from 17.8% to 10.6% after five weeks, $C_{18:0}$ increased during eight weeks, $C_{18:1}$ decreased from 16.4% to 14.4% after three weeks and increased to 24.7% after eight weeks but $C_{18:2}$ decreased during the whole germination period from 28.2% to 7.4%. Neutral lipid and glycolipid increased during eight weeks germination but phospholipid decreased

rapidly during the germination period. Study of the molecular species and fatty acid distributions of triglycerides obtained from cotyledons of soybean seedling was carried out by Yoshida (1984). There were greater reductions in molecular species of triglycerides containing saturated fatty acids (palmitic or/and stearic) throughout germination of soybean seeds. These triacylglycerols were hydrolysed slightly faster than other species and they became very minor components by 12 d. He suggested that the mechanism of initial triacylglycerol hydrolysis may be different in various molecular species.

4.3 Lipid metabolism in germinating seeds

In many seeds disappearance of fats is accompanied by the appearance of carbohydrates. This reaction apparently proceeds as follows. First, the fatty acid undergoes β-oxidation. The acetyl-CoA formed is converted to malate via the glyoxylate shunt. The malate thus formed is converted to carbohydrate by a number of reactions. All these reactions occur in the cotyledons or endosperm of fat-containing seeds such as soybean, castor bean and groundnuts (Mayer and Polijakoff-Mayber, 1982). Lipoxidase is also believed to play a part in fatty acid oxidation. This enzyme, which also occurs in seeds, is supposed to break the fatty acid chain into two smaller parts by peroxidative attack at a double bond. The fatty acids formed by hydrolysis from the glycerides are further metabolised by the glyoxylate cycle or directly utilised in cell metabolism

(Bewley and Black, 1978, and Mettler and Beevers, 1980). In developing seed tissue, be it cotyledonous or endosperm tissue, there is now considerable evidence that special organelles identified as proplastids contain all the necessary enzymes to generate ATP, NADPH, NADH, as well as the enzymes to convert pyruvate to acetyl-CoA and the fatty acid enzymes to utilise acetyl-CoA for oleic acid synthesis. During the first few days of germination fat reserves are used as respiratory substrates by conversion to fatty acids and sucrose. The conversion of fat to sucrose in endosperm tissue of the castor bean seed (Noggle and Fritz, 1983) begins with the hydrolysis of fats to glycerol and long-chain fatty acids through the action of lipase. Glycerol is converted to triose phosphate and further metabolised. The LCFAs are converted to acetyl-CoA by the process of β-oxidation, which with the addition of Coenzyme A, consumption of ATP, and the reduction of NAD^+ to NADH. Acetyl-CoA formed by β-oxidation is not metabolised via the tricarboxylic (TCA) cycle but rather by the glyoxylic shunt. The relation of the glyoxylate pathway to the tricarboxylate acid cycle on the one hand and the conversion of fats to carbohydrates is shown in Figure 2.

In castor bean seeds, fats are stored in subcellular organelles known as 'glyoxysomes' and the enzymes in fat hydrolysis, β-oxidation, and the glyoxylic cycle are all contained in this organelle (Mettler and Beevers, 1980). Further conversion of acetyl-CoA to sucrose and fatty acids occurs in four metabolic phases: (1) formation of citric

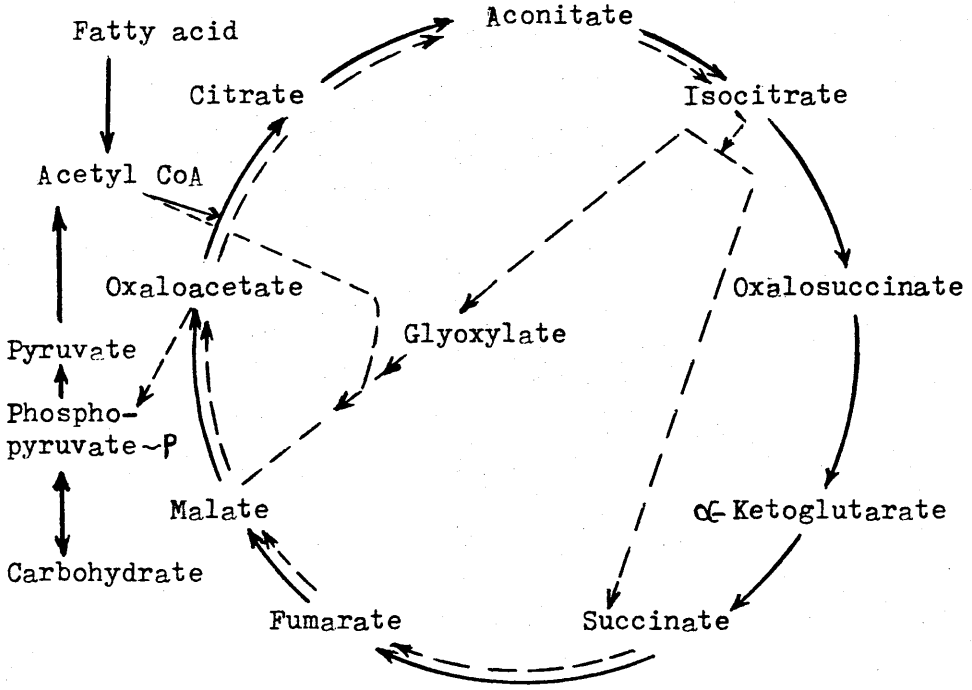


Figure 2. The reaction between the TCA cycle (solid line) and the glyoxylate shunt (broken line) and the possible way of conversion of fat to carbohydrate.

Source: Mayer and Polijakoff-Mayber (1982).

acid, succinic acid, and malic acid in glyoxysomes via the glyoxylic acid cycle; (2) metabolism of succinic acid to malic acid in mitochondria; (3) conversion of malic acid to sucrose in the cytosol by a reversal of glycolysis; and (4) conversion of citric acid (produced in glyoxysomes) to fatty acids in proplastids. Many of the enzymes catalysing these reactions are not present in dry seeds but must be synthesised de novo after water imbibition. Clearly the early stages of seed germination require numerous metabolic substrates supplied by seed reserves and involve considerable metabolic activity in forming new cellular material.

5. Subcellular localisation of lipids and fatty acids

The steps of membrane isolation include homogenisation, separation, purification and identification. Homogenisation is the first and crucial step to disaggregate the tissue in order to obtain a suspension of single subcellular organelles. Differential centrifugation permits the separation of the major subcellular organelles and membrane systems. Centrifugation of the homogenate at about 1000 g for 10 min provides a nuclear fraction, which contains besides nuclei, unbroken cell, and various large cell fragments, depending on the conditions adopted during homogenisation. Centrifugation of the supernatant remaining after collection of the nuclear fraction at about 10000 g for 15 min sediments the mitochondrial fraction that also contain lysosomes. Finally, centrifugation of the supernatant overlying the mitochondrial

fraction pellet at about 100,000^{g} for 2 - 4 h yields a microsomal fraction and a supernatant containing smooth and rough endoplasmic reticulum (ER), the golgi apparatus (GA) complex and the plasma membrane (PM) (Evans, 1982). The purification and the identification of the separated membrane fragments and organelles is carried out by quantitative measurement of enzymic markers, supported by chemical analysis and electron microscopy.

5.1 The problems of isolating lipid membranes

Most of the problems involved in the isolation of membranes are due to the active enzymes that attack the lipids of these membranes. The problems of membrane isolation can be summarised as follows:

(i) Hydrolytic enzymes can directly attack the lipid components of membrane structure leading to structural breakdown (Galliard, 1974a).

(ii) Free fatty acids produced by the action of lipolytic acylhydrolases can inhibit the activities of organelles such as mitochondria (Dalgaron & Birt, 1963) and may also stimulate the enzymatic breakdown of membrane lipids, for example linoleic acid causes a dramatic increase in the hydrolysis of mitochondrial lipids catalysed by a lipolytic acylhydrolase found in plants.

(iii) Many plant tissues contain active enzymes that attack the lipids of membranes such as phosphatidohydrolase (catalyses the hydrolysis of phospholipids to phosphatidic acid), lipase (attacking water-insoluble neutral lipids, Barron, 1964), phospholipase β activity (causes liberation

of both fatty acids from diacylphospholipids, Galliard, 1973), galactolipase (removes both fatty acids from mono- and digalactodiglycerides (Kates, 1970), and the group of lipoxygenase enzymes (catalyse the formation of hydro-peroxides from unsaturated fatty acids especially linoleic and linolenic acids (Galliard and Phillips, 1971)).

5.2 Conditions to minimise lipid degardation during isolation of membranes

The following conditions minimise lipid damage during isolation of membranes:

(i) The usual precautions during isolation are working at low temperature and gentle homogenisation in the presence of an osmoticum, and these are most effective in reducing the quantity of harmful agents and enzymes released from liposomes and peroxisomes. On the other hand, a rapid isolation method that removes required organelles from a crude homogenate containing these enzymes would be advantageous (Maddy, 1976).

(ii) High pH (7.5 - 8) reduces the activity of most lipid-degarding enzymes such as phospholipases (Punnett, 1959).

(iii) Chelating agents. Addition of ethylene diamine tetracetic acid (EDTA) to extraction media inhibits the activity of some lipases (Galliard, 1974a).

(iv) Antioxidants. Dithiothreitol (DTT) can reduce the activity of some enzymes, including polyphenol oxidase (Waldron, 1984).

(v) Bovin Serum Albumin (BSA). The beneficial effect of BSA is due to its ability to bind FFA of endogenous origin or fatty acids formed by the action of acylhydrolase enzymes

during extractions. A concentration of BSA between 0.1 and 1% (w/v) has been claimed for optimal activities of chloroplasts (Friedlander and Neumann, 1968) and mitochondria (Ikuma, 1970).

(vi) The membranes should either be frozen as quickly and deeply as possible under protection of nitrogen or they should be stored at a low temperature (-20°C) and homogenised in a polar lipid solvent containing an antioxidant, preferably in a nitrogen atmosphere (Maddy, 1976).

In spite of these difficulties there is an increasing amount of knowledge in the literature concerning the isolation and identification of lipid membranes (Golowick and Kaplan, 1974; Hall and Roberts, 1975; Nagahashi and Beevers, 1978; Evans, 1982, and Smith and Stein, 1982).

5.3 Markers of membranous cell components

Biochemical and morphological markers could be considered as two basic criteria for the identification of isolated membranous cell components. The most reliable method for membrane identification is the use of biochemical markers and, in particular, enzyme markers. Cell components contain numerous enzymes, but the selection of specific ones as markers depends on the organelle specificity. According to DeDuve (1971) and Morré et al. (1979) a marker must be found exclusively in a single population of cell components and distributed homogeneously within that population. Markers should be stable and the amounts present can be measured easily and with confidence. The common enzyme markers of plant subcellular organelles and their derivative parts are

described by Quail (1979).

For the endoplasmic reticulum (ER), NAD-cytochrome c reductase and NADPH-cytochrome c reductase have been reported in the microsomal fraction and they are most reliable markers to identify the ER in plants (Sottocasa et al., 1967; Hall and Roberts, 1975; Nagahashi and Beevers, 1978). The Golgi apparatus (GA) is identified by inosine diphosphatase, IDPase, an enzyme of uncertain biological significance (Baydoun, 1980), and by glycosyl transferases (e.g. galactosyl transferase) (Bergeron et al., 1973; Vischer and Reutter, 1978, and Morré et al., 1979).

The plasma membrane (PM) is identified by Adenosine Triphosphatase (ATPase) which is activated by Mg^{2+} and further stimulated by monovalent cations, especially K^{+}, and has been reported to be associated with the PM preparations from roots of several higher plants (Nagahashi et al., 1978; Travis and Booz, 1979, and Scharschmidt et al., 1979).

The most commonly used markers for the mitochondria (M) in all cells are succinate dehydrogenase (Kawasaki and Sato, 1979; Narahara et al., 1979) and cytochrome c oxidase (Klaus and Kindl, 1979). The outer mitochondrial membrane is characterised by monoamine oxidase activity (Wibo et al., 1981).

5.4 Lipid storage organelles

The lipid reserves in plants are generally present in special organelles, which are intracellular particles. These subcellular particles have been variously named 'microsomes', 'spherosomes', 'oleosomes', 'oil bodies' and 'lipid-containing vesicles'.

5.4.1 Nomenclature and variability

The name 'microsomes' was applied to lipid-rich cytoplasmic particles but botanists changed the name to 'spherosomes', a term first used by Dangeard (1921). Frey-Wyssling *et al.* (1963) studied the origin of spherosomes and oil droplets in the cytoplasm of rape and mustard cotyledon cells. They concluded that spherosomes originated as vesicles formed from the endoplasmic reticulum and, by accumulating netural lipids, developed into oil bodies (passing through a number of transition stages). Sorokin (1967) concluded from light microscopic observations that spherosomes, which occur in both low and high lipid tissues, were fairly constant in size, 0.8 - 1.0 μm in diameter, and reacted strongly with phospholipid stains but poorly with neutral lipid stains. Oil droplets occurred in plants with a higher-lipid content and reacted strongly with neutral lipids and varied in size from 0.3 to 3. 0 μm in diameter. She claimed that spherosomes were surrounded by a unit membrane which prevented the spherosomes from coalescing, whereas the oil bodies easily coalesced during preparation of microscopic specimens. She therefore suggested that spherosomes and oil droplets represent distinctly different entities. Yatsu *et al.* (1971) isolated spherosomes from onion and cabbage as well as oil droplets from cottonseed. They found that spherosomes and oil droplets were not only morphologically very similar, even at the ultrastructural level, but also chemically very similar, being rich in lipids with very little phospholipid

and little protein. Cabbage spherosomes varied in size (0.4 - 2 μm in diameter) but cottonseed spherosomes were 2 - 3 times larger in diameter. They suggested the name 'oleosomes' for these particles. Several workers have since adopted this nomenclature (Kleinig et al., 1978; Wanner and Theimer, 1978). Mollenhauer and Totten (1971 a,b) claimed that there are two distinct classes of lipid-containing vesicles in the cotyledons of pea, bean and soybean: the "simple vesicles", generally 0.5 - 3 μm in diameter and containing $C_{18:3}$ acid rich triglycerides with phospholipids as minor components, and the "composite vesicles" which are smaller (0.1 - 1 μm in diameter) and contain a molar ratio of phospholipids to triglycerides of 1:10. Schwarzenbach (1971) used the term 'spherosomes' for the oil bodies of castor beans, identified a precursor organelle, and preferred the term 'prospherosomes' which are surrounded by a unit membrane. Smith (1974) found the spherosomes and oil bodies of Crambe abyssinica to be separate entities with different sites of origin and functions within the cell. Hall et al. (1974) concluded that spherosomes are easily recognised as spherical particles between 0.5 - 1.0 μm in diameter, although they may be as large as 2.5 μm in diameter, and that they are surrounded by a single unit membrane and have a fine granular internal structure under the electron microscope. They suggested that spherosomes probably originated from the endoplasmic reticulum and it is possible that oil droplets develop from spherosomes by replacement of the granular stroma by oil.

Jelsema et al. (1977) isolated the lipid reserve bodies from the aleurone layer of wheat. They classified spherosomes into "light spherosomes" which contained more triglycerides and "heavy spherosomes" which contained more phospholipids and proteins. Salisbury and Ross (1978) concluded that lipids are always stored in specialised bodies within the cell. They preferred the term 'oleosomes' because it indicates that they contain lipid and distinguishes them from 'peroxisomes' and 'glyoxysomes' which are also rather spherical and range in size around 1 μm in diameter. They reported that oleosomes are indeed half-membrane-bound organelles (Yatsu and Jacks, 1972) whose polar, hydrophilic surfaces are exposed to the exterior and whose nonpolar, hydrophobic surfaces face the lipids at the inside. They mentioned that probably the endoplasmic reticulum expands, forcing these halves apart and ultimately leading to a full-grown oleosome. Noggle and Fritz (1983) mentioned that plant cells contain spherosomes which are small spherical bodies ranging in size from 0.7 to 0.9 μm in diameter. They noted that these bodies which are visible under the light microscope, were found to be rich in lipids, and under electron microscope were not bounded by the usual unit membrane but rather by what appeared to be a single layer of phospholipids and proteins.

5.4.2 Chemical composition and biosynthetic activities of spherosomes

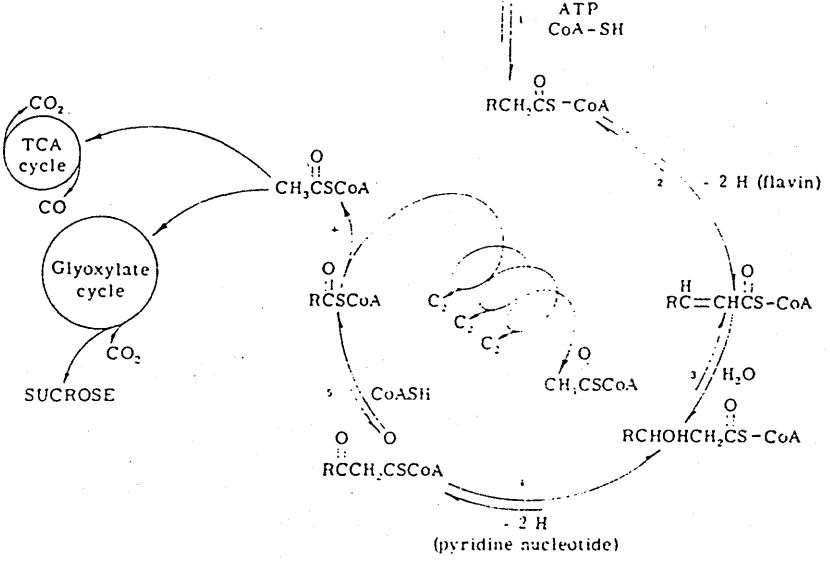
Jacks et al. (1967) isolated spherosomes (fat pad) and mitochondria (pellets) from the cotyledons of germinated

peanuts. On examination at fine structural level the spherosome diameter was found to be in the range 1 - 2 μm. These spherosomes were composed of 98.1% total lipid (triglycerides), 0.77% phospholipids and 1.27% protein by dry weight. They found that spherosomes of germinating peanuts are the principal site of lipid storage in the seed. On the other hand, Yatsu et al. (1971) found 99.65% total lipids (triglycerides), 0.09% phospholipids and 0.22% protein in repeatedly-washed preparations of peanut spherosomes. In a study of spherosomes from castor beans, Ory et al. (1968) examined by a combination of biochemical and electron microscopic techniques the fat pad prepared from ungerminated castor beans. Their results confirmed the presence of spherosomes, the fat storage organelles of the cells concentrated in these preparations. They found that lipase activity is localised in the spherosomes derived from endosperm tissue of ungerminated castor beans. Harwood et al. (1971) isolated oil bodies from castor seed and found that they could synthesise fatty acids from [^{14}C] acetate and [^{14}C] malonyl-CoA and triglyceride from [^{14}C] oleoyl-CoA. The oil body inclusions are important sites of the biosynthesis of triglycerides in maturing castor bean endosperm.

Trelease (1969) isolated lipid bodies from maize scutella; these consisted mostly of triglycerides with small proportions of sterols, phospholipids and several non-polar lipids. The fat fraction of sunflower seed contained 89.3% lipid, 4.3% protein and 6.4% others by dry weight (Gurr et al., 1974).

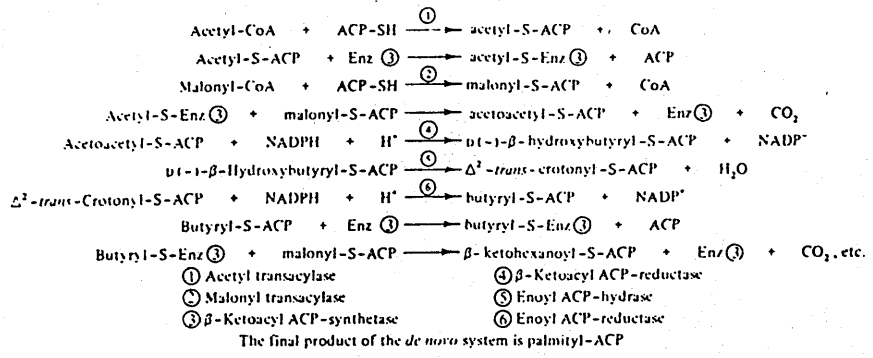
In the study of spherosomes from cottonseed Yatsu et al. (1971) found that the spherosomes contained 98.8% lipid and 0.63% protein. Gurr (1980) found that the chemical composition of oil bodies isolated from cottonseed contained 98.4 triglycerides, 0.4 phospholipids, 0.6 protein and others 0.6 g/100 g oil bodies. He reported that spherosomes are rich in phospholipids and protein and contain acid β-glycerophosphatase but that oil bodies are rich in triglycerides with smaller amounts of protein and phospholipids and their function is to store the triglycerides accumulating during seed development. Kleinig et al. (1978) found that spherosomes from carrot suspension culture cells contained 97% triglycerides and 1 - 2% protein.

Esau (1977) showed that the reaction to osmium tetroxide indicates the presence of lipids (triglycerides) in the fat globules of plants. These globules are organelles (spherosomes) enclosed in a unit membrane or oil droplets having no bounding membrane, suggesting that the globules originated as lipid-containing vesicles cut off from the endoplasmic reticulum and are later converted into oil droplets. She found that the seed lipid reserve is a triglyceride which is hydrolysed in situ by lipase to glycerol and fatty acids. The fatty acids can be used for the synthesis of phospholipids and glycolipids which are required as constituents of organelles, but most are converted to sugars and transported to the seedling body for growth. Harwood and Russell (1984) reported that the plant spherosome although rich in lipids (mainly phospholipid) does not have a role in energy storage but, acts instead as the precursor site for the formation of storage granules.



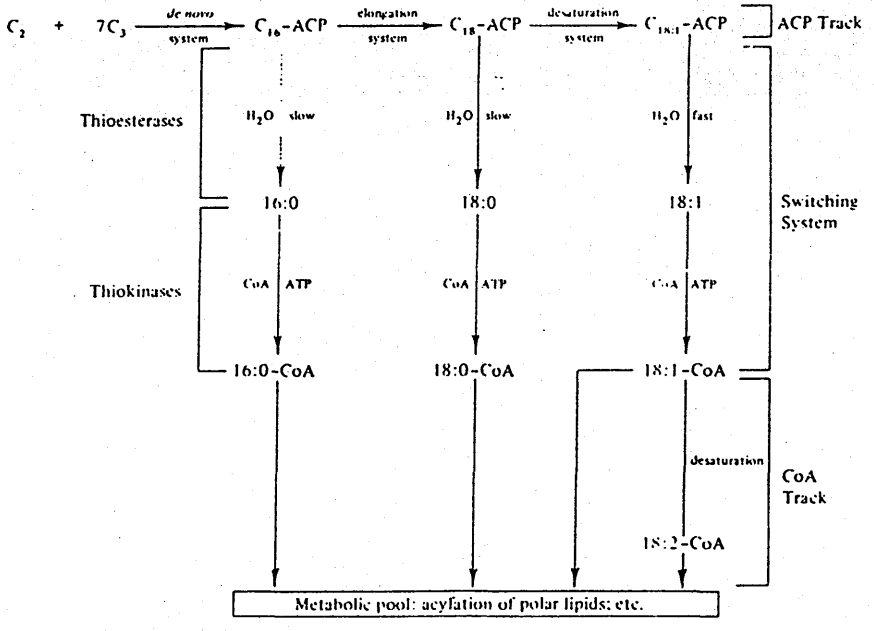
1 Fatty acid thiokinases
2 Fatty acyl-CoA dehydrogenases
3 Enoyl hydrase
4 β-Hydroxyacyl dehydrogenase
5 β-Ketoacyl thiolase

B



① Acetyl transacylase
② Malonyl transacylase
③ β-Ketoacyl ACP-synthetase
④ β-Ketoacyl ACP-reductase
⑤ Enoyl ACP-hydrase
⑥ Enoyl ACP-reductase

The final product of the *de novo* system is palmityl-ACP



A: Degardation of FA by β-oxidation

B: The systems involved in the production of major FA found in plant cells (From Stumpf, 1976)

6. Rationale and Scheme of Research Programme

Despite considerable interest in the economic and biochemical aspects of lipids and fatty acids in oilseeds, there is remarkably little information available on the changes taking place in the levels of these components during the processes of germination and seedling emergence. This thesis describes a project on the analyses of fatty acids in *H. annuus* and *G. barbadense* seeds at various phases of germination.

The first section of the Results and Discussion details the germination characteristics of both species, an essential prerequisite to the overall study. The second section is concerned with lipid content, both total lipids and the three lipid classes - neutral, phospholipid and glycolipid. In the third section, long-chain fatty acids from the total lipid as well as from each of the lipid classes are investigated. Short-chain fatty acids are dealt with in the fourth section. Finally, in the fifth section, studies have been carried out on lipid storage organelles (spherosomes or oleosomes) with comparative studies on mitochondria and to a lesser extent in the endoplasmic reticulum. Throughout the second, third and fourth sections of the Results, emphasis has been placed on time-course experiments, and the sub-division of seeds into their component parts.

MATERIALS AND METHODS

1. Plant Materials

Commercial sunflower (Helianthus annuus L.) and cotton (Gossypium barbadense L.) seeds were used in this study. Sunflower seeds (fruits), H. annuus cv. Tall single were obtained from Daggs, the gardeners' shop, 16 Bath Street, Glasgow; cotton seeds, G. barbadense cv. Giza 66 were obtained from the Cotton Research Institute, Agriculture Research Centre, Giza, Egypt. Seeds of both species were stored in darkness at 5°C in a cold room until required. Seed characteristics of both species are illustrated in Plate 1.

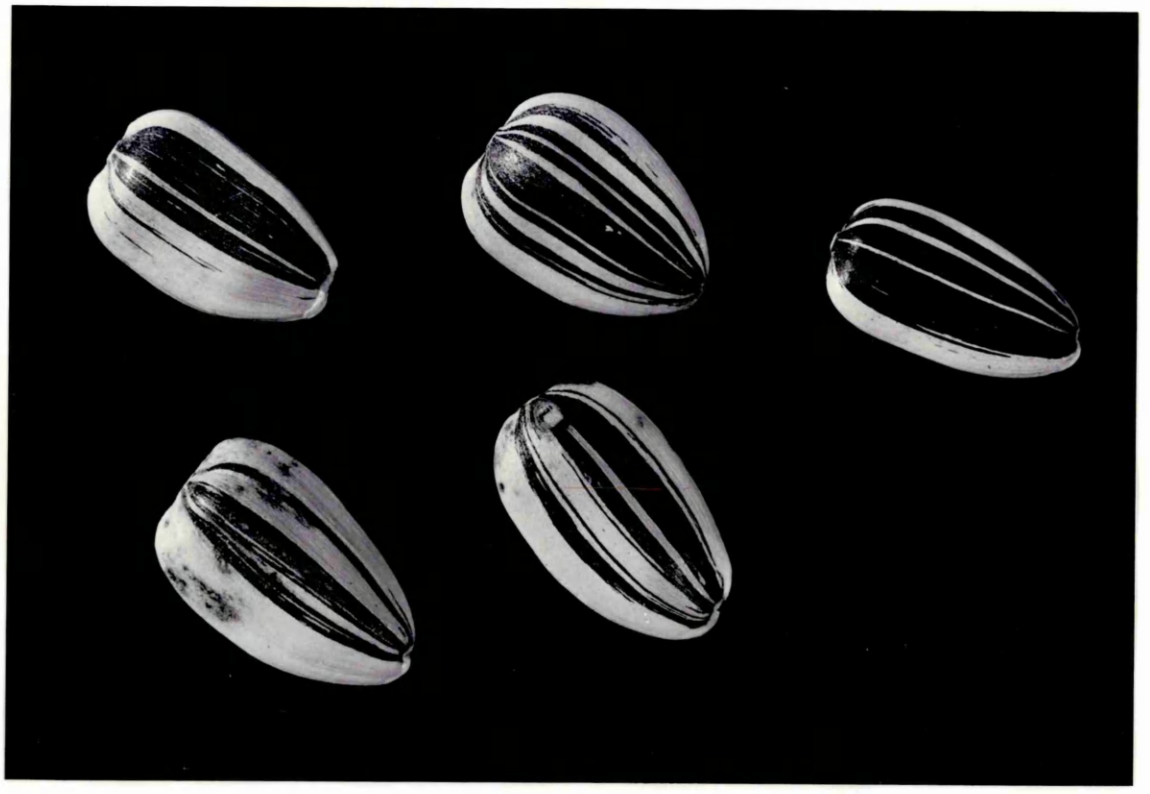
2. Chemicals and gases

Chemical	Source
BSA (Bovine Serum Albumin) 98-99%, essential fatty acid globulin free	Sigma Chemical Co., Ltd., London, U.K.
Diazald (n-methyl-n-nitroso-P-toulene sulfonamide)	Sigma
Diolein (Glyceroldioleate)	Sigma
DTT (Dithiothreitol, Cleland's reagent; DL-DTT)	Sigma
EDTA (Ethylenediaminetetraacetate)	Sigma
FAME standard (Fatty acid methyl ester)	
(i) SCFAME (Short-chain fatty acid methyl ester; C_6-C_{12})	Sigma

Plate 1 Selected seed characteristics of *Helianthus annuus* and *Gossypium barbadense*.

Species	Dry wt. (mg seed^{-1})	Length (mm)	Colour
Helianthus annuus	86.40 ± 10	12.11 ± 0.4	black to white through different shades of brown, and longitudinal pale (white or cream) striping
Gossypium barbadense	96.80 ± 11	10.11 ± 0.3	black seed coat covered by short fibres

A



B

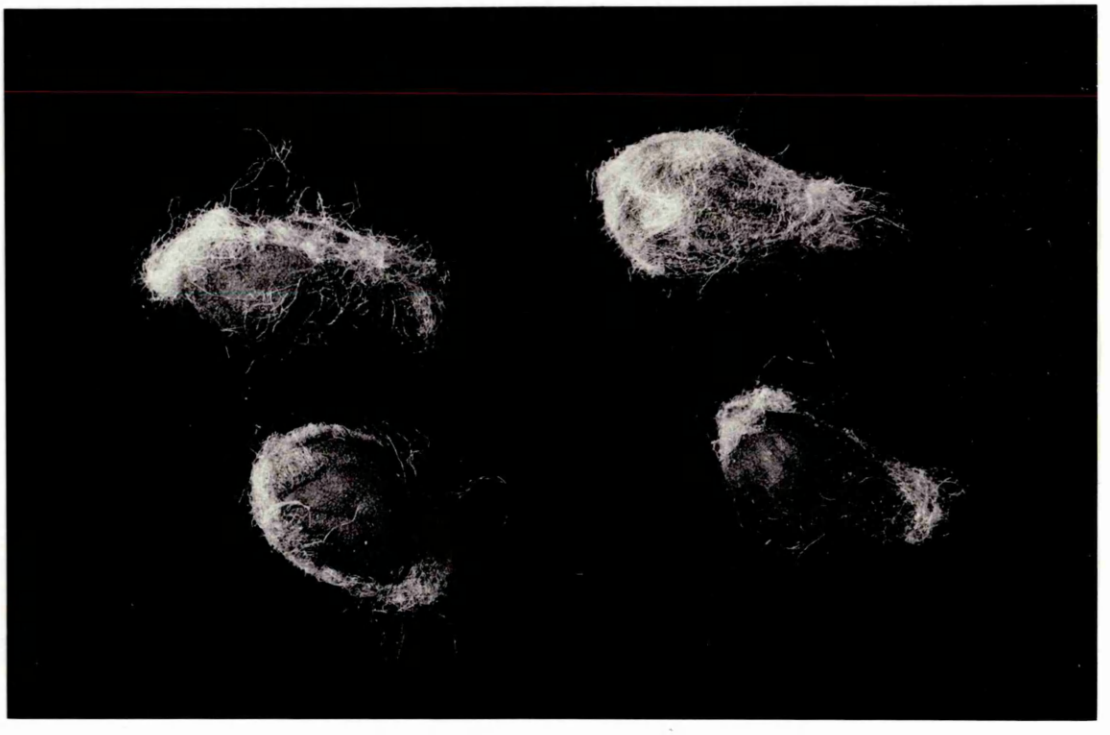
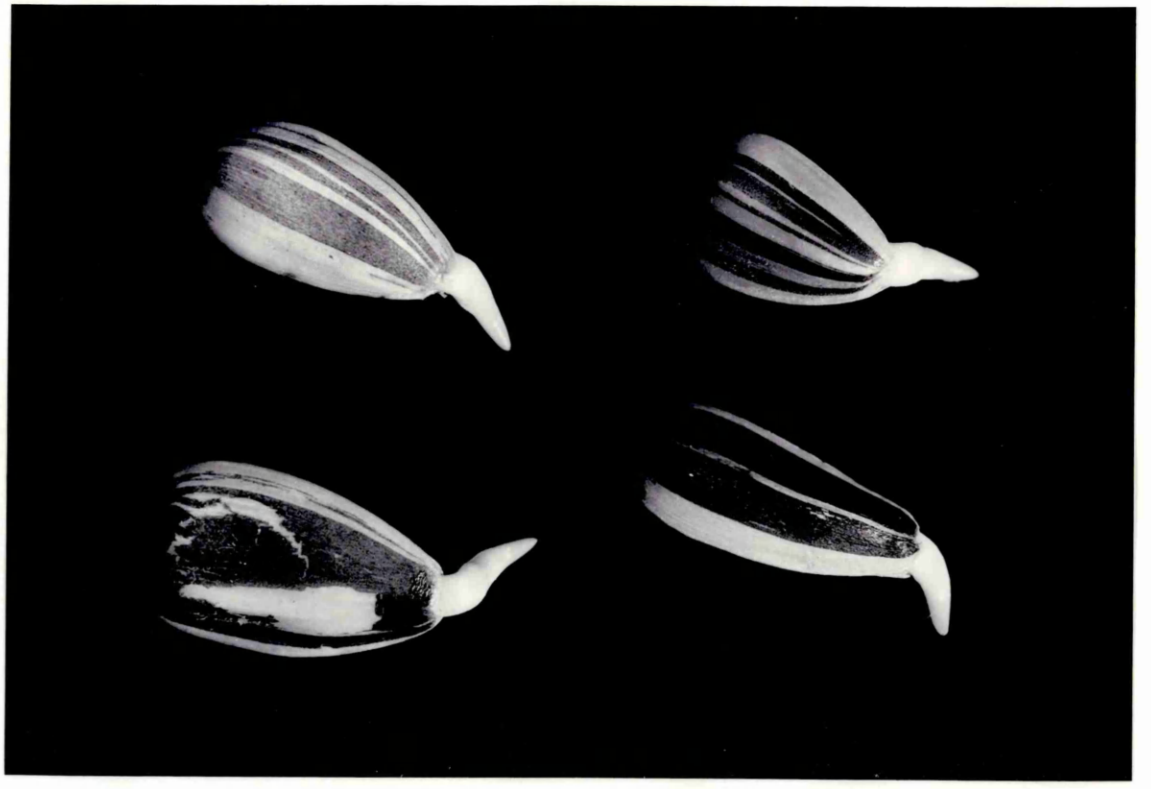


Plate 1.1 Dry seeds, X 3.1

A: Sunflower (Helianthus annuus)

B: Cotton (Gossypium barbadense)

A



B

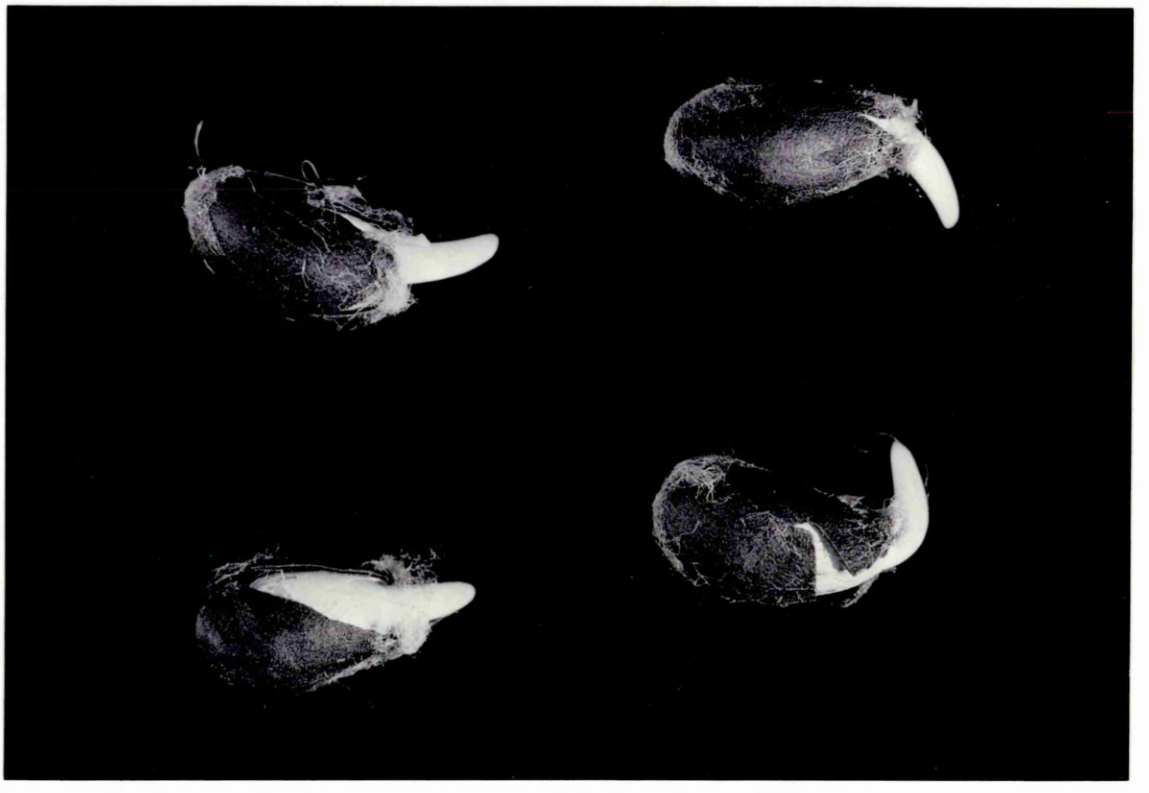
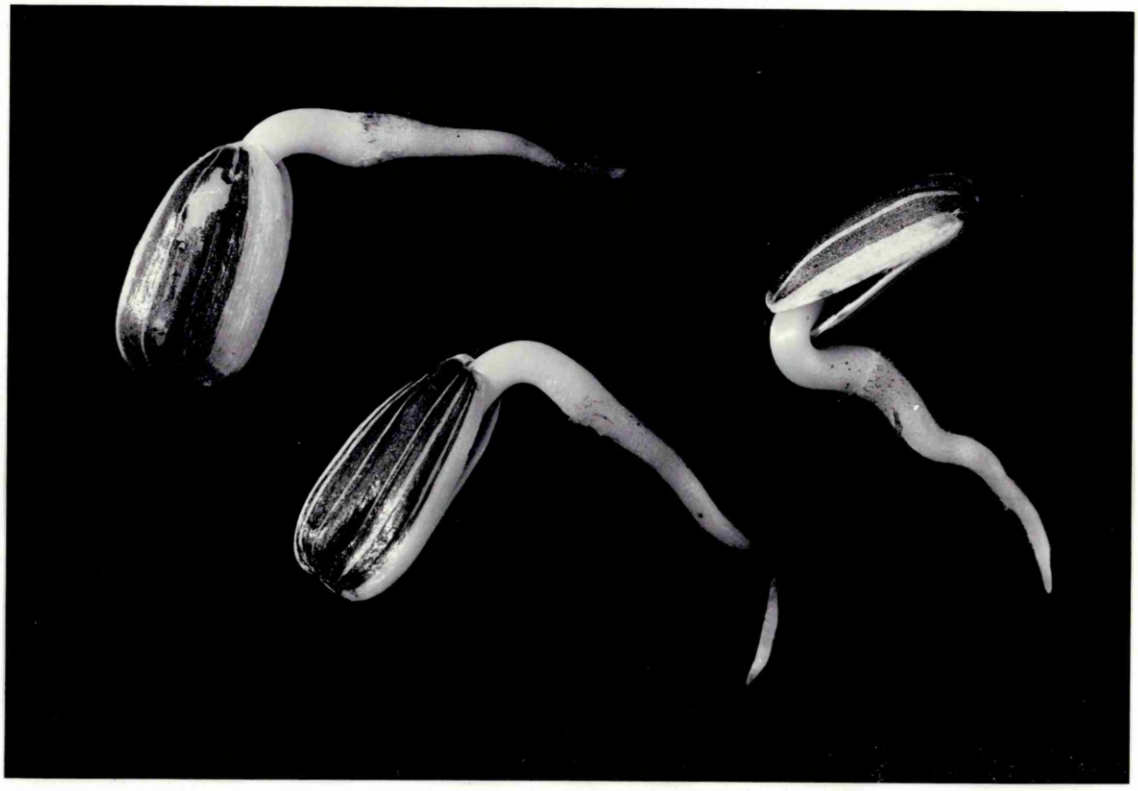


Plate 1.2 1 d germinating seeds, X 3.1

A: Sunflower (Helianthus annuus)

B: Cotton (Gossypium barbadense)

A



B

Plate 1.3 3d germinating seeds, X 3.1

A: Sunflower (Helianthus annuus)

B: Cotton (Gossypium barbadense)

(ii) LCFAME (Long-chain fatty acid methyl ester)	
- C_{14}, C_{16}, $C_{16:1}$, C_{18}, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$	Sigma
- C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and C_{20}	Greyhound, Chromatography and Allied Chemicals, U.K.
NADH (β-Nicotinamide adenine dinucleotide, reduced form)	Sigma
L-α-Phosphatidylcholine (from fresh egg yolk)	Sigma
L-α-Phosphatidylethanolamine (from fresh egg yolk)	Sigma
Silicic acid (230-400 mesh ASTM)	E. Merck, Darmstadt, FRG.
Succinic acid	Sigma
2,3,5-triphenyl-tetrazolium chloride	B.D.H. Chemicals Ltd., Poole, England, U.K.
Tris-HCl buffer	Sigma
Triolein (Glyceroltrioleate)	Sigma

Gases

Air, Argon, Helium, Hydrogen and Nitrogen (oxygen free)	British Oxygen Company (B.O.C.), Glasgow, U.K.

All other chemicals used in this work were either analytical grade or redistilled before use, and supplied by (i) B.D.H. Chemicals Ltd., Poole, England, U.K., (ii) Steetley Chemicals Limited, Berk House, Hampshire, U.K., (iii) Hopkins and Williams Ltd., Chadwell Heath, U.K., (iv) May and Baker Ltd., Dagenham, U.K. and (v) Sigma Chemical Co. Ltd., London, U.K.

3. Methods

3.1 Germination tests

Prior to carrying out germination tests, viability was estimated using triphenyl tetrazolium chloride.

3.1.1 Viability

The viability of sunflower and cotton seeds was determined by the tetrazolium test employing 1% aqueous solution of 2',3',5'-triphenyl tetrazolium choride at pH 6.4 (Wikander, 1977). In this test, the colourless tetrazolium is reduced in living cells by the action of dehydrogenase enzymes to form a red water-insoluble formazan product. Lakon (1942, 1949) and Porter et al. (1947) have shown that the coloration of the seed embryo by tetrazolium is an indication of its viability.

The seeds to be tested were soaked in H_2O for 24 h and each seed was bisected longitudinally through the embryo so that each half had a part of the cotyledons and the radicle. One half of each seed (100 seeds; 3 replicates) was then placed in a petri dish and the solution of tetrazolium chloride was poured over the half seeds until they were completely immersed. The petri dishes were kept for 12 h in a dark cabinet at 25°C. At the end of this immersion period each half seed was examined to determine the degree to which the parts of the embryo were stained carmine-red. The staining of the entire embryo was taken as the criterion of viability. The percentage viability was calculated from:

$$\% \text{ viability} = \frac{S}{\Sigma (S+U+E)} \times 100$$

Where S is the number of embryos completely stained, U is

the number of embryos unstained or partially stained, and E is the number of abnormal seeds.

3.1.2 Standard germination

Seeds of Helianthus and Gossypium were placed in 9 cm diameter plastic petri dishes on one sheet of seed test paper (Whatman Grade 181, size 9 cm). Twenty seeds were placed in each dish and soaked with 5 cm^3 of distilled water (page 99) Seeds of Helianthus were germinated for 72 h in darkness at 25 ± 1°C (page101) and Gossypium were germinated for 72 h in darkness at 30 ±1°C (page101) in a thermostatically controlled incubator. The seeds of both species were dissected every 8 h up to 72 h, from the start of each experiment, into cotyledons, radicle and testa and pericarp. The seed tissues were dried to constant weight in an oven and ground in a coffee blender (Moulinex type 228, France).

3.2 Lipid content

3.2.1 Extraction of total lipids

The total lipids, including the whole conjugated fatty compounds were extracted by chloroform-methanol (2:1 by volume) and "Folch" wash as described by Christie (1982) for the procedure of Folch et al. (1957). This method yields approximately 95-99% recovery of lipids.

Ground seed tissue (1g) was homogenised in a Ten Bruek (France) hand homogeniser for 3 min with 30 cm^3 of extraction solvent. The mixture was filtered and the solid residue resuspended in 30 cm^3 solvent and homogenised for 3 min. The combined filtrates were transferred to a measuring cylinder and one quarter of the total volume of the filtrate of

0.88% potassium chloride in water was added, the mixture was transferred to a separating funnel, shaken and allowed to settle. The upper layer was discarded and to the lower layer one quarter of its volume of water-methanol (1:1,v/v) was added, and separated again. The bottom layer now contained the partially purified lipid. The solvent was removed under vacuum on a rotary evaporator at 30°C and under nitrogen again, and the total lipid content was determined. The lipid was stored in a small volume of chloroform at -20°C until required for further analysis.

3.2.2 Extraction of neutral lipids

Ground seed tissue (10 g) was placed in the thimble of a soxhlet apparatus and extracted with petroleum ether (b.p. 40-60°C) for 4 h according to the A.O.C.S. official method (1973). The meal was re-extracted twice for 4 h each, the extracts were transferred to a preweighed flask, and the solvent was evaporated using a rotary evaporator at 30°C. The neutral lipid content was expressed as percentage of the total initial weight of seed tissue.

3.2.3 Isolation of lipid classes

The lipid classes were isolated by column chromatography on silicic acid using a method based on that described by Rouser et al. (1967). Silicic acid (5 g), 230-400 mesh ASTM (E. Merck, Darmstadt) was slurried with 15 cm^3 chloroform. This slurry was poured into a glass column, 20 x 250 mm, with glass wool and a disc of filter paper on the sintered disc at the base of the column. Before use, the adsorbent was washed several times using chloroform and allowed to settle,

and the column was tested with standard mixtures of glycerides and phospholipids. Total lipid (50-100 mg) obtained from seed tissues and membranes was dissolved in 5-10 cm^3 chloroform and applied to the silicic acid column. The netural lipids were recovered by elution with 150 cm^3 chloroform, glycolipids with 200 cm^3 acetone and phospholipids with 150 cm^3 of methanol. These fractions were collected in preweighed flasks and solvents were evaporated in a rotary evaporator at 30°C and distilled off completely under nitrogen at room temperature. Each fraction was expressed as a weight percentage of the total lipid applied to the column of silicic acid.

3.2.4 Purification of lipid classes

The lipid fractions were separated by thin-layer chromatography (TLC) as described by Luddy et al. (1968).

Neutral lipids and phospholipids were purified on TLC prior to analyses by GC-FID or GC-MS, using the solvent systems petroleum ether-diethyl ether-acetic acid (85:15:1 by volume) and chloroform-methanol-water (65:25:4 by volume) respectively.

Glass TLC plates (Anachem Ltd., 15 Power Court, Luton, Beds. LU1 5JJ) precoated with silica gel (0.25 mm) Merck HF were pre-washed in chloroform. Samples were spotted on the plate 2 cm from the base, and the plate was then placed in a tank containing the eluting solvent and the chromatogram was developed for 18 cm. The plate was dried and sprayed with iodine to render the lipid visible. Zones containing purified lipid fractions were scraped into a vial containing chloroform for further analysis.

NL (diolein and triolein) and PL (PC and PE) were used as standards on the side of each plate which were developed under nitrogen.

3.3 Saponification of lipids

The alkaline hydrolysis (saponification) of fats produces water-soluble alkali salts of the constituent fatty acids of the glycerides of these lipids and non-saponifiable neutral compounds. The procedure according to Meara (1955) was adopted and modified as follows. Lipid material was placed in a 5 cm^3 vial and 1 N alcoholic potassium hydroxide solution (prepared by dissolving potassium hydroxide in absolute methanol) added (1:10;lipid material:alcoholic potassium hydroxide;w/v). The lipid was saponified by refluxing for 6-8 h at 60°C, and the contents of the vial transferred to a centrifuge tube (40 cm^3), and 5 cm^3 distilled water and 30 cm^3 diethyl ether were added. The tube was centrifuged at 1000 g for 10 min using MSE bench centrifuge until the two layers separated. The ethereal layer from the top layer (non-saponifiable material, NSM) contained sterols. The aqueous alcoholic soap solution (saponifiable material, SM) from the lower layer was acidulated by concentrated hydrochloric acid, and the solution was transferred into a centrifuge tube, centrifuged at 1000 g and extracted three times with 20 cm^3 diethyl ether each time. The combined ether extracts were dried over anhydrous sodium sulphate. Finally, the ether was evaporated using rotary evaporator at 30°C, and the fatty acids stored in a small volume of chloroform at -20°C until required for further analysis.

3.4 Extraction of short-chain fatty acids (SCFA)

The short-chain fatty acids C_6-C_{12} from sunflower and cotton seed tissues were extracted by co-distilling steam

method as described by A.M.M. Berrie (Botany Department, University of Glasgow, personal communication).

Acidulated water (150 cm^3) was added to 5 g dried seed tissues (3 replicates) in a round-bottomed flask using concentrated phosphoric acid to adjust the pH to 2-3 (Fig. 3). About 120-130 cm^3 distillate was collected from the boiler; the acids distilling along with the steam. Phosphoric acid was added to give the distillate a pH of about 2.5. After partition three times against dichloromethane in a separating funnel (about 75 cm^3 of solvent each time), the combined fractions were evaporated to approximately 2 cm^3 in a rotary evaporator at less than 35°C, and transferred to a glass vial. The extracts were stored in a small volume of dichloromethane at -20°C for further analysis by GC and GC-MS.

3.5 Methylation of fatty acids

Before separating the fatty acids of a lipid by GC and GC-MS, it is necessary to prepare the comparatively volatile methyl-ester derivative of the fatty acids. Diazomethane reacts rapidly with fatty acids forming methyl esters. Methyl derivatives of extracts were prepared using ethereal diazomethane as described by Schlenk and Gellerman (1960). The base (approx 1 cm) of a 50 ml reactant flask (Fig. 4) was covered with about 2 g diazald (n-methyl-n-nitroso-p-toulene sulfonamide), 24 cm^3 of diethyl-ether was added followed by 2-3 cm^3 of methoxy-ethanol and 2-3 cm^3 of concentrated potassium hydroxide (40%). The reaction vessel was immersed in warm water, keeping the recovery arm in a flask of chilled ether. When the contents of the recovery flask became yellow

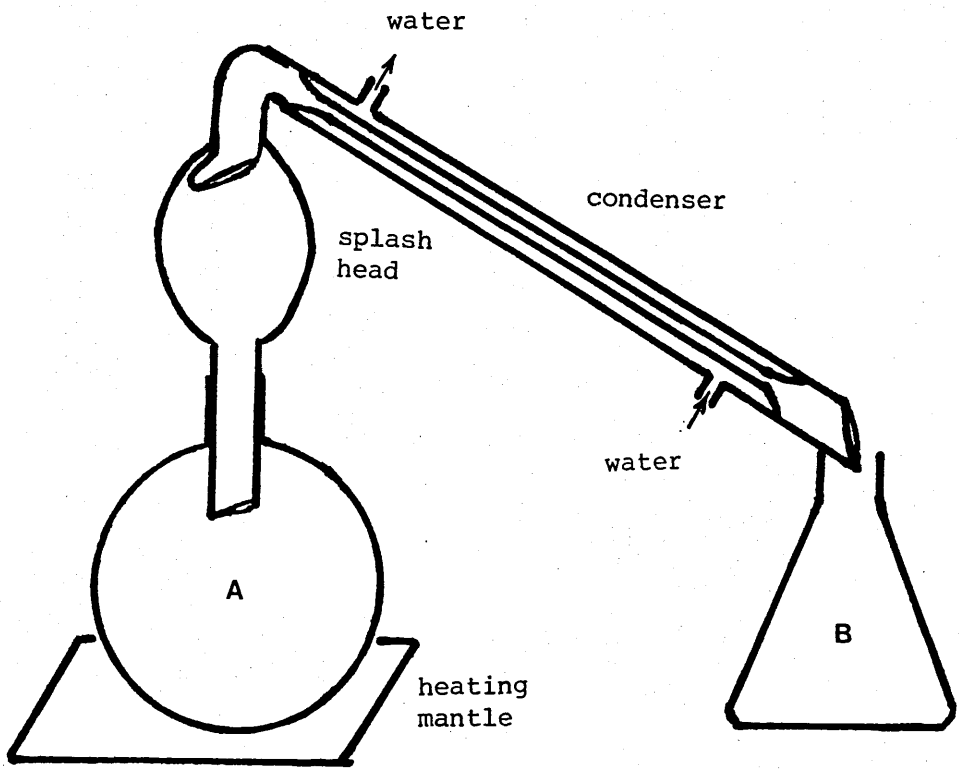


Figure 3 Apparatus used for the extraction of SCFA.

A: 5g dried seed + phosphoric acid (pH 2-3) + 150 cm^3 water

B: distillate (120 - 130 cm^3)

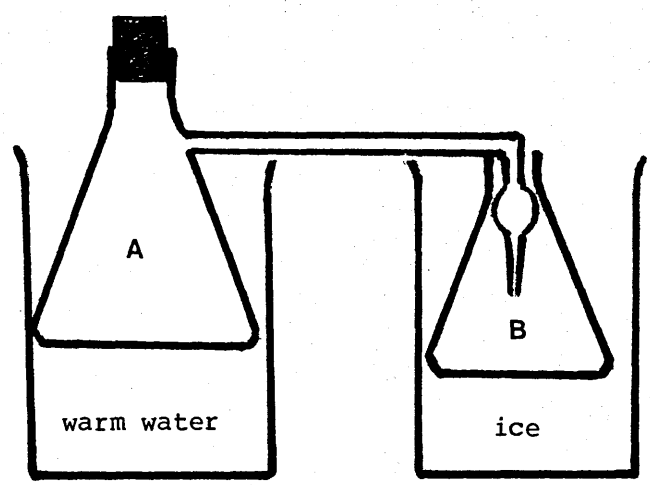


Figure 4 Apparatus used for the preparation of diazomethane

A: reactant flask covered with 2g diazald + ether + 2 cm^3 methoxy-ethanol + 2 cm^3 KOH (40%)

B: recovery flask covered with about 25 cm^3 diethyl ether

it was assumed that diazomethane was codistilling with ether in the reaction flask and the reaction was considered complete when no further distillate was obtained. Samples were methylated by the addition of excess ethereal diazomethane. The vials were stoppered and allowed to stand for 1 - 2 h. If the yellow colour of the solution remained, it was assumed that methylation was complete and no more diazomethane was added, otherwise more was added and then after methylation, solvent was removed under nitrogen at room temperature.

Fatty acids methyl esters were quantified by GLC-FID using heptadecanoic (C_{17}) and hendecanoic (C_{11}) acids methyl esters as internal standards for LCFA and SCFA analyses respectively. Internal standards were used in representative samples.

3.6 Operating parameters used for the analysis of long-chain fatty acid methyl esters (LCFAME) and short-chain fatty acid methyl esters (SCFAME)

Instrument	Column	Temp. programme	Carrier gas	Recorder
GC-FID Gas chromatograph, Model Pye 104 with hydrogen flame ionization detector (W.G. Pye and Co. Ltd., Cambridge, England)	9 ft x 4 inch SP 2330 packed column (Supelcoport)	Initial temp 130°C no hold 5°C min^{-1} to final temp 250°C. Injector 250°C.	H_2-Head pressure [14 p.s.i.] Flow rate of 25-30 Cm^3s^{-1} FID 1/16. Air supply to FID 24 Pound per square inch (p.s.i.)	1 mV full-scale deflection (F.S.D.), chart speed 5 mm min^{-1}
Gas chromatograph, Model F17 (Perkin Elmer) with flame ionization detector (FID).	BP 15 capillary column (equivalent to OV 225) 25 m in length, bonded phase with vitreous silica (SGE)	Initial temp 150°C no hold 5°C min^{-1} to final temp 250°C for LCFAME. Initial temp 65°C no hold 5°C min^{-1} to final temp 230°C for SCFAME.	H_2-Head pressure [18 p.s.i.] Flow rate of 35-40 Cm^3s^{-1} FID 1/16. Air supply to FID 32 p.s.i.	
	OV 351 capillary column 25 m in length and 0.31 mm in diameter (Phase Sep)	Initial temp 110°C no hold 5°C min^{-1} to final temp 230°C for LCFAME and initial temp 55°C 2 min hold 5°C min^{-1} to final temp 230°C for SCFAME.	SCFA;H_2[18 p.s.i.], Flow rate 40 Cm^3s^{-1}. FID 1/16. Air supply to FID 32 p.s.i. LCFA;H_2[14 p.s.i.] Flow rate 45-50 Cm^3s^{-1}. FID 1/32. Air supply 24 p.s.i.	
	BP20 capillary column 25 m in length, bonded phase with vitreous silica (SGE)	Initial temp 130°C no hold 4°C min^{-1} to final temp 220°C Injector 250°C.	H_2[18 p.s.i.] Flow rate 40 Cm^3s^{-1} FID 1/16. Air supply to FID 24 p.s.i. Split approx 1:60.	

Instrument	Column	Temp. programme	Carrier gas	Recorder
GC-MS AEI (Kratos) MS-30 (Kratos, Urmston, Manchester, U.K.) mass spectrometer interfaced via a single jet separator with a Perkin Elmer Sigma-3 gas chromatograph (Perkin Elmer)	BP1 capillary column 12 m in length, bonded phase (SGE)	Initial temp 65°C no hold 10°C min^{-1} to final temp 220°C.	He as carrier gas Flow rate 40 $Cm^{-3}s^{-1}$	
	BP15 capillary column 25 m (SGE)	Initial temp 155°C no hold 5°C min^{-1} to final temp 250°C	He as carrier gas Flow rate 30$Cm^{-3}s^{-1}$	
	OV351 capillary column 25 m (Phase Sep)	Initial temp 120°C, 2 min hold 5°C min^{-1} to final temp 250°C	Flow rate 30 $Cm^{-3}s^{-1}$	
	3 ft x ¼ inch SP 2330 packed column (Supelcoport)	Initial temp 140°C, 4 min hold 4°C min^{-1} to final temp 200°C	Flow rate 30 $Cm^{-3}s^{-1}$	

3.7 Isolation of membranes

3.7.1 Isolation of spherosomes and mitochondria

Cotyledons were removed from the dry seeds and seedlings of both Helianthus and Gossypium after 1, 2 and 3 d germination. The cotyledons were chopped to small pieces and ground gently in a mortar for 1 - 2 min using liquid nitrogen and homogenisation medium. Spherosomes were isolated using a method similar to that described by Jacks et al. (1967) except that the homogenisation medium consisted of 0.25 M sucrose, 50 mM Tris-hydrochloric acid buffer pH 7.4, 2 mM ethylenediaminetetraacetate (EDTA), 1 mM diothiothreitol (DTT), 10 mM potassium chloride (KCl), 1.1 mM magnesium chloride ($MgCl_2$) and 1 g bovine serum albumin (BSA). After filtration through four layers of muslin, the homogenate was centrifuged at 1000 g for 5 min at 5°C using High speed 18 MSE (Measuring and Scientific Equipment Ltd., Manor Royal, Crawley, Sussex, England) centrifuge. The supernatant was transferred to another centrifuge tube and centrifuged at 15000 g at 5°C for 20 min to produce a creamy band (fat pad or spherosomes) on the supernatant liquid and pellet (Fig. 5). The fat pad was removed with a spatula and the supernatant liquid was centrifuged again at 15000 g at 5°C for 20 min. The resultant pellets were combined with the previous pellets, resuspended in 10 cm^3 of homogenisation medium and was designated "the mitochondrial fraction". The washed spherosomes (fat pad) were resuspended in 10 cm^3 of the homogenisation medium. Spherosomal fractions prepared for chemical analysis were washed with distilled water instead of the homogenisation medium.

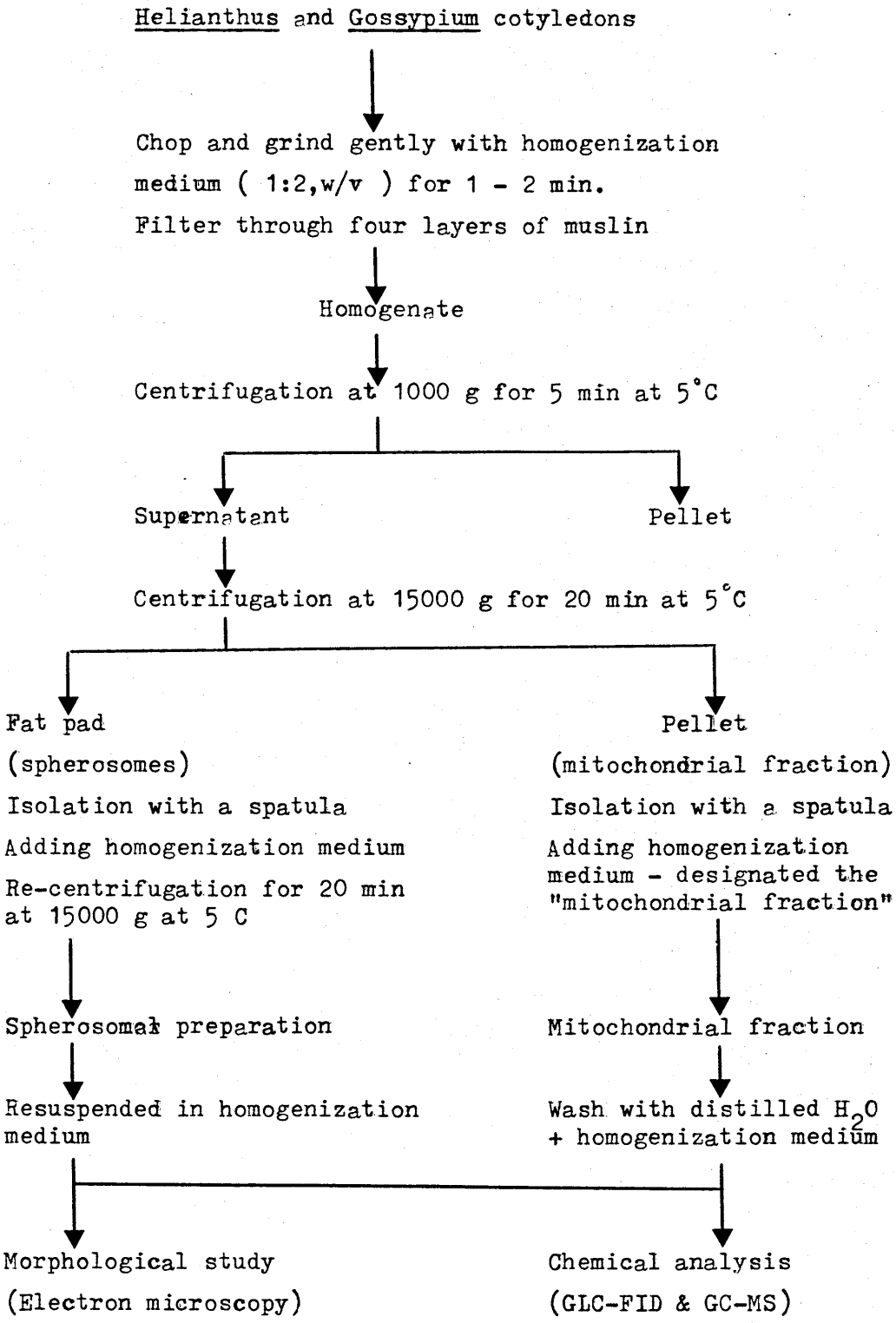


Figure 5. Isolation of spherosomes and mitochondria

3.7.2 Preparation of membrane fractions

The techniques used to obtain endoplasmic reticulum and mitochondrial fractions from the radicle of sunflower and cotton seedlings were based on a modification of the method of Baydoun (1980) from the method of Bowles and Kauss (1976). Fresh radicles (10 g) were excised and mixed using scissors, washed twice in the homogenisation medium (8% sucrose by weight, 50 mM Tris-HCl buffer pH 7.4, 1 mM EDTA, 1 mM DTT, 0.1 mM $MgCl_2$, 10 mM K Cl and 1 g BSA), suspended in 10 cm^3 of homogenisation medium, and ground gently in an ice-chilled mortar for 2 - 3 min using a squashing action. The suspension was filtered through three layers of muslin, and the filtrate centrifuged at 800 g for 10 min in an MSE centrifuge (Model High Speed 18). The supernatant was made up to 13 cm^3 by addition of homogenisation medium and immediately layered on a discontinuous sucrose density gradient. This was prepared in a 38 cm^3 cellulose nitrate tube by layering in succession 5 cm^3 of 50, 40, 30, 20 and 10% (w/w) sucrose solutions using pasteur pipettes. The tube was centrifuged at 100000 g for 4 h using Sorvall centrifuge (Du Pont Company Instrument Products, Biochemical Division, Newtown, Connecticut) at 4°C. The particulate material at each sucrose interface was carefully collected.

The procedure for homogenisation and fractionation is summarised in Fig. 6.

3.7.3 Marker enzyme assays

3.7.3.1 NADH-cytochrome C reductase

The assay mixture contained 200 µl of 0.2 M sodium

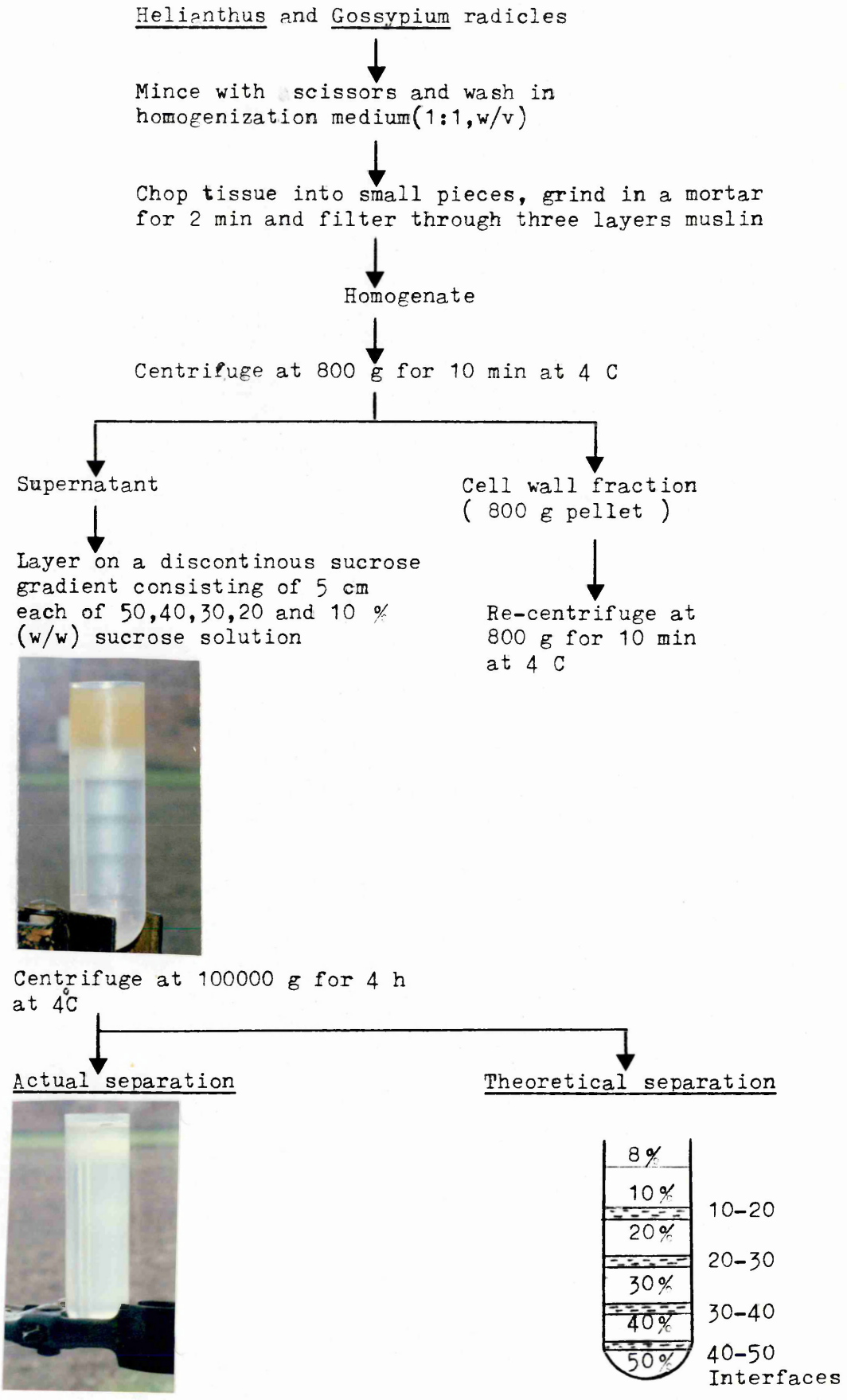


Figure 6. Preparation of membrane fractions

phosphate buffer, pH 7.4, 100 µl of 5 mg ml^{-1} cytochrome C, 10 µl of 10 mM potassium cyanide, 275 µl of water and 20 µl of membrane fraction, as described by Bowles and Kauss (1976). The reaction was started by the addition of 100 µl NADH (3 mg ml^{-1}) and followed by measurement of the rate of increase in absorbance at 550 nm at 25°C using a spectrophotometer (SP8 500 UV, Scientific and Industrial Equipment Division, Philips). Enzymic activity was estimated from the equation:

$$OD = E \times C \times L$$

where OD is the optical density from the standard graph, E is the extinction coefficient which equals 18.5 mM^{-1} cm^{-1}, C is the rate of increase of cytochrome C concentration per min in mM units (enzyme activity) and L is the path length of light through a 1 cm cuvette.

3.7.3.2 Succinate dehydrogenase

The assay mixture contained 2.4 cm^3 of 0.1 M sodium phosphate buffer, pH 7.5, 1.7 mM potassium cyanide, 0.1 cm^3 of 1% (w/v) phenanzine methyl sulphate, 0.1 cm^3 of 0.15 mM 2,6-dichlorophenol-indophenol and 0.1 cm^3 of membrane fraction according to the method of Veeger et al. (1969). The reaction was started by the addition of 0.3 cm^3 of 0.4 M sodium succinate followed by measurement of the decrease in absorbance at 600 nm at 25°C. Enzymic activity was calculated using an extinction coefficient for 2,6-dichlorophenol-indophenol of 21 $mM^{-1}cm^{-1}$.

3.7.4 Electron microscopy

3.7.4.1 Negative staining

The isolated membrane fractions were examined by negative

staining. A drop of each membrane fraction was placed on a formvar/carbon-coated 300-mesh grid. The liquid removed with the edge of a piece of filter paper. A drop of methylamine tungstate (1% v/v) was added and allowed to stain for 30 s. This was then removed with filter paper. Examination of the grids was carried out using a Philips EM 301 microscope (Eindhoven, Netherlands).

3.7.4.2 Thin-sectioning

Clotted spherosomes (approx. 1 cm^3) were fixed in 3% gluteraldehyde in 0.2 M sodium cacodylate buffer for 12 - 16 h, then rinsed in 0.2 M cacodylate buffer (three changes over a period of three hours). Half the samples were post-fixed in 1% osmium tetroxide in 0.2 M cacodylate buffer. Both sets of samples were block-stained in 2% aqueous uranyl acetate for two hours, dehydrated in an acetone series and embedded in Spurr resin for 24 h at 60°C. Sections were cut on an LKB Ultratome III, picked up on 300-mesh copper grids, stained in lead citrate (Reynolds, 1963) for 2 min, and viewed in the electron microscope.

3.8 Statistical analysis

The statistical analyses used during the present investigation included standard error of the mean for each series of experiments and was calculated using the formula:

$$SE = \pm \sqrt{\frac{SD}{n}}$$

$$SD = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n - 1}}$$

where:

SE = standard error

SD = standard deviation

n = number of values (replicates)

x = individual value of observation

Each experiment was repeated 3 - 5 times. The standard errors of means were represented graphically as a vertical bar from the point of the mean value, and the bar denotes one SE value.

RESULTS AND DISCUSSION

1. Germination behaviour

Helianthus and *Gossypium* seeds were tested under several conditions in order to establish the optimum conditions for germination.

1.1 Viability

The results of germination tests must be assessed taking into account seed viability. Table 14 shows the results of an experiment in which the viability of *Helianthus* and *Gossypium* seeds was assayed by the tetrazolium test. The viability of *Helianthus* seeds was calculated to be 93 - 91% (1982 - 1985) for the same batch. The viability of *Gossypium* seeds was calculated to be 85% for the first batch (1982), and 83% for the second and third batches (1984) from the same variety (Giza 66). According to these results it is reasonable to expect that experiments on *Helianthus* and *Gossypium* will give germination values closely approaching those of the viability values. These results demonstrate that viability as assessed by the tetrazolium test is retained to a large extent under the storage conditions of low temperature (5°C) and darkness. Berrie (1984) mentioned that viability is lost with time and the rate of viability loss depends on the temperature and humidity of storage. The tetrazolium assay does not distinguish between non-dormant and dormant seeds - the ultimate criterion for non-dormant, viable seeds is germination itself.

Table 14. Determination of viability of Gossypium and Helianthus seeds treated with 1% tetrazolium chloride.

Species	% Viability
Gossypium barbadense	
1982 batch	85
1983 batch	83
1984 batch	83
Helianthus annuus	
(Single batch)	
1982	93
1983	92
1984	91
1985	91

1.2 Water effect

During germination the first event is the uptake of water by the seed. The effect of water on germination percentages was tested by germinating batches each of 20 seeds of Helianthus and Gossypium in 1,2,3,4,5,6,7 or 8 cm^3 distilled water in 9 cm plastic Petri dishes using a single sheet of seed test paper (5 replicates for each volume). The germination of the seeds was at 25°C in darkness for 7 days. Counts were made daily until no change in radicle emergence was observed.

The results (Table 15) showed that the germination percentage was zero at 1 cm^3 water for both Helianthus and Gossypium. Increasing the volume of water to 3 cm^3 led to increases in sunflower seed germination up to 72% and up to 31% in cotton seeds. Germination increased with increasing water volume, reaching 92% in sunflower and 82% in cotton for 5 cm^3 water. Increasing the volume of water to 8 cm^3 was associated with a slight decrease in germination in both species. The larger volumes of water may reduce germination for several reasons such as 'water injury' which could lead to the formation of a mucilage coat. Heydecker and Cheterman (1971) reported that increasing the volume of water in the Petri dish is related to an increase in the bacterial population around the seeds.

1.3 Temperature effect

Different seeds have different temperature ranges within which they germinate. Helianthus and Gossypium seeds were germinated in 5 cm^3 distilled water in 9 cm Petri dishes using

Table 15. Percentage germination of *Gossypium* and *Helianthus* at 25°C in darkness in different quantities of water.

Time (days)	*Gossypium barbadense* Water (cm^3)								*Helianthus annuus* Water (cm^3)							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
1	0	0	5	10	55	57	59	53	0	0	30	45	56	56	55	51
2	0	0	14	55	76	77	74	68	0	5	65	68	66	68	69	61
3	0	0	22	63	82	80	74	68	0	6	72	81	92	86	83	62
4	0	5	31	63	82	80	74	68	0	6	72	83	92	90	84	62
5	0	5	31	63	82	80	74	68	0	6	72	83	92	90	85	63
6	0	5	31	63	82	80	74	68	0	6	72	83	92	90	85	63
7	0	5	31	63	82	80	74	68	0	6	72	83	92	90	85	63

a single sheet of seed test paper (Whatman Grade 181) and kept in the dark at different constant temperatures of 10,20, 25,30,35,40 and 50°C with 5 replicates each of 20 seeds at each temperature. Germination was recorded up to 7 d until no change in the germination was observed for all temperatures. Visible emergence of the radicle was taken as the criterion for germination.

The results presented in Table 16 showed that germination of Helianthus seeds was 84% at 20°C but increased, producing a maximum value of 92% at 25°C, and the germination percentage decreased to 10% at 50°C. The germination of Gossypium seeds was 73% at 20°C, increased producing a maximum value of 82% at 30°C, and the germination percentage decreased to 54% at 50°C. These results agree with El-Abyed and Saleh (1971) and Abdel Magid and Osman (1977) who have shown that 30°C was favourable for the germination of Egyptian cottonseeds. Bhatti (1974) found that 27°C was the optimum temperature for the germination of cottonseeds, but Goldsworthy and Fisher (1984) recently showed that the optimum temperature is 34°C for the germination of G. hirsutum.

These results of experiments (1.2 and 1.3) showing that the optimum condition for Helianthus germination at 25°C and at 30°C for Gossypium in darkness, each of 20 seeds and 5 cm^3 water in 9 cm plastic Petri dish using a single sheet of seed test paper.

1.4 Imbibition

The initial absorption of water by the seed is called the

Table 16. Percentage germination of Gossypium and Helianthus in darkness at different constant temperatures.

Time (days)	Gossypium barbadense							Helianthus annuus						
	Temperature (°C)													
	10	20	25	30	35	40	50	10	20	25	30	35	40	50
1	0	13	21	55	55	45	50	0	48	52	60	70	45	10
2	0	47	50	77	75	62	54	5	62	62	76	73	55	10
3	0	72	73	82	78	63	54	5	82	92	90	73	55	10
4	5	73	75	82	78	63	54	8	84	92	90	73	55	10
5	5	73	75	82	78	63	54	8	84	92	90	73	55	10
6	5	73	75	82	78	63	54	8	84	92	90	73	55	10
7	5	73	75	82	78	63	54	8	84	92	90	73	55	10

imbibition phase. Imbibition is dependent on several factors: availability of water in the medium, temperature, kind of seed, and the presence of seed coats. The time course of imbibition was followed in Helianthus and Gossypium seeds. Twenty seeds (5 replicates) of each sunflower and cotton were placed to imbibe in 9 cm diameter plastic Petri dishes with 5 cm^3 distilled water on one sheet of seed test paper. The Petri dishes were kept in the dark at 25°C (sunflower) or 30°C (cotton). Each seed was weighed dry before placing in the Petri dish and at specific times (6,12, 24,36 and 54 h) they were removed from the dish, excess surface water dried with filter paper, and reweighed to 0.5 mg accuracy and replaced in the Petri dish. The imbibition curve (Figure 7) is expressed as percentage increase in the initial fresh weight of seeds at different times using the equation:

$$\text{Percentage increase in weight} = \left(\frac{\text{weight at different times}}{\text{initial weight}} \times 100 \right) - 100$$

In both Helianthus and Gossypium seeds, most imbibition took place within the first 24 h. At the end of the imbibition period the original weight of Helianthus seeds had increased by 100% up to 36 h, Gossypium had imbibed 60% of its original weight up to 36 h. During the imbibition period (54 h) the Helianthus seed imbibed more water than Gossypium seeds. Mayer and Poljakoff-Mayber (1982) noted that lettuce seeds increased their original weight by 170% up to 48 h and sunflower seeds 54% up to 10 h.

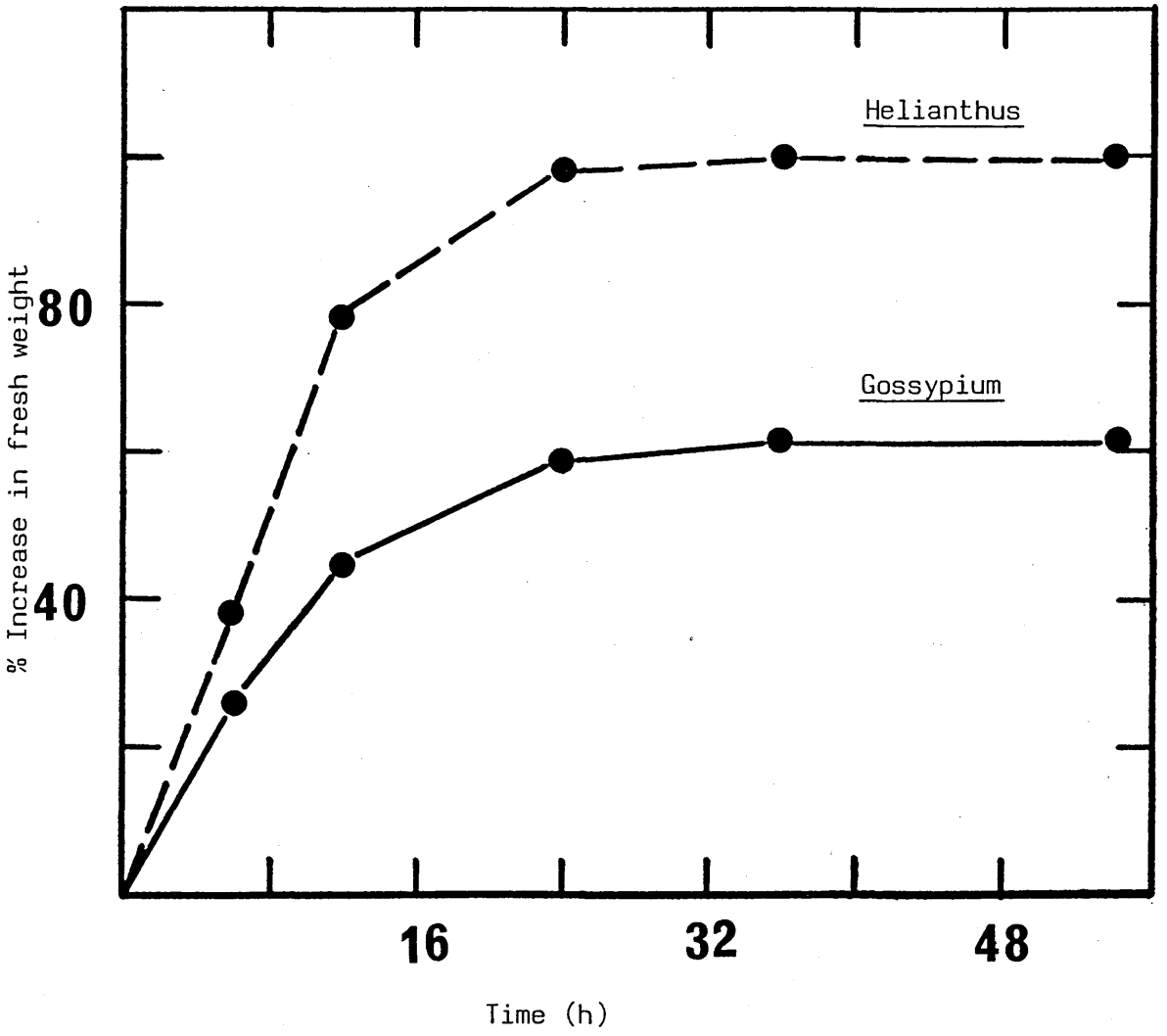


Figure 7 Time course of imbibition in Helianthus and Gossypium seeds.

1.5 Delinting effect

Germination of linted cottonseeds in Petri dishes is seemingly delayed compared with delinted seeds, an effect perhaps related to an increase in fungal and bacterial contamination around the seeds. On the other hand, the presence of a hard seed coat impermeable to water or to gases may be the cause of germination imbibition and any chemical treatments to remove short fibres may remove a waxy layer from the seed coat and thereby increase germination. The removal of short fibres and testa of Gossypium has been noted previously to increase germination percentages (Halloin, 1976). The effect of short fibres and hard seed coats on the germination of cottonseeds was examined. Cottonseeds were delinted by immersing in concentrated sulphuric acid (60%) for 2 min, then washed in diluted alkaline solution (1% potassium hydroxide), and finally washed several times in distilled water. Batches of 20 seeds were germinated in plastic Petri dishes for 7 d under favourable optimum conditions (5 cm^3 distilled water at 30°C) in darkness.

The germination percentage of delinted seeds increased during first day up to 70% than that lint seed was 55% (Figure 8). After 3 d, the germination percentage of lint (normal) seeds increased up to 82% more than those of delinted seeds (72%). Marani and Amirav (1970) found that acid delinting increases the permeability of the seed coat and acid-delinted seeds showed a higher rate of water absorption and the highest percentages of germination at a relatively low temperature (15°C). Bhatti (1974) found that sulphuric acid treatment hastened germination, but decreased the ultimate numbers of seeds germinating.

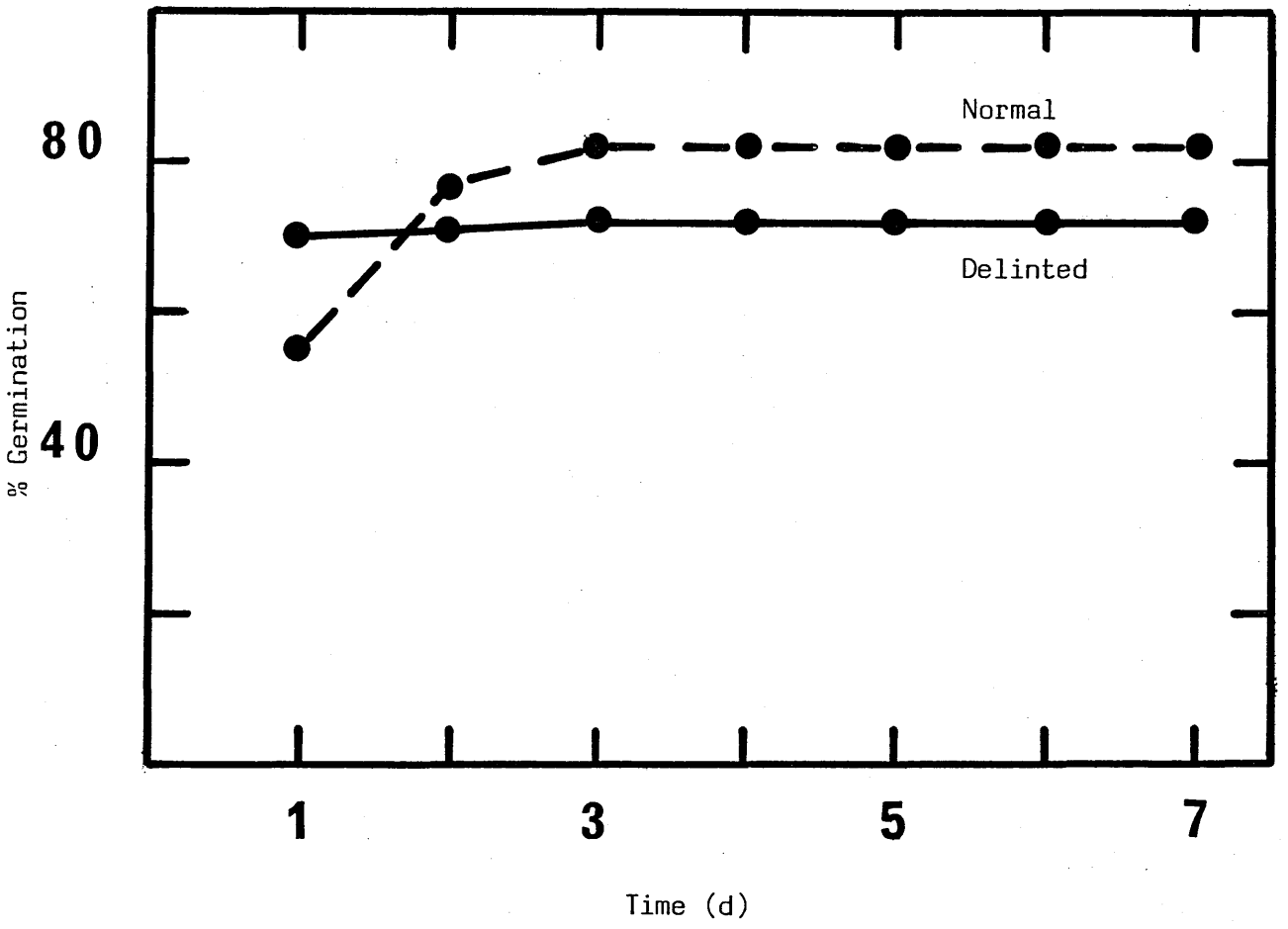


Figure 8 The effect of chemical delinting on germination of Gossypium seeds.

2. Lipid content of Helianthus and Gossypium

2.1 Total lipid content

The seeds of Helianthus were germinated at 25°C and Gossypium at 30°C for 72 h in darkness (previous section; 1.2 and 1.3). At zero time (dry seeds) and thereafter at 8 h intervals samples (whole seeds, cotyledons, radicles, pericarp and testa) were taken for the determination of total lipid (Tables 17 - 19 and Figures 9 - 11).

The total lipid content was higher in whole dry seeds of Helianthus (329 mg g^{-1} dry seed) than in Gossypium seeds (244 mg g^{-1} dry seed). Previously published results report oil contents for sunflower seeds of 40% (Astrit and Rudolf, 1971), 44% (Robertson and Windham, 1981), 38.2 - 39.4% (Beard and Geng, 1982). Campbell (1983) recognised two classes of sunflower; a high-oil class with 40% and low-oil class with 30%. If these classes are real, sunflower used in these experiments belongs to the low-oil class; on the other hand it is possible that the differences between classes may be due to sampling errors and variations resulting from specific environmental conditions experienced by the growing crop. Previously published results report oil contents for cottonseeds of 20% (Eckey, 1954), 22.7 - 26.4% in Egyptian cottonseeds - G. barbadense (Labaneiah, 1970), and 12-24% in American cottonseeds - G. hirsutum (Duffus and Slaughter, 1980). The cottonseed samples used in the experiments reported in this thesis are therefore quite typical.

Total lipid content decreased during germination of Helianthus and Gossypium seeds (Table 17 and Figure 9). In Helianthus seeds, the lipid contents decreased to 259 mg g^{-1} dry seed after 24 h and to 242 mg g^{-1} dry seed after 48 h and to 232 mg g^{-1} dry seed after 72 h. In Gossypium seeds, the lipid contents decreased to 205 mg g^{-1} dry seed after 24 h and to 189 mg g^{-1} dry seed after 48 h and to 172 mg g^{-1} dry seed after 72 h.

In a study of lipid contents in flaxseed during germination, Zimmerman and Klosterman (1965) found only a slight decrease in the oil content (neutral lipid) during the first 18 h of germination but oil content decreased by 53% (compared with total lipid in dry seeds) after 90 h germination. Bhatia et al. (1978) have shown that in germinating sunflower seeds there was a 10% to 25% decrease in lipid content in dry seeds between 1 - 5 d germination. Canella and Caetana (1982) published that total lipid content of sunflower seeds decreased from 39% (dry seeds) to 30% for up to 6 d germination.

I. Helianthus

In seed tissues of Helianthus (Table 18 and Figure 10), the total lipid content decreased by about a third in the cotyledons during germination, from 302 mg g^{-1} dry seed to 205 mg g^{-1} dry seed during the 8 h to 72 h germination period, but increased in the radicle from 10 mg g^{-1} dry seed after 8 h to 18 mg g^{-1} dry seed after 48 h and to 16 mg g^{-1} dry seed after 72 h, the total lipid content changed slightly

Table 17. Total lipid contents during germination of whole *Gossypium* and *Helianthus* seeds.

Species	Total lipid (mg g^{-1} dry seed)									
	Germination time (h)									
	Dry	8	16	24	32	40	48	56	64	72
G. barbadense	244±14	240±16	222±22	205±8	204±16	201±13	189±12	188±16	178±8	172±14
H. annuus	329±17	318±14	283±8	259±15	256±12	252±8	242±6	238±15	234±14	232±14

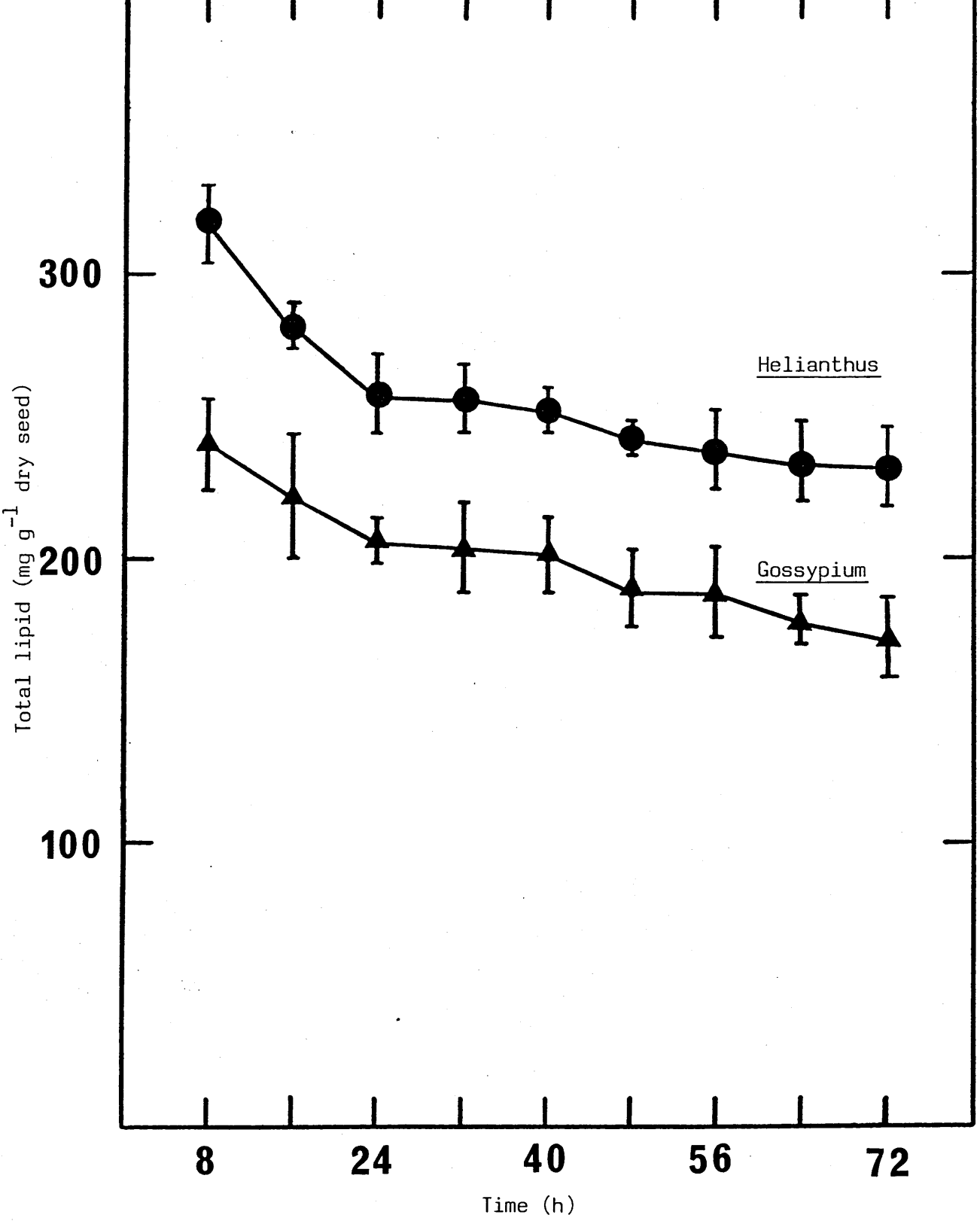


Figure 9 Total lipid contents during germination of Helianthus and Gossypium seeds.

Table 18. Total lipid contents of seed tissues during germination of Helianthus.

Values are means ± SE

Germination time (h)	Total lipid (mg g^{-1} seed)		
	Cotyledons	Radicle	Pericarp+testa
8	302 ± 7	10 ± 1	4 ± 1
16	266 ± 14	14 ± 1	4 ± 0.5
24	248 ± 8	16 ± 2	3.4 ± 0.4
32	238 ± 7	16 ± 2	4 ± 0.3
40	237 ± 9	13 ± 2	2 ± 0.3
48	224 ± 4	18 ± 1	1.3 ± 0.2
56	211 ± 3	16 ± 3	2.4 ± 0.5
64	210 ± 8	18 ± 3	2 ± 0.4
72	205 ± 5	16 ± 2	3 ± 0.5

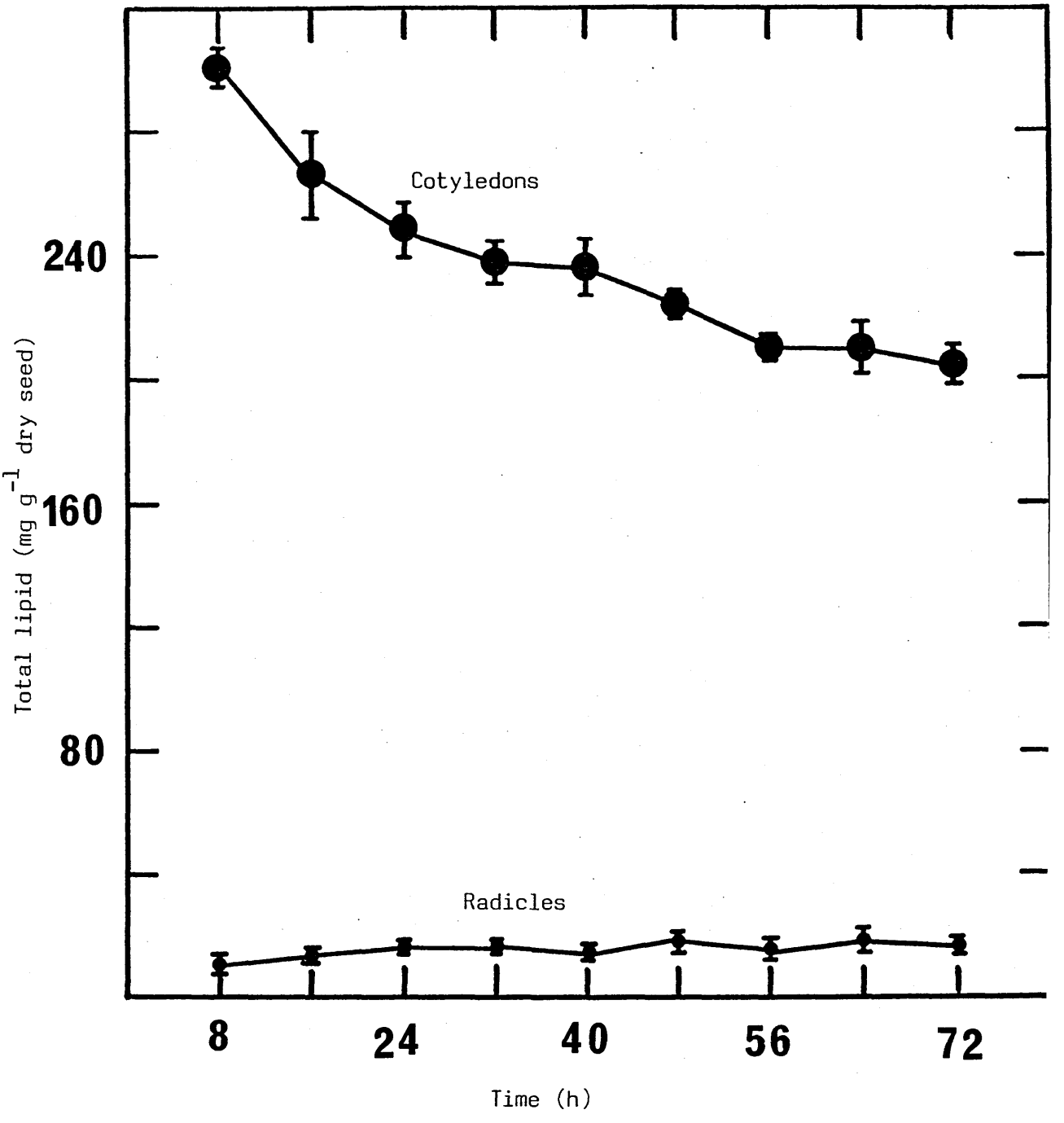


Figure 10 Total lipid contents during germination of Helianthus.

during germination in the pericarp and testa from 4 mg g^{-1} dry seed after 24 h to 1.3 mg g^{-1} dry seed after 48 h and to 3 mg g^{-1} dry seed after 72 h.

II. Gossypium

In the seed tissues of Gossypium (Table 19 and Figure 11), the total lipid content decreased in the cotyledons from 201 mg g^{-1} dry seed to 152 mg g^{-1} dry seed during the germination period from 8 h to 72 h; the total lipid content of the radicle changed slightly from 15 mg g^{-1} dry seed after 24 h to 17 mg g^{-1} dry seed after 72 h, but no statistically significant change occurred during this period in the testa (about 2 mg g^{-1} dry seed).

The results of these experiments confirm broadly those of Abdel Magid and Osman (1977) who showed that oil content in the cotyledons of cottonseeds decreased from 35% to less than 5% after 5 d germination, and Bewley and Black (1978) who found that during the first 11 d after imbibition of peanut, the oil content in cotyledons decreased to 55% of the total lipid in dry seeds.

Table 19. Total lipid contents of seed tissues during germination of Gossypium.

Values are means ± SE

Germination time (h)	Total lipid (mg g^{-1} seed)		
	Cotyledons	Radicle	Testa
8	201 ± 16	15 ± 2	2.5 ± 0.4
16	199 ± 9	15 ± 1	2 ± 0.2
24	188 ± 7	15 ± 1	2 ± 0.4
32	187 ± 7	17 ± 1	2 ± 0.4
40	181 ± 4	17 ± 1	2 ± 0.3
48	173 ± 7	16 ± 1	1.8 ± 0.4
56	170 ± 12	16 ± 2	2.7 ± 0.8
64	168 ± 4	17 ± 2	1.8 ± 0.4
72	152 ± 9	17 ± 2	1.5 ± 0.4

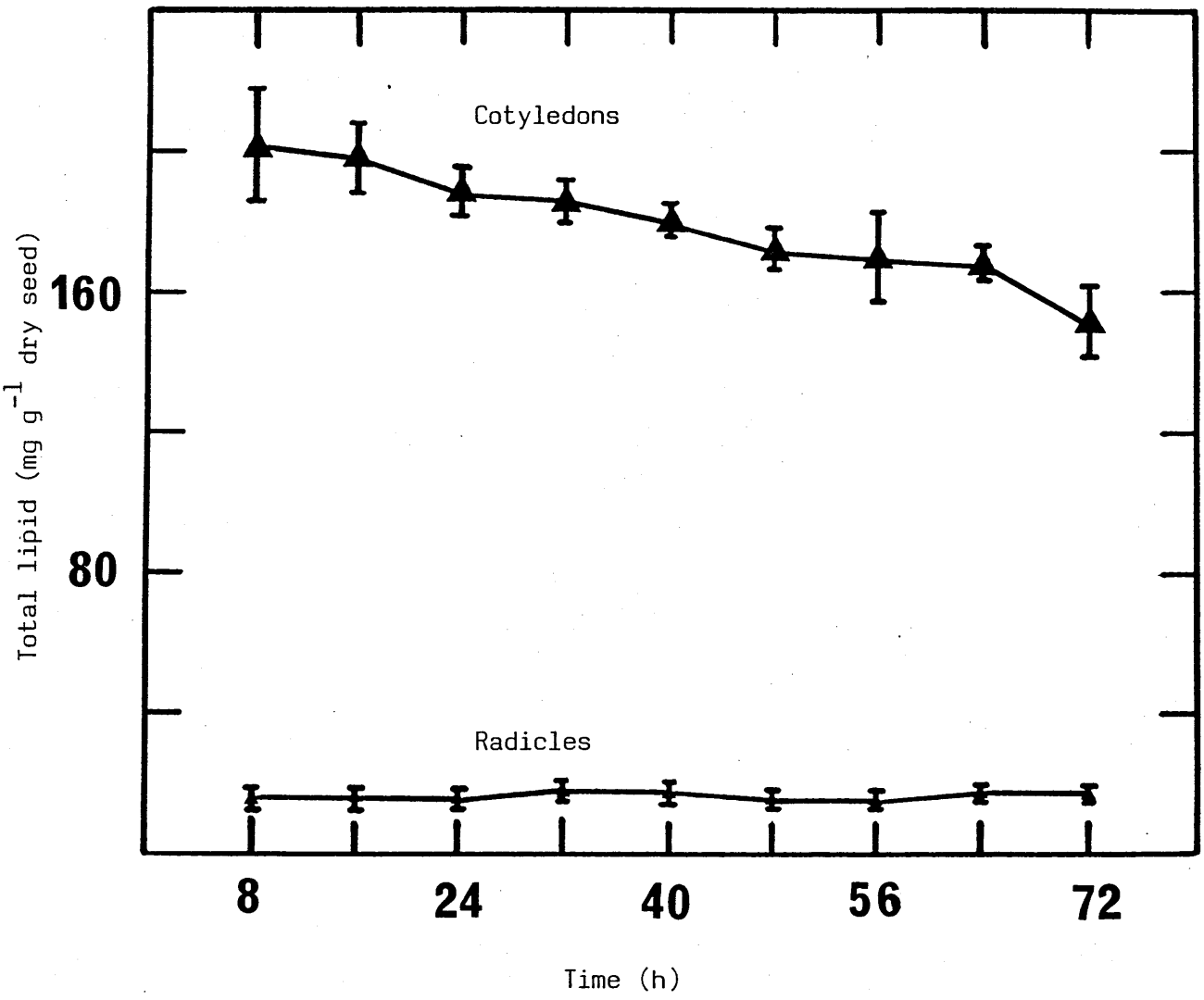


Figure 11 Total lipid contents during germination of Gossypium.

2.2 Lipid classes

The lipid classes extracted from cotyledons and radicles of both Helianthus and Gossypium after 1, 2 and 3 d germination were isolated by column chromatography on silicic acid to provide two main classes: neutral lipids (glycerides) and polar lipids (phospholipid and glycolipid). These fractions were expressed as a weight percentage of the total lipid applied to the column of silicic acid. In parallel experiments, neutral lipids of both species were extracted using a soxhlet apparatus and petroleum ether, 40 - 60°C bp. and quantitatively determined during germination (Figures 12 and 13).

I. Helianthus

In seed tissues of Helianthus, the neutral lipid fraction extracted from the cotyledons during 3 d germination was 82 - 89% of the total lipid and the polar lipid fraction constituted 11 - 18% of total lipid. In the cotyledons, the neutral lipid fraction decreased from 204 mg g^{-1} dry seed after 1 d to 178 mg g^{-1} dry seed after 3 d; similarly, polar lipid (phospholipid) decreased from 41 mg g^{-1} dry seed after 1 d to 23 mg g^{-1} dry seed after 3 d, but the glycolipid fraction increased slightly from 3 mg g^{-1} dry seed after 1 d to 4 mg g^{-1} dry seed after 3 d germination.

In the radicle of Helianthus the neutral lipid fraction was 77 to 85%, and the polar lipid 15 to 23%, of the total lipid during a three-day germination period. The neutral lipid extracted from the radicle changed slightly from 13.6 mg g^{-1} dry seed after 1 d to 14.6 mg g-1 dry seed after 2 d and to

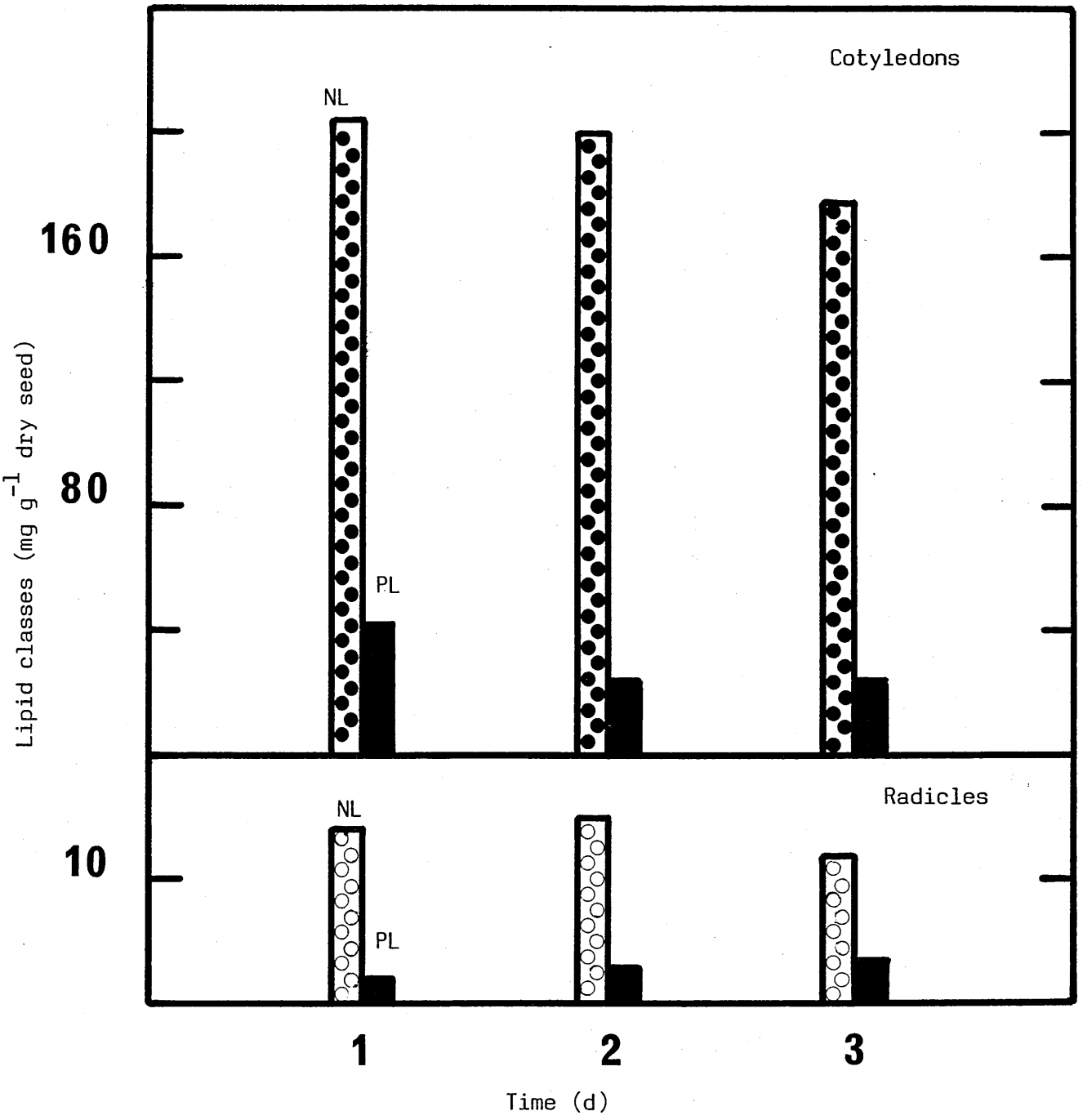


Figure 12. Neutral (NL) and phospholipid (PL) during germination of Helianthus.

12.4 mg g^{-1} dry seed after 3 d, but no significant changes were noted in the polar lipid (phospholipid) fraction (2 mg g-1 dry seed after 1 d to 3.3 mg g^{-1} dry seed after 3 d) and in the glycolipid fraction of radicles (about 0.4 mg g^{-1} dry seed) during germination.

II. Gossypium

In seed tissues of Gossypium, the neutral lipid fraction extracted from the cotyledons during germination constituted 79 - 88% of the total lipids, and the polar lipid 12-21%. The neutral lipid of cotyledons decreased from 166 mg g^{-1} dry seed after 1 d to 120 mg g^{-1} dry seed after 3 d, but the polar lipid (phospholipid) fraction increased from 11 mg g^{-1} dry seed after 1 d to 20 mg g^{-1} dry seed after 3 d; likewise the glycolipid fraction increased from 0.7 mg g^{-1} dry seed after 1 d to 1.3 mg g^{-1} dry seed after 3 d germination.

In the radicle of Gossypium during a three-day germination period, the neutral lipid constituted from 77 - 88% of the total lipid, and the balance consisted of polar lipid. The neutral lipid of the radicle did not change during germination (about 13 mg g^{-1} dry seed), but the polar lipid (phospholipid fraction) increased from 2.2 mg g^{-1} dry seed after 1 d to 4 mg g^{-1} dry seed after 3 d germination.

In a study of lipid classes of Helianthus during germination, Grewal et al. (1978) found that in whole dry sunflower seeds polar lipids constituted about 2.5% of the total lipid, of this figure phospholipid constituted 76% and the balance was glycolipid.

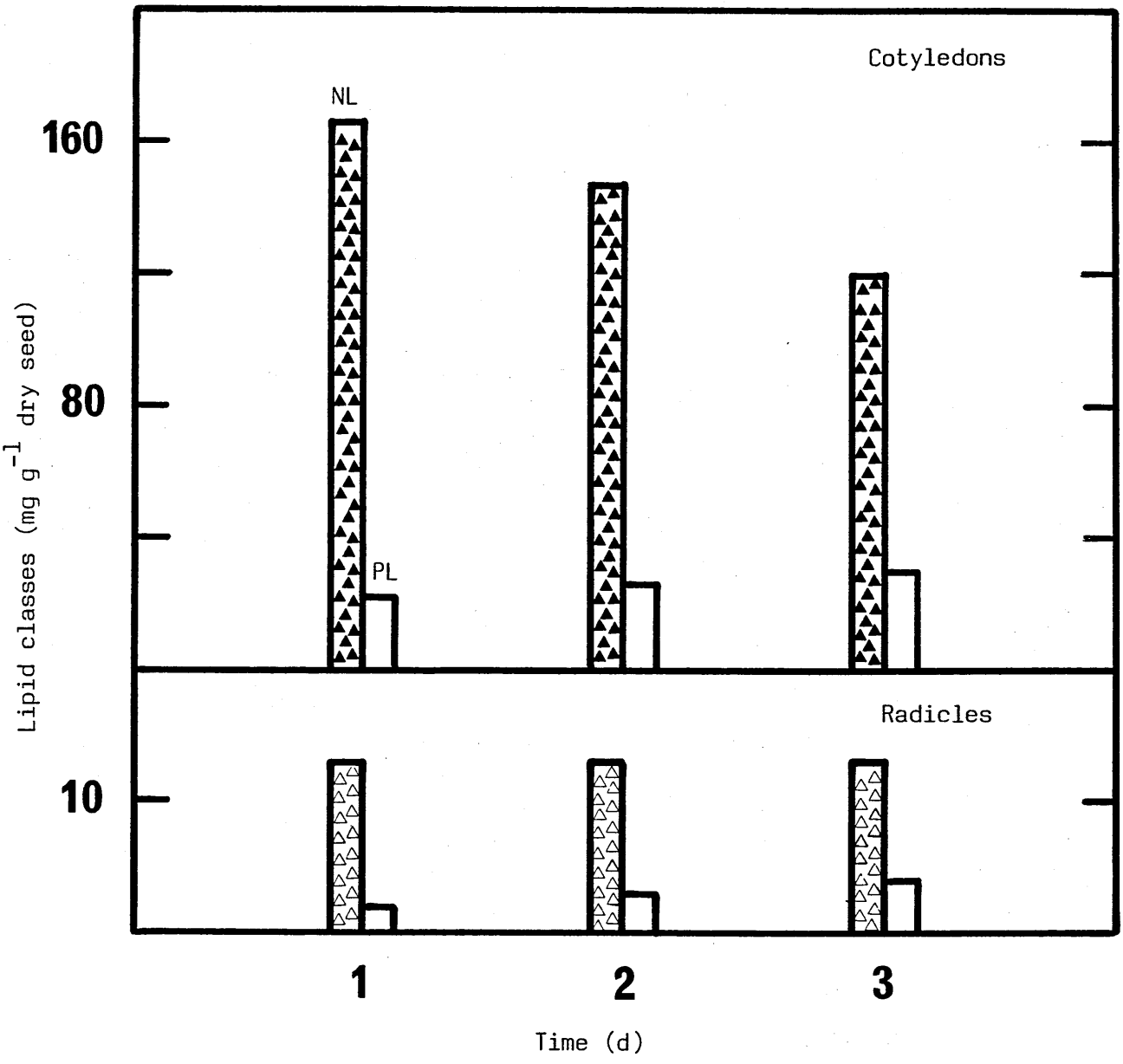


Figure 13 Neutral (NL) and phospholipid (PL) during germination of Gossypium.

Comparable studies have been carried out on lipid classes of Gossypium by Gunstone and Qureshi (1965) who published that lipid content in the whole dry cottonseeds was 20%, of which 86% was neutral lipid, and Achaya (1975) who showed that the content of phospholipid of cottonseeds (G. hirsutum) varied from 1.3 - 1.5% of the total lipid in the kernel and 1% in the whole seed but varied from 0.9 - 2.7% in Indian cottonseeds (G. herbaceum). Doman et al. (1982) noted that the neutral lipid extracted from the cotyledons of cottonseeds decreased from 20 to 14 mg per cotyledon pair during the period from initial soaking up to 48 h germination.

3. Long-chain fatty acid methyl esters (LCFAME)

3.1 Total LCFAME

Eight long-chain fatty acids, myristic ($C_{14:0}$), palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$) and arachidic ($C_{20:0}$), were determined in dry seeds and in germinating seed tissues every 8 h up to 72 h in both Helianthus and Gossypium using GC-FID and GC-MS (details of reference mass spectra are given in the Appendix). The results of the analyses are presented as fatty acid contents relative to $C_{18:2}$ as 100 (Tables) and as mg g^{-1} dry seed (Figures).

In the dry seeds of Helianthus, the saturated fatty acids C_{16}, C_{18} and C_{20} were calculated to be present in the amounts 19 mg g^{-1}, 16 mg g^{-1} and 2 mg g^{-1} dry seed respectively. In terms relative to the content of linoleic acid, they were present at 9, 7 and 1 respectively. The unsaturated fatty acids $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were present at 78 mg g^{-1}, 211 mg g^{-1} and 1 mg g^{-1} dry seed respectively, with contents of $C_{18:1}$ and $C_{18:3}$ relative to $C_{18:2}$ at 37 and 0.5.

In the dry seeds of Gossypium, the results were somewhat different from those obtained with Helianthus. Thus, C_{14} was detected in Gossypium at 2 mg g^{-1} dry seed, with a relative level of 2. C_{16} and C_{18} were present at 61 mg g^{-1} and 7 mg g^{-1} dry seed with relative levels of 55 and 6, respectively. C_{20} was not detected. $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were quantified at 56 mg g^{-1}, 111 mg g^{-1} and 0.2 mg g^{-1} dry seed with relative levels of $C_{18:1}$ and $C_{18:3}$ at 51 and 0.2 (Tables 20 and 21, and Figure 14).

Table 20. Fatty acid contents in dry Gossypium and Helianthus seeds.

Values are means ± SE. ND = not detected

	Fatty acids - mg g^{-1} seed (% of total lipid indicated in brackets)							
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}
G. barbadense	1.95±0.2	61.24±8.3	3.66±0.6	6.83±1.2	56.36±11.5	110.53±30.5	0.24±0.1	ND
	(0.80)	(25.10)	(1.50)	(2.80)	(23.10)	(45.30)	(0.10)	ND
H. annuus	ND	19.41±6.4	ND	15.79±4.1	78.30±12.2	211.22±27.1	0.98±0.2	1.68±0
	ND	(5.90)	ND	(4.80)	(23.80)	(64.2)	(0.30)	(0.51

Table 21. Fatty acid contents in dry Gossypium and Helianthus seeds.

Species	Fatty acids relative to $C_{18:2}$(100)							
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20}
G. barbadense	1.76	55.41	3.31	6.18	50.99	100	0.22	—
H. annuus	—	9.19	0.00	7.48	37.07	100	0.46	0.80

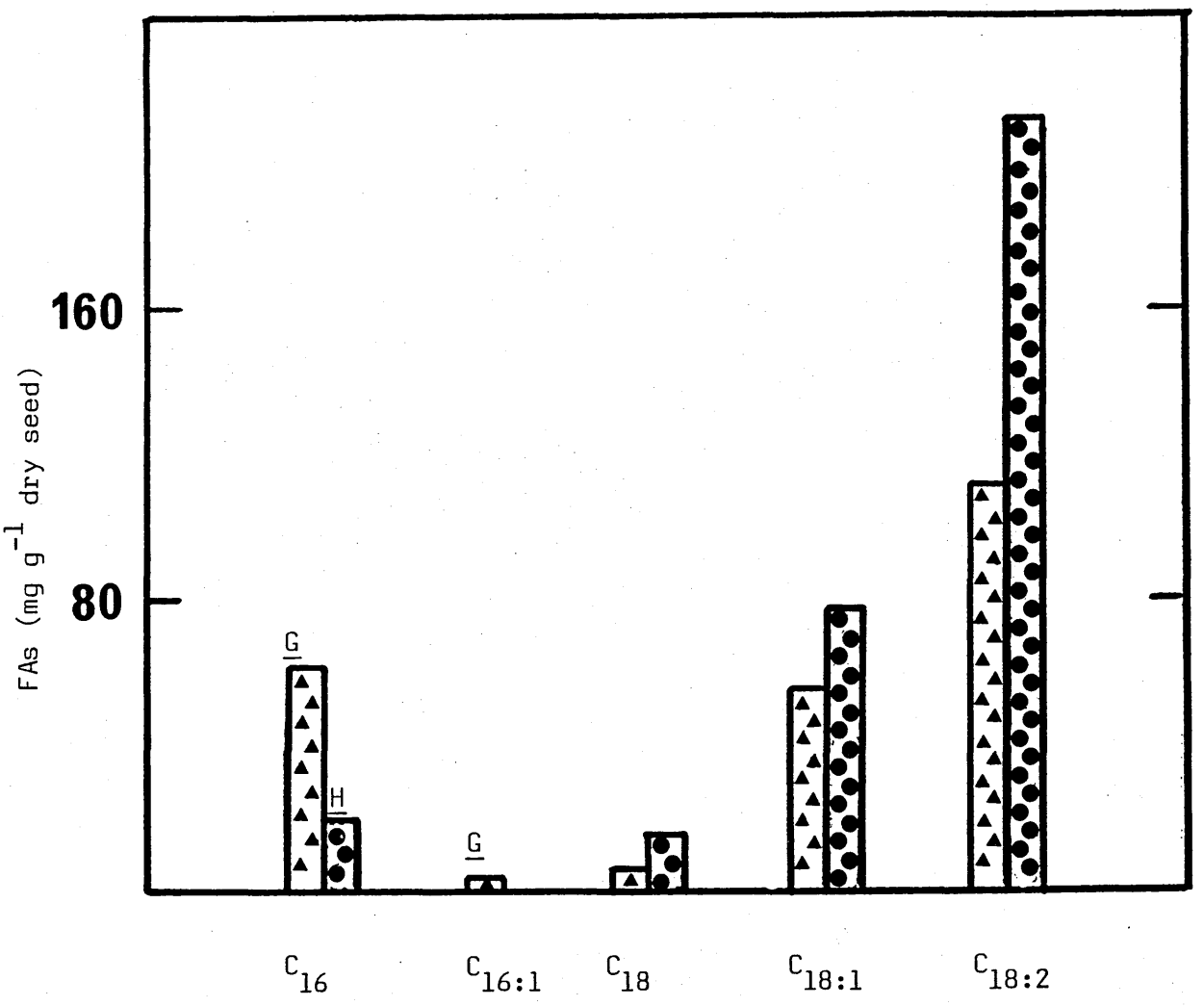


Figure 14 LCFAME in dry Helianthus (H) and Gossypium (G) seeds.

The unsaturated FA $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ constituted about 88% and 70% of the total lipids in Helianthus and Gossypium respectively.

Throughout the analyses, variations in the calculated amounts of LCFA were noted, especially in $C_{18:3}$ and C_{20} contents which were present in near trace amounts. $C_{18:2}$ was always found to be present in quantitites greater than other FAs.

The fatty acid composition of oil from dry whole sunflower seed has been examined in detail by Kinman and Earle (1964), Robertson (1972), Dorrell and Whelam (1978), Beard and Geng (1982) and Campbell (1983). C_{16} varied between 5 - 8%, C_{18} acid 2 - 6%, $C_{18:1}$ acid, 18 - 40%, $C_{18:2}$ acid, 50 - 80% of the total lipid content.

Comparable analyses have been carried out on the fatty acid composition of dry whole cottonseed oil, Williams (1966), Sreenivasan (1968), Achaya (1975), Duffus and Slaughter (1980) and Raie et al. (1983). C_{16} acid constituted 20 - 26%, C_{18} acid 2 - 4%, $C_{18:1}$ acid 18 - 32%, $C_{18:2}$ acid 30 - 55% of the total lipid content.

In this study, changes in fatty acid composition during germination of Helianthus and Gossypium were determined in the cotyledons, radicles, pericarp and testa. The major fatty acids were $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ in both species (Tables 22 - 27 and Figures 15 - 22).

I. Helianthus annuus

C_{16} in the cotyledons decreased from 45 mg g^{-1} dry seed at 8 h to a general level of 20 mg g^{-1} dry seed with a peak of 32 mg g^{-1} dry seed at 24 h. When the results are presented as

relative to $C_{18:2}$, a similar pattern emerges. A decrease from 41 at 8 h to around 20 thereafter with evidence of a small peak (27) at 24 h (Table 22). In the radicle, the absolute levels of C_{16} varied slightly at around 5 mg g^{-1} dry seed, but relative to $C_{18:2}$, there were two pronounced peaks at 24 h (165), and 64 h (192). The levels of C_{16} in the pericarp and testa of Helianthus were low, less than 2 mg g^{-1} dry seed, throughout the germination period. Large changes in the values relative to $C_{18:2}$ in the pericarp and testa were noted, ranging from a high of 150 at 8 h to a low of 33 at 48 h with peaks at 32 h and 64 h.

C_{18} contents in Helianthus cotyledons ranged between 23 mg g^{-1} (8 h) and 13 mg g^{-1} dry seed (16 h) with values relative to $C_{18:2}$ in cotyledons varying between 21 (8 h) and 10 (16 h). In both methods of presenting the results, there is evidence of a peak at 56 h. In the radicle, absolute quantities of C_{18} varied between 1 and 3 mg g^{-1} dry seed; evidence of peaks at 24 h and 64 h was substantiated by the fact that the relative values were about 93 and 116 at these times compared with an average of approximately 26 at the other sampling times (Table 23). In the pericarp and testa C_{18} levels were lower (0.7 mg g^{-1} dry seed) than in the radicle.

There was a general decline in $C_{18:1}$ content during imbibition and germination in the cotyledons of Helianthus, decreasing from 100 mg g^{-1} at 8 h to about 50 mg g^{-1} dry seed at 72 h. This decline was reflected in the values relative to $C_{18:2}$ in the cotyledons (92 at 8 h to 47 at 72 h). In the radicle, $C_{18:1}$ varied between 2 mg g^{-1} at 8 h to 6 mg g^{-1} dry seed at 48 h.

Pronounced peaks in relative values were detected at 24 h (169) and 64 h (174). In the pericarp and testa the highest $C_{18:1}$ content was at 8 h (1 mg g^{-1} dry seed, a value of 131 relative to $C_{18:2}$) and the lowest at 48 h (0.3 mg g^{-1} dry seed, relative value of 57).

Over the 72 h germination period, $C_{18:2}$ varied in cotyledons between 105 at 56 h and 140 mg g^{-1} dry seed at 40 h. In the radicle, $C_{18:2}$ levels varied between 3 mg g^{-1} at 64 h and 8 mg g^{-1} dry seed at 32 h, with levels greater than 6 mg g^{-1} dry seed between 32 and 56 h. In the pericarp and testa, the highest content of $C_{18:2}$ was about 2 mg g^{-1} dry seed at 16 h; during the period 40 - 72 h the level did not exceed 0.6 mg g^{-1} dry seed.

The unsaturated fatty acid $C_{18:3}$ appeared in trace levels in the cotyledons of Helianthus at 8, 16 and 24 h, and in the radicle at 8 and 16 h (Tables 22 - 24 and Figures 15 - 18).

The content of $C_{18:2}$ in the cotyledons of Helianthus remains essentially constant during germination. As a consequence the changes of absolute amounts in other acids reflect the change in the relative to $C_{18:2}$. The constancy of $C_{18:2}$ in the cotyledons is also true for C_{18}. However, both C_{16} and $C_{18:1}$ decline from their initial values, occurring in the first 16 h in the case of C_{16}, but with $C_{18:1}$ the decline continued until about 56 h before in both cases the contents of these fatty acids levelled out. Whilst the amounts of the individual acids in the radicles appear not to change substantially during germination, the amounts relative to $C_{18:2}$ fluctuate considerably (Table 23). However, a considerable degree of

Table 22. Long-chain fatty acids extracted from Helianthus cotyledons during germination.

Germination time (h)	Fatty acids relative to $C_{18:2}$(100)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
8	—	41.28	—	21.29	91.84	100	4.10
16	—	18.63	—	9.90	71.50	100	2.21
24	—	26.62	—	16.29	57.68	100	2.19
32	—	17.36	—	13.91	57.50	100	—
40	—	13.07	—	11.20	40.83	100	—
48	—	15.57	—	11.03	48.06	100	—
56	—	19.96	—	20.86	51.48	100	—
64	—	15.54	—	13.30	51.22	100	—
72	—	20.48	—	13.36	46.96	100	—

Table 23. Long-chain fatty acids extracted from Helianthus radicles during germination.

Germination time (h)	Fatty acids relative to $C_{18:2}$ (100)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
8	—	35.98	—	24.07	64.29	100	5.65
16	—	46.29	—	20.51	76.17	100	2.12
24	—	165.47	—	93.17	169.06	100	—
32	—	30.80	—	19.53	47.18	100	—
40	—	28.10	—	16.92	39.88	100	—
48	—	46.12	—	26.06	85.07	100	—
56	—	30.94	—	16.44	53.81	100	—
64	—	192.24	—	115.94	174.10	100	—
72	—	135.65	—	61.63	142.90	100	—

Table 24 Long-chain fatty acids extracted from Helianthus pericarp and testa during germination.

Germination time (h)	Fatty acids relative to $C_{18:2}$ (100)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
8	—	150.00	—	87.50	131.25	100	—
16	—	45.81	—	28.39	63.23	100	—
24	—	70.53	25.26	56.84	95.79	100	—
32	—	135.96	—	74.16	67.42	100	—
40	14.75	67.21	14.75	34.43	60.66	100	—
48	—	32.65	4.08	20.41	57.14	100	—
56	—	77.94	13.24	38.24	66.18	100	—
64	30.23	123.26	27.91	58.14	106.98	100	—
72	—	119.05	—	50.00	107.14	100	—

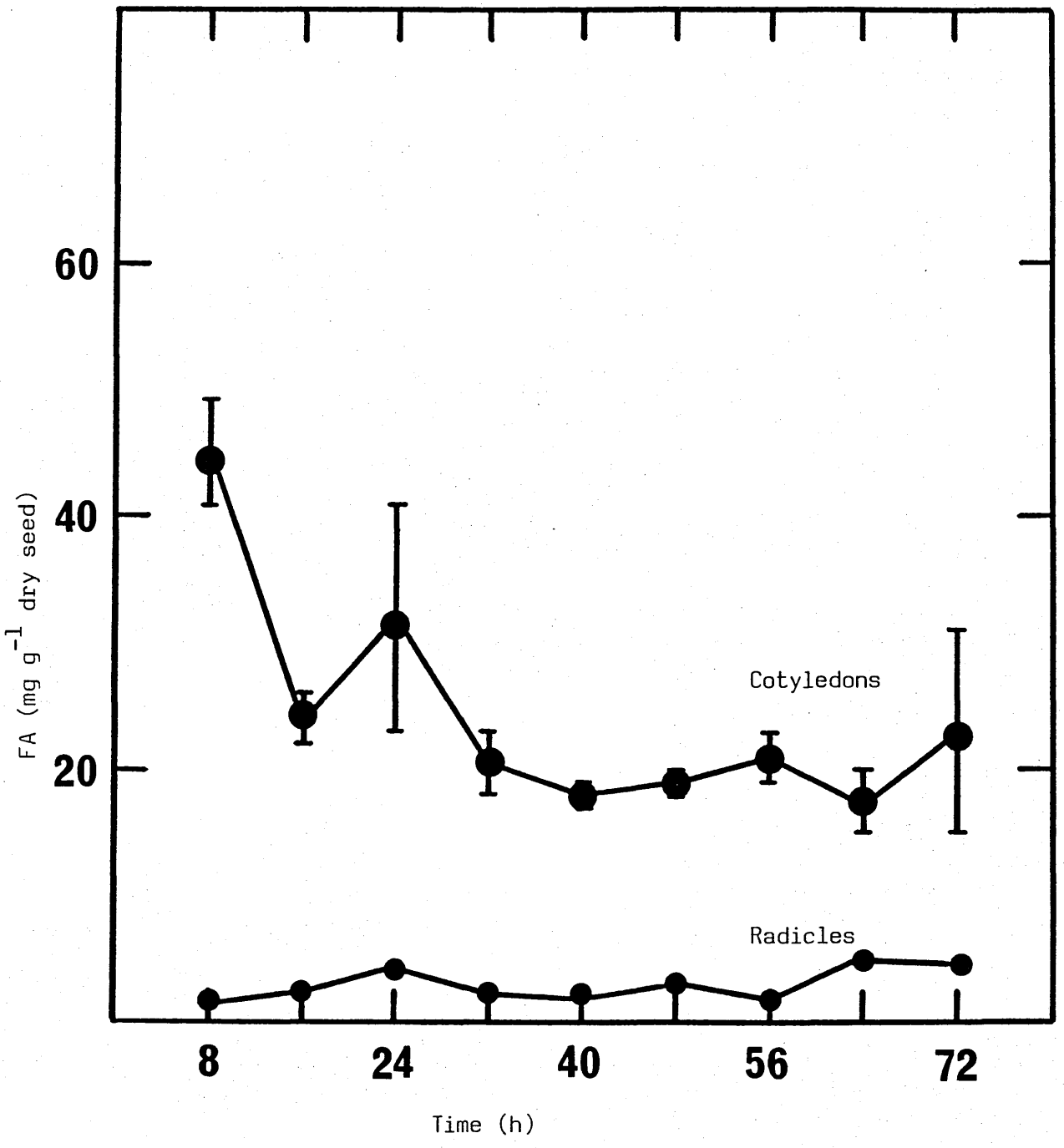


Figure 15 FAME C_{16} during germination of Helianthus.

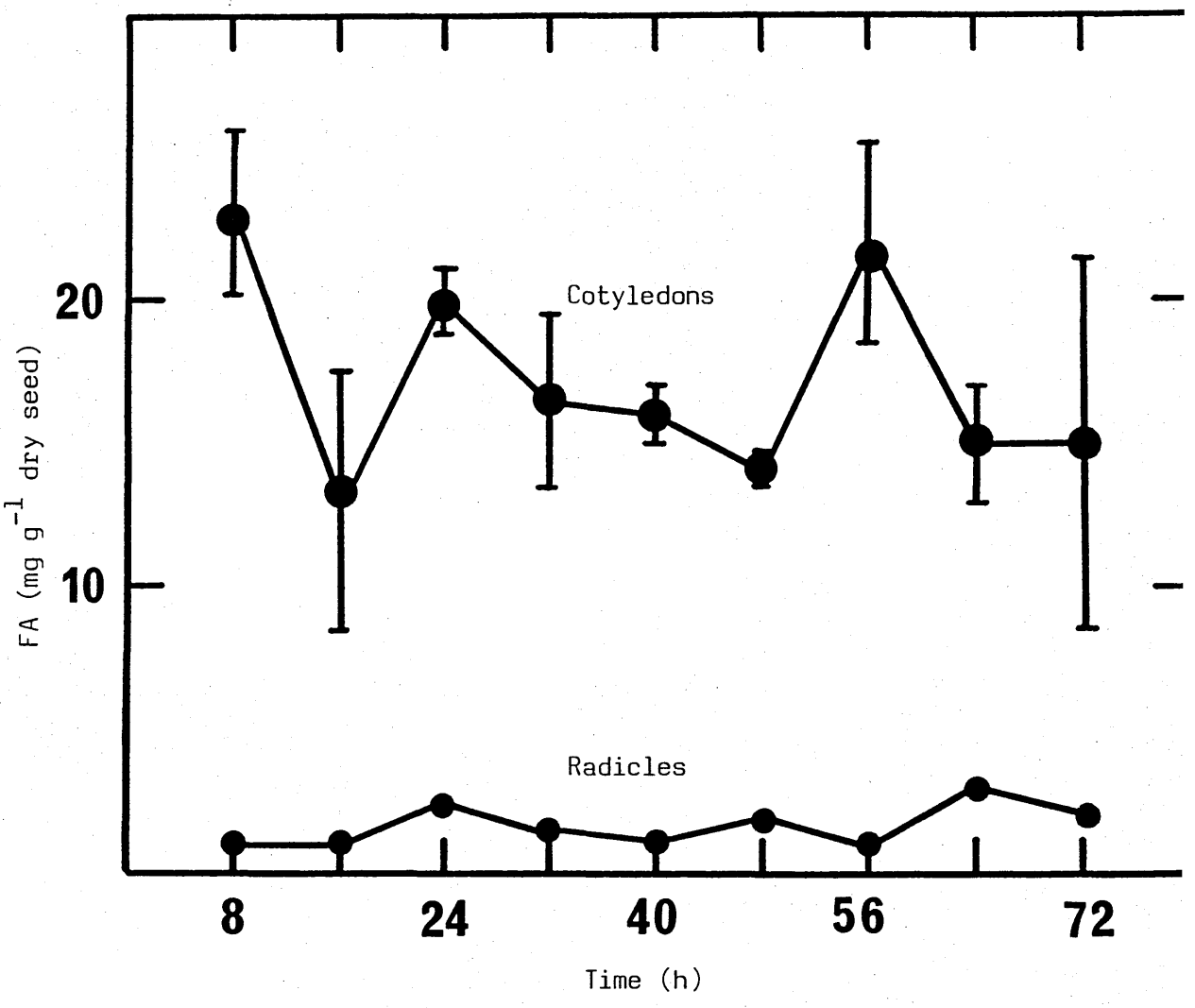


Figure 16 FAME C_{18} during germination of Helianthus.

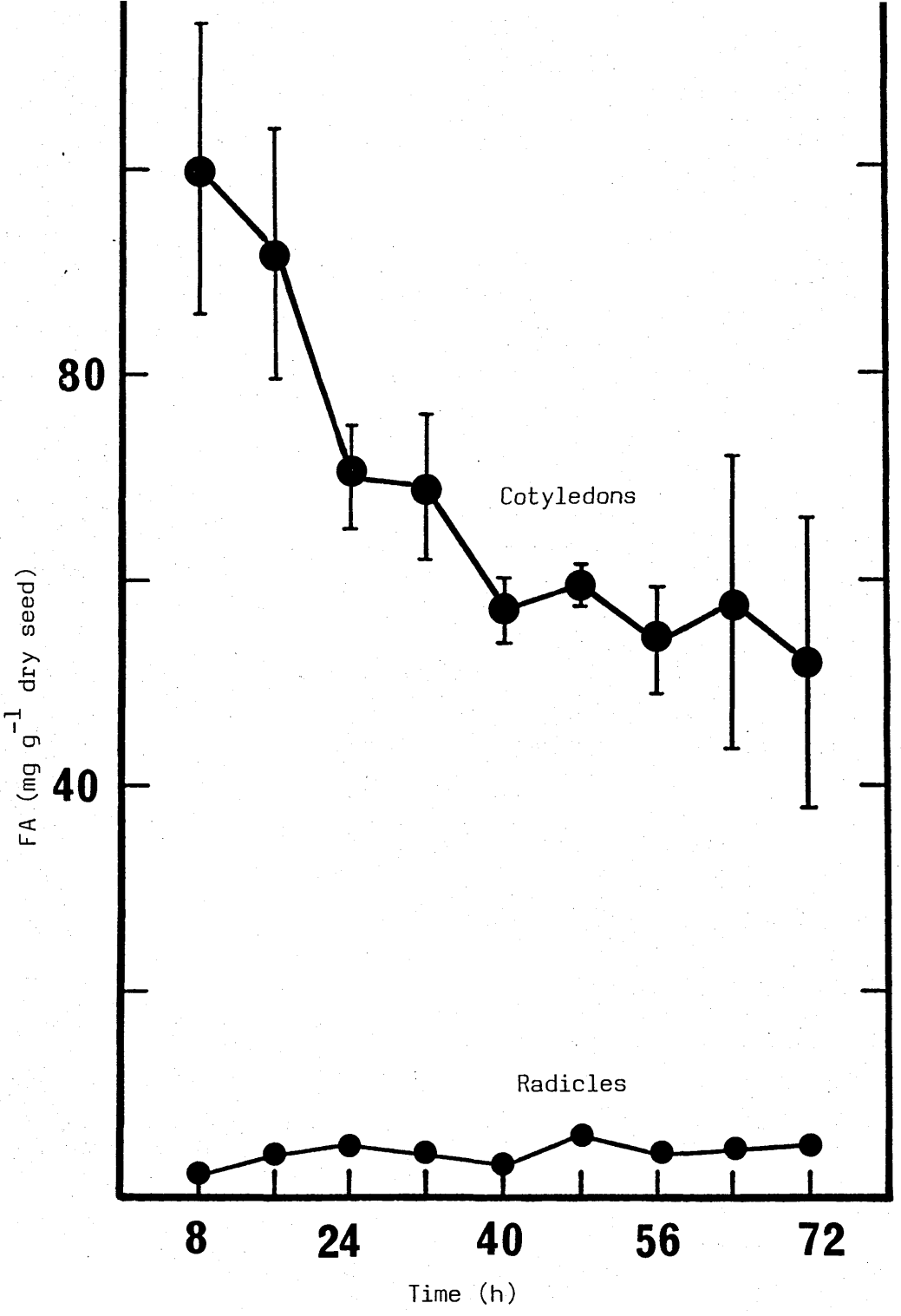


Figure 17 FAME $C_{18:1}$ during germination of Helianthus.

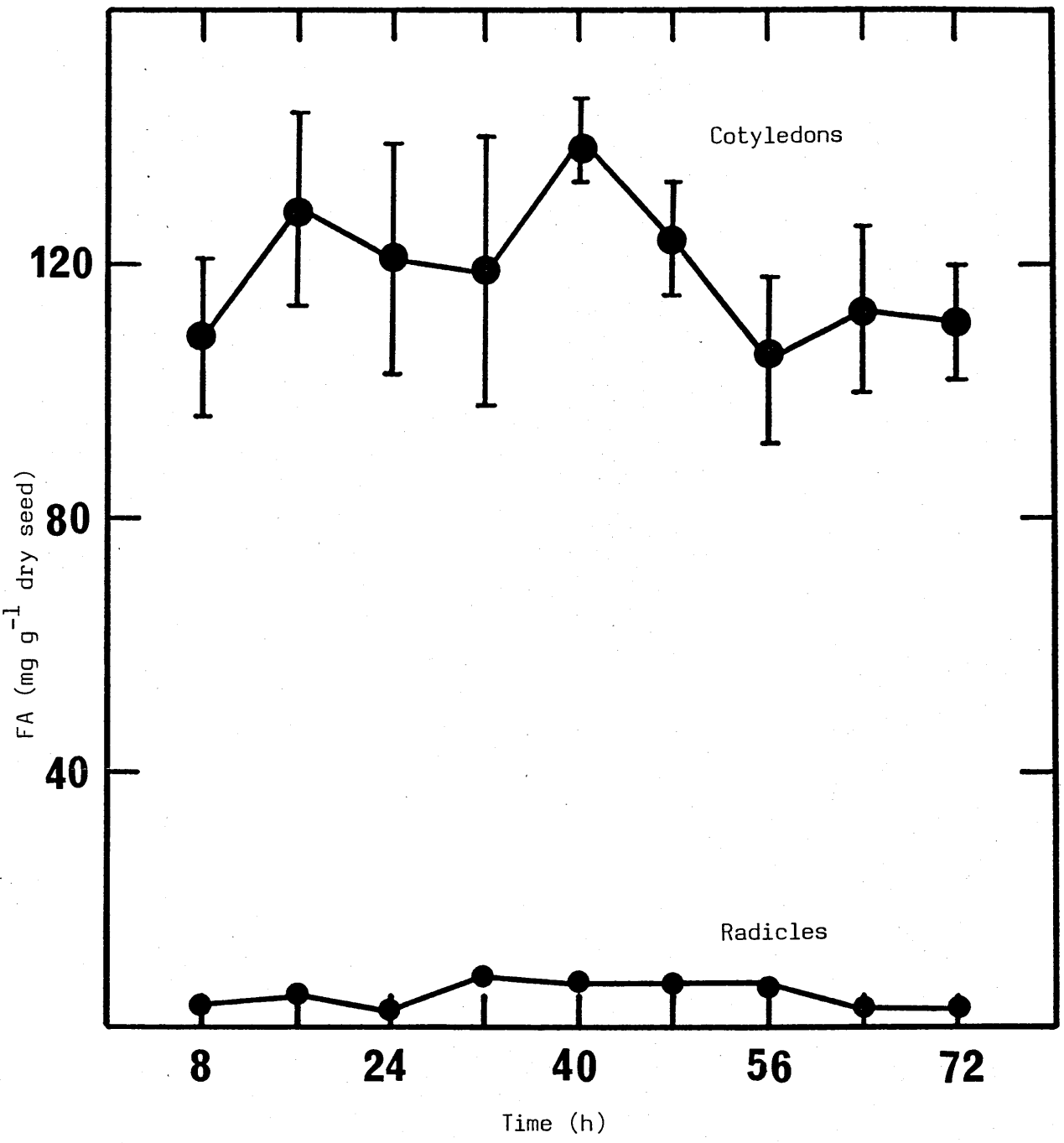


Figure 18 FAME $C_{18:2}$ during germination of Helianthus.

caution is required in offering an interpretation of these findings because of the small average values associated with large standard errors.

Until more precise analyses are carried out by employing a substantially greater number of replicates, little can be said to try to correlate the observed changes in fatty acids content in the radicles with stages of germination. There may be at 24 h a decrease in $C_{18:2}$ with an increase in the others so giving the very high relative amounts. These high relative amounts decline immediately to the pre-24 h values, but high relative amounts are found at 64 and 72 h due principally to a decline in $C_{18:2}$ though there are small increases in the amounts of the other acids.

II. Gossypium barbadense

The saturated fatty acid $C_{14:0}$ (myristic) was detected in extracts of cotyledons at 8, 16, 40, 48 and 56 h at levels of about 1 - 2 mg g^{-1} dry seed. It was present in radicle extracts only at 8 h (0.2 mg g^{-1} dry seed), but was not detected in the testa during germination.

C_{16} content in the cotyledons varied between 43 mg g^{-1} (at 48 h) and 73 mg g^{-1} dry seed (at 32 h); when the results are considered as values relative to $C_{18:2}$ in the cotyledons, then values greater than 100 were noted at 24, 56, 64 and 72 h, with values of less than 70 at 8 h and 48 h. In the radicle C_{16} levels varied slightly between 5 mg g^{-1} at 8 h and 8 mg g^{-1} dry seed at 48 h. Relative to $C_{18:2}$, however, there were pronounced peaks at 16 h (543) and 56 h (147). In the

testa, C_{16} levels were low (0.3 - 1.4 mg g^{-1} dry seed).

Two peaks in C_{18} content in the cotyledons were noted at 40 h (13.2 mg g^{-1} dry seed, relative value 19) at 56 h (12.5 mg g^{-1} dry seed, relative value 37); at other sampling times the content varied between 7.2 - 9.3 mg g^{-1} dry seed. In the radicle, there was a pronounced peak of C_{18} content at 16 h of 4 mg g^{-1} dry seed (relative value 357); at all other times the C_{18} content was less than 1 mg g^{-1} dry seed. In the testa, C_{18} content was low (0.1 - 0.5 mg g^{-1} dry seed); the highest level was detected at 32 h (relative value 185).

The unsaturated fatty acid $C_{16:1}$ (palmitoleic) content increased in the cotyledons of Gossypium after 48 h from an average of 2.5 mg g^{-1} to an average of 5.0 mg g^{-1} dry seed. In values relative to $C_{18:2}$ in the cotyledons, the highest contents were at 56 h (19), 64 h (10) and 72 h (11). In the radicle $C_{16:1}$ changed slightly (0.1 - 0.3 mg g^{-1} dry seed, relative values 1 - 7). In the testa $C_{16:1}$ appeared in trace amounts (approx. 0.1 mg g^{-1} dry seed) at 48, 56, 64 and 72 h.

There was a pronounced decline in the content of the unsaturated fatty acid $C_{18:1}$ at 32 h (24 mg g^{-1} dry seed; average level at other sampling times 46 mg g^{-1} dry seed) in the cotyledons of Gossypium. This was substantiated by analysis of the relative values, although there is a marked peak (147) at 56 h. In the radicle $C_{18:1}$ content varied between 2 - 4 mg g^{-1} dry seed, but inspection of the values relative to $C_{18:2}$ reveals a large peak (343) at 16 h. In the testa $C_{18:1}$ content was low (0.2 - 0.5 mg g^{-1} dry seed) throughout the germination period.

There was decrease in the content of the unsaturated fatty acid $C_{18:2}$ at 48 h; average level was 70 mg g^{-1} dry seed from 8 - 48 h and 41 mg g^{-1} dry seed from 56 - 72 h in the cotyledons. In the radicle $C_{18:2}$ decreased markedly at 16 h to approx. 1 mg g^{-1} dry seed; the average level at other sampling times was 6 mg g^{-1} dry seed. In the testa $C_{18:2}$ constituted about 0.2 - 0.7 mg g^{-1} dry seed during the 72 h germination period.

The unsaturated fatty acid $C_{18:3}$ was present in the cotyledons of Gossypium at 24, 40 and 64 h, ranging from 4 - 11 relative to $C_{18:2}$, and from 3 - 7 mg g^{-1} dry seed in absolute amounts. In the radicle, $C_{18:3}$ was present at lower amounts at 24, 56, 64 and 72 h, ranging from 6 - 15 relative to $C_{18:2}$, from 0.4 - 0.7 mg g^{-1} dry seed. In the testa, $C_{18:3}$ was not detected (Tables 25 - 27 and Figures 19 - 22).

The results obtained for the contents of the LCFA in Gossypium cotyledons are extremely variable as can be seen for the large values associated with the standard errors. Nonetheless, it may be possible to make an interpretation of the findings. In the case of $C_{18:2}$ in this species there appears to be a gradual decrease at 8 h through to 56 h, though the decrease may in fact be continuous over the whole period of observation. All the other acids seem to remain constant in amounts during the 72 h period. Because of the substantial change in $C_{18:2}$ content, relative content of the other acids appear to change dramatically, especially when there is a counter change, as measured, e.g. as at 56 h (Table 25).

In the relatively few studies of fatty acids during

Table 25. Long-chain fatty acids extracted from Gossypium cotyledons during germination.

Germination time (h)	Fatty acids relative to $C_{18:2}$ (100)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
8	1.40	64.40	3.56	10.23	65.68	100	—
16	2.52	96.80	4.29	11.84	72.13	100	—
24	—	104.41	2.64	11.90	75.21	100	11.41
32	—	94.21	3.35	10.36	31.40	100	—
40	3.23	82.26	3.67	19.28	65.84	100	4.40
48	2.93	66.72	6.78	14.33	63.64	100	—
56	3.28	188.36	19.40	37.31	147.16	100	—
64	—	117.83	9.98	17.62	88.11	100	6.79
72	—	130.49	10.73	21.46	98.78	100	—

Table 26. Long-chain fatty acids extracted from Gossypium radicles during germination.

Germination time (h)	Fatty acids relative to $C_{18:2}$ (100)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
8	2.90	64.49	2.17	2.75	32.90	100	——
16	——	543.48	0.00	356.52	343.48	100	——
24	——	63.89	1.18	6.94	47.22	100	7.64
32	——	95.95	2.99	5.55	29.99	100	——
40	——	95.31	3.13	14.84	40.63	100	——
48	——	139.62	6.60	12.45	47.17	100	——
56	——	146.74	5.43	11.96	36.96	100	15.22
64	——	109.62	2.88	8.65	50.00	100	13.46
72	——	66.67	3.03	9.09	45.45	100	6.06

Table 27. Long-chain fatty acids extracted from Gossypium testa during germination.

Germination time (h)	Fatty acids relative to $C_{18:2}$ (100)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
8	—	69.84	—	15.87	31.75	100	—
16	—	38.89	—	30.56	45.83	100	—
24	—	150.00	—	100.00	100.00	100	—
32	—	529.63	—	185.19	185.19	100	—
40	—	379.31	—	68.97	137.93	100	—
48	—	57.14	14.29	14.29	57.14	100	—
56	—	220.00	20.00	20.00	40.00	100	—
64	—	500.00	50.00	50.00	100.00	100	—
72	—	350.00	50.00	50.00	200.00	100	—

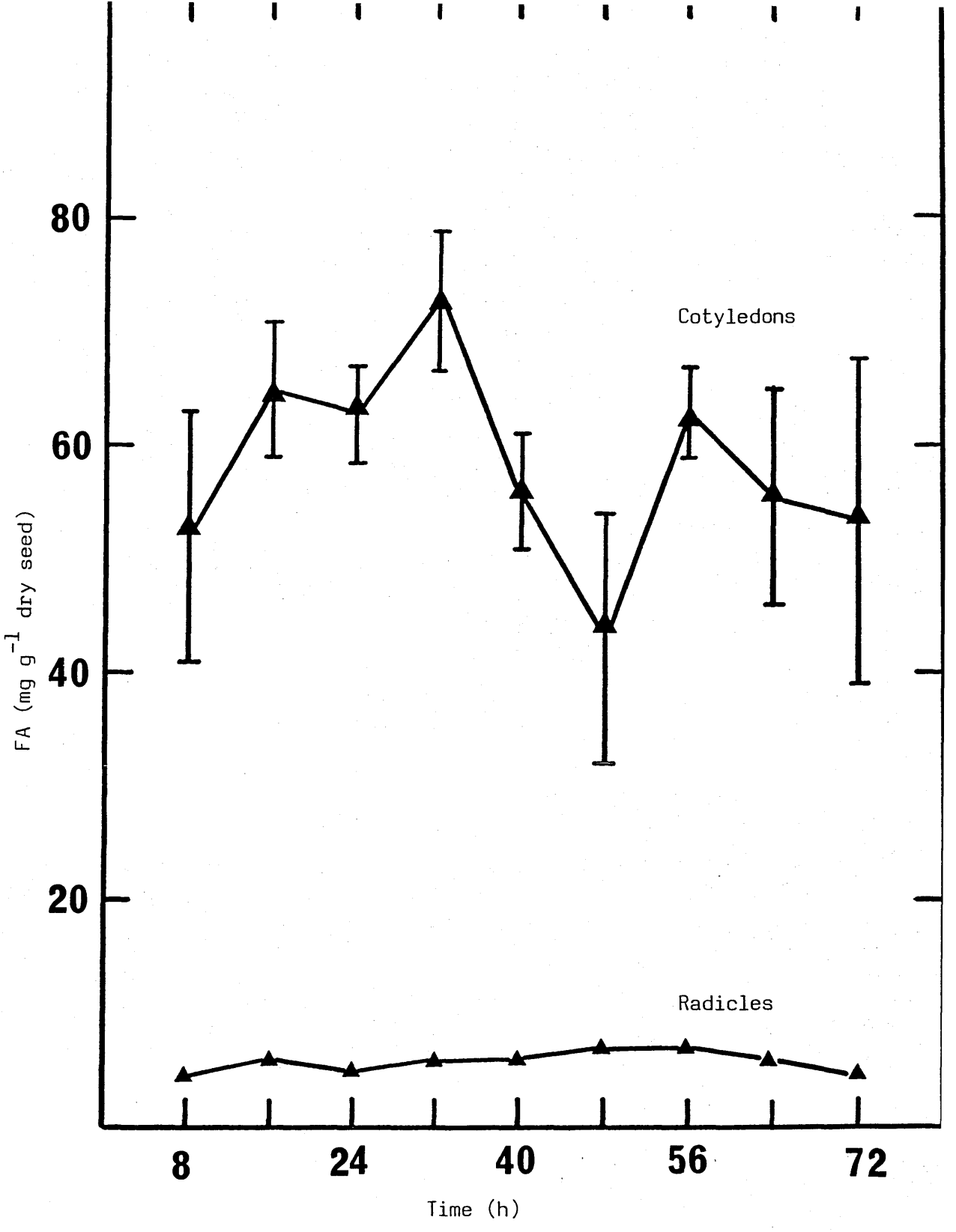


Figure 19 FAME C_{16} during germination of Gossypium.

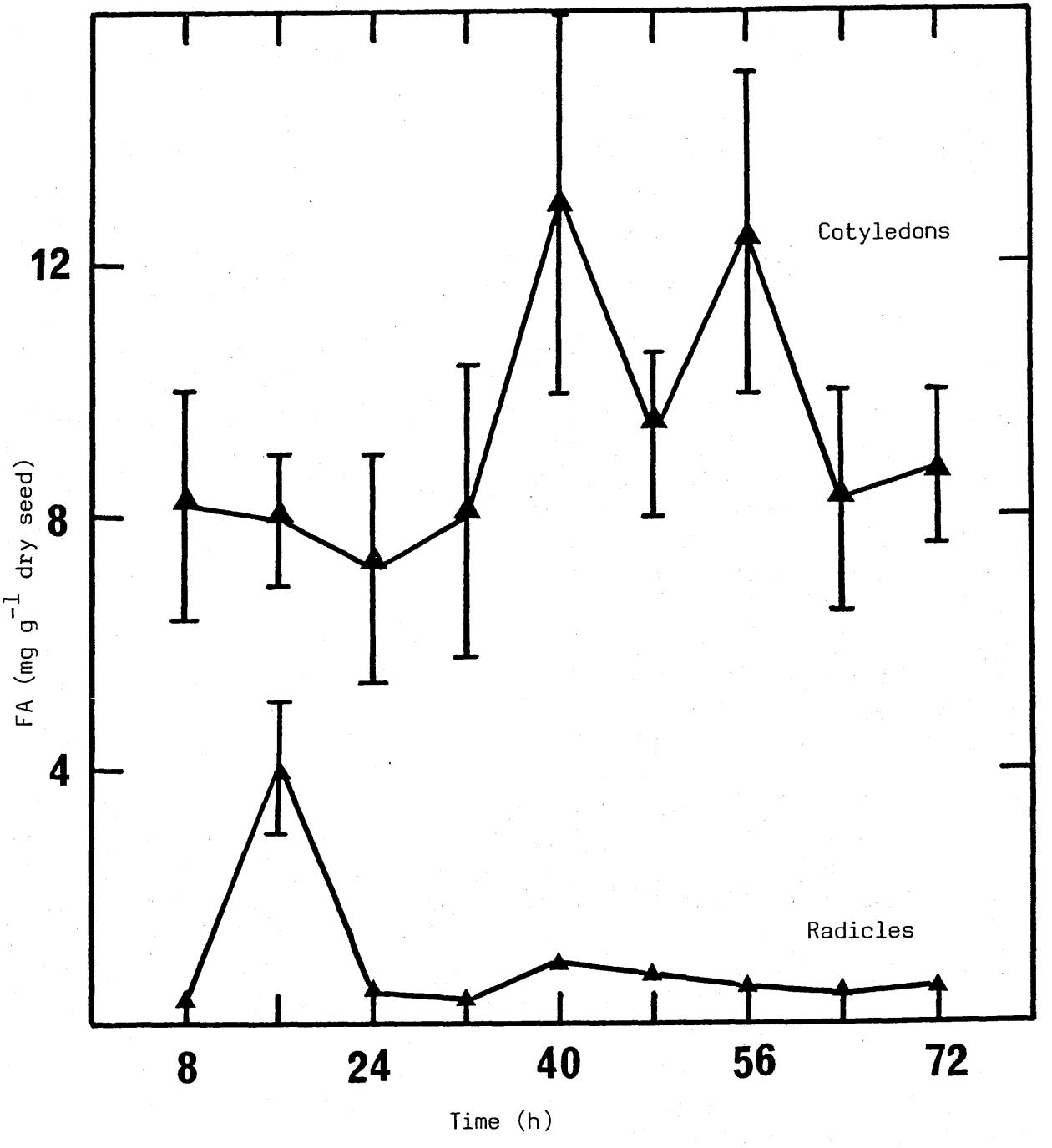


Figure 20 FAME C_{18} during germination of Gossypium.

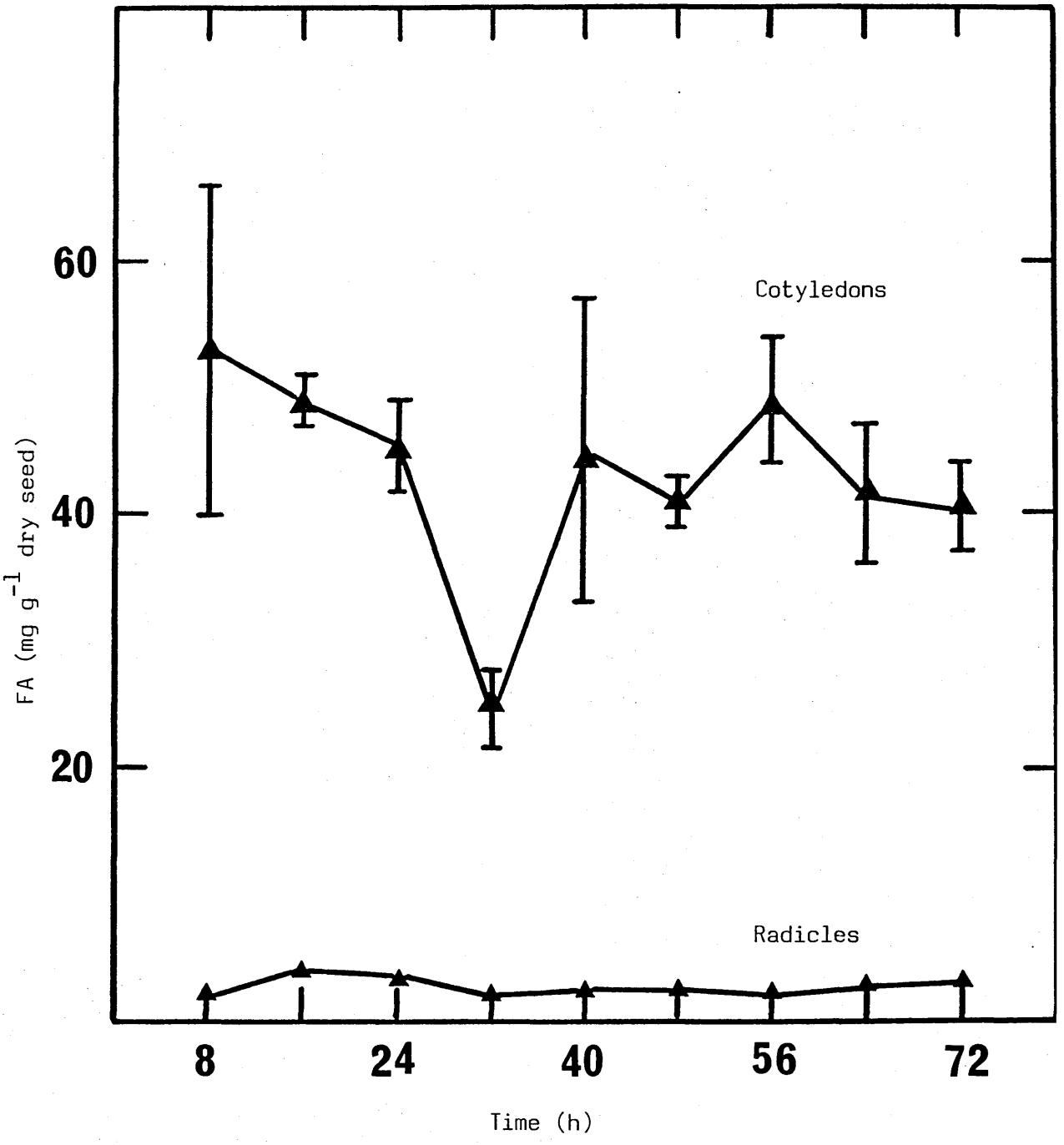


Figure 21 FAME $C_{18:1}$ during germination of Gossypium.

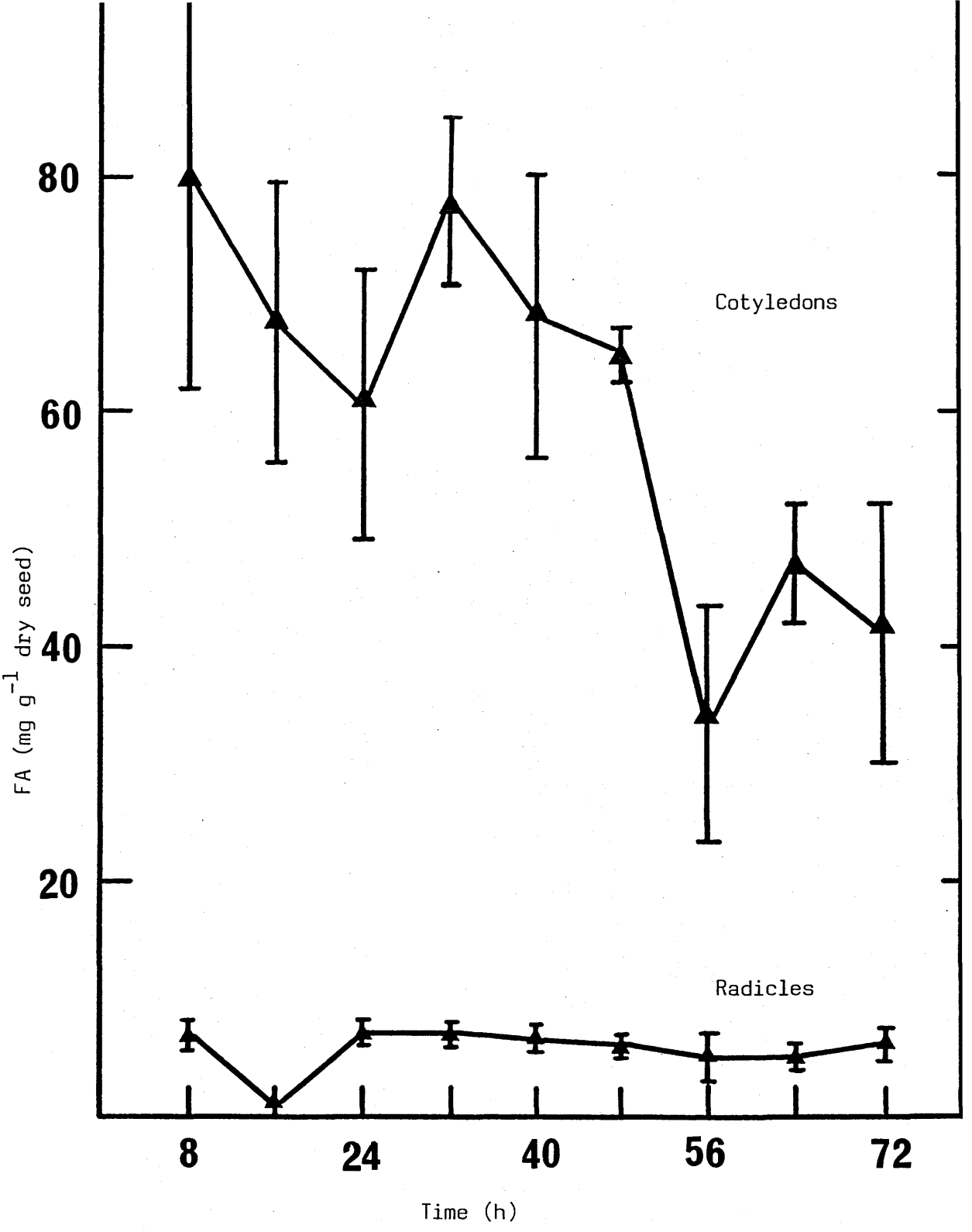


Figure 22 FAME $C_{18:2}$ during germination of Gossypium.

3.2 Long-chain fatty acids of lipid classes

Neutral and polar lipids were extracted from the cotyledons and radicles of Helianthus and Gossypium after 1, 2 and 3 d germination. Long-chain fatty acids C_{16}, $C_{16:1}$, C_{18}, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ from these lipid fractions were quantitatively determined during three days germination using GC-FID and GC-MS.

Helianthus annuus

The quantitites of saturated fatty acids C_{16} and C_{18} of neutral lipid extracted from the cotyledons of Helianthus did not vary during 1, 2 and 3 d germination period, constituting 14 - 18 mg g^{-1} dry seed (C_{16}) and 12 - 14 mg g^{-1} dry seed (C_{18}). The unsaturated fatty acid $C_{18:1}$ decreased from 59 mg g^{-1} at 1 d to 44 mg g^{-1} dry seed after 3 d (relative values 54 to 46 respectively). $C_{18:2}$ levels did not vary during the three sampling times (average content of 105 mg g^{-1} dry seed). Similarly, there were no changes in the contents of the saturated fatty acids C_{16} and C_{18} of neutral lipid extracted from the radicles of Helianthus. They constituted about 2 - 4 mg g^{-1} dry seed (C_{16}) and 1 - 2 mg g^{-1} dry seed (C_{18}). Likewise the unsaturated fatty acids $C_{18:1}$ (4 mg g^{-1} dry seed) and $C_{18:2}$ (2 - 5 mg g^{-1} dry seed) did not vary over the three-day period (Tables 28 - 29 and Figure 23).

The C_{16}, C_{18}, $C_{18:1}$ and $C_{18:2}$ contents of phospholipids extracted from the cotyledons of Helianthus decreased after 2 d. C_{16} declined from 12 to 5 mg g^{-1} dry seed (relative values 94 to 61), C_{18} declined from 5 to 1 mg g^{-1} dry seed (relative values

Table 28 Long-chain fatty acids of netural lipids extracted from Helianthus cotyledons during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	16.71	—	12.72	53.77	100	2.10
2	12.62	—	11.13	46.29	100	—
3	18.94	—	14.17	45.79	100	—

Table 29 Long-chain fatty acids of netural lipids extracted from Helianthus radicles during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	227.62	—	116.57	211.05	100	—
2	43.20	—	19.30	79.41	100	—
3	92.90	—	42.90	106.34	100	—

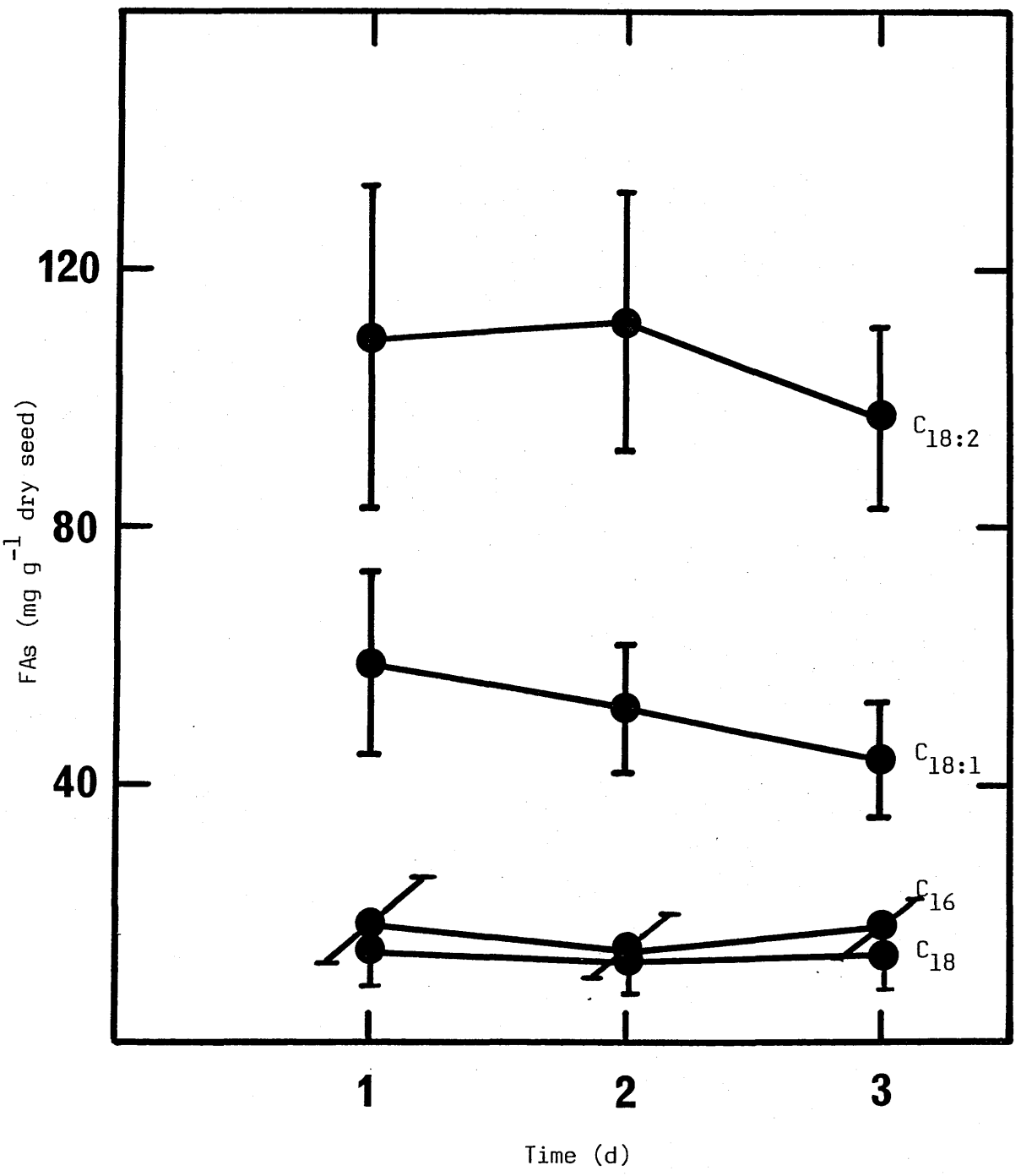


Figure 23 LCFAME of cotyledonary neutral lipid during germination of Helianthus.

40 to 13), $C_{18:1}$ declined from 10 to 8 mg g^{-1} dry seed (yet relative value increased from 77 to 92), and $C_{18:2}$ declined from 13 to 8 mg g^{-1} dry seed. The lower values were sustained at day 3. The contents of the saturated acids C_{16} and C_{18} of phospholipids extracted from radicles increased from 0.3 to 1.1 mg g^{-1} dry seed (C_{16}; relative values 37 to 136), and 0.1 to 0.5 mg g^{-1} dry seed (C_{18}; relative values 13 to 54). For the unsaturated fatty acids $C_{18:1}$ and $C_{18:2}$, there were no changes in their content in phospholipids from radicles; both varied between 0.7 - 1.1 mg g^{-1} dry seed (Tables 30 - 31 and Figure 24).

The change of FA of phospholipids as presented (viz. in relation to the dry weight of the seed) does not truly reflect the situation as FAs metabolise to phospholipid themselves and additionally the PL decrease in amount during the period of germination (Figure 24). In this instance the relative amounts of individual FA more correctly indicates the relative change in the composition of FA of PL (Table 30).

The absolute fatty acid content of glycolipids extracted from the cotyledons of Helianthus all showed a decline at 2 d, similar to that noted for fatty acids in phospholipids but at 3 d, $C_{18:1}$ and $C_{18:2}$ increased. However, at three sampling times their contents did not exceed 2.8 mg g^{-1} dry seed. When considered in level of values relative to $C_{18:2}$ there was an increase in C_{16}, C_{18} and $C_{18:1}$ at day 2. In glycolipids extracted from Helianthus radicles, the fatty acid levels were low, and varied from 0.1 to 0.6 mg g^{-1} dry seed and the relative values of C_{16}, C_{18} and $C_{18:1}$ showed a decline at day 2 (Tables

Table 30. Long-chain fatty acids of phospholipids extracted from Helianthus cotyledons during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	94.93	—	39.97	76.74	100	—
2	61.81	—	12.53	92.24	100	—
3	40.54	—	13.82	88.90	100	—

Table 31. Long-chain fatty acids of phospholipids extracted from Helianthus radicles during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	37.35	—	13.25	87.95	100	—
2	87.84	—	60.81	148.65	100	—
3	135.80	—	54.32	90.12	100	—

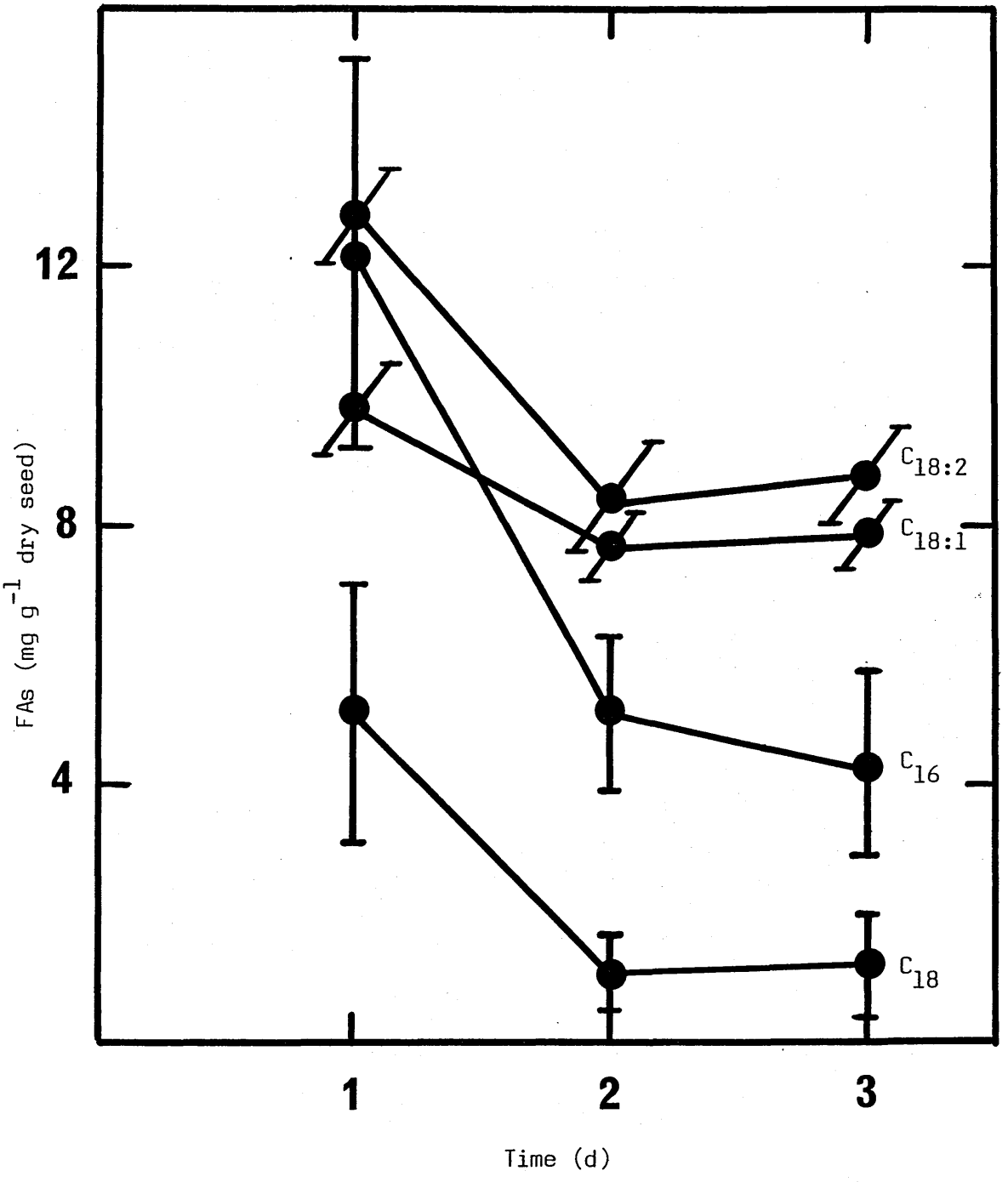


Figure 24 LCFAME of cotyledonary phospholipid during germination of Helianthus.

32 ÷ 34).

In a study of polar lipids of sunflower seeds, Grewal et al. (1978) found that total polar lipids constituted 2.5% of the total lipid. The fatty acids of these polar lipids presented in Table 35 compare with polar lipids in Helianthus used in the experiments reported in this thesis.

Gossypium barbadense

The quantities of saturated fatty acid C_{16} of neutral lipid extracted from the cotyledons of Gossypium decreased from 51 mg g^{-1} dry seed (relative value of 85) at 1 d to 36 mg g^{-1} dry seed (relative value of 75) at 2 d and changed to 43 mg g^{-1} dry seed (yet a large peak, relative value 123) at 3 d. C_{18} increased during 3 d germination from 6.2 mg g^{-1} to 8.3 mg g^{-1} dry seed (relative values 10.3 to 23.7). The unsaturated fatty acids $C_{18:1}$ and $C_{18:2}$ decreased from 42 mg g^{-1} at 1 d to 27 mg g^{-1} dry seed at 3 d and from 61 mg g^{-1} at 1 d to 35 mg g^{-1} dry seed at 3 d respectively, with relative value of $C_{18:1}$ averaging 71. There were no changes in the contents of the saturated fatty acids C_{16} and C_{18} of neutral lipid extracted from the radicles of Gossypium. They constituted about 4 mg g^{-1} dry seed (C_{16}) and 0.5 - 0.7 mg g^{-1} dry seed (C_{18}). Likewise the unsaturated fatty acids $C_{18:1}$ (2.3 - 2.8 mg g^{-1} dry seed) and $C_{18:2}$ (5 mg g^{-1} dry seed) did not vary over the three-day period. The $C_{16:1}$ appeared in the radicles at 1 d (about 2 mg g^{-1} dry seed), with relative value 37 and in a trace amount at 2 d, but $C_{18:3}$ appeared in a trace amount (0.2 mg g^{-1} seed) during the germination period (Tables 36 - 37 and Figure 25).

Table 32. Long-chain fatty acids of glycolipids extracted from Helianthus cotyledons and radicles during germination.

Values are means + SE. ND = not detected.

Germinated seed tissues (d)	Fatty acids (mg g^{-1} seed)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1 d						
Cotyledons	0.62 ± 0.2	ND	0.68 ± 0.2	1.12 ± 0.4	2.32 ± 0.9	ND
Radicle	0.25 ± 0.1	ND	0.38 ± 0.2	0.24 ± 0.1	0.22 ± 0.1	ND
2 d						
Cotyledons	0.38 ± 0.1	ND	0.26 ± 0.1	0.28 ± 0.1	0.39 ± 0.2	ND
Radicle	0.11 ± 0.1	ND	0.14 ± 0.1	0.44 ± 0.2	0.58 ± 0.3	ND
3 d						
Cotyledons	0.26 ± 0.1	ND	0.13 ± 0.1	1.21 ± 0.4	2.75 ± 1.1	ND
Radicle	0.10 ± 0.01	ND	0.10 ± 0.01	0.21 ± 0.1	0.22 ± 0.2	ND

Table 33 Long-chain fatty acids of glycolipids extracted from Helianthus cotyledons during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	26.72	—	29.31	48.28	100	—
2	97.44	—	66.67	60.26	100	—
3	11.27	—	4.73	44.00	100	—

Table 34 Long-chain fatty acids of glycolipids extracted from Helianthus radicles during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	113.64	—	172.73	109.10	100	—
2	18.97	—	24.14	75.86	100	—
3	47.72	—	47.72	95.45	100	—

Table 35. Comparison of polar lipids in Helianthus.

FA	Polar lipids extracted from Helianthus (% of total pL)			
	Cotyledons + Radicles during germination			Dry whole seeds
	1 d	2 d	3 d	(Grewal et al., 1978)
C_{16}	29.3	20.0	16.0	14.8
C_{18}	12.2	4.0	4.0	3.0
$C_{18:1}$	24.4	31.2	31.2	13.3
$C_{18:2}$	34.1	44.8	48.8	68.7

Table 36. Long-chain fatty acids of netural lipids extracted from Gossypium cotyledons during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	84.79	___	10.25	68.91	100	___
2	75.22	___	15.96	66.44	100	___
3	122.67	___	23.65	77.35	100	___

Table 37. Long-chain fatty acids of netural lipids extracted from Gossypium radicles during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	82.56	37.36	9.96	51.92	100	4.41
2	84.01	0.39	10.60	54.14	100	3.85
3	70.08	___	12.55	44.59	100	5.41

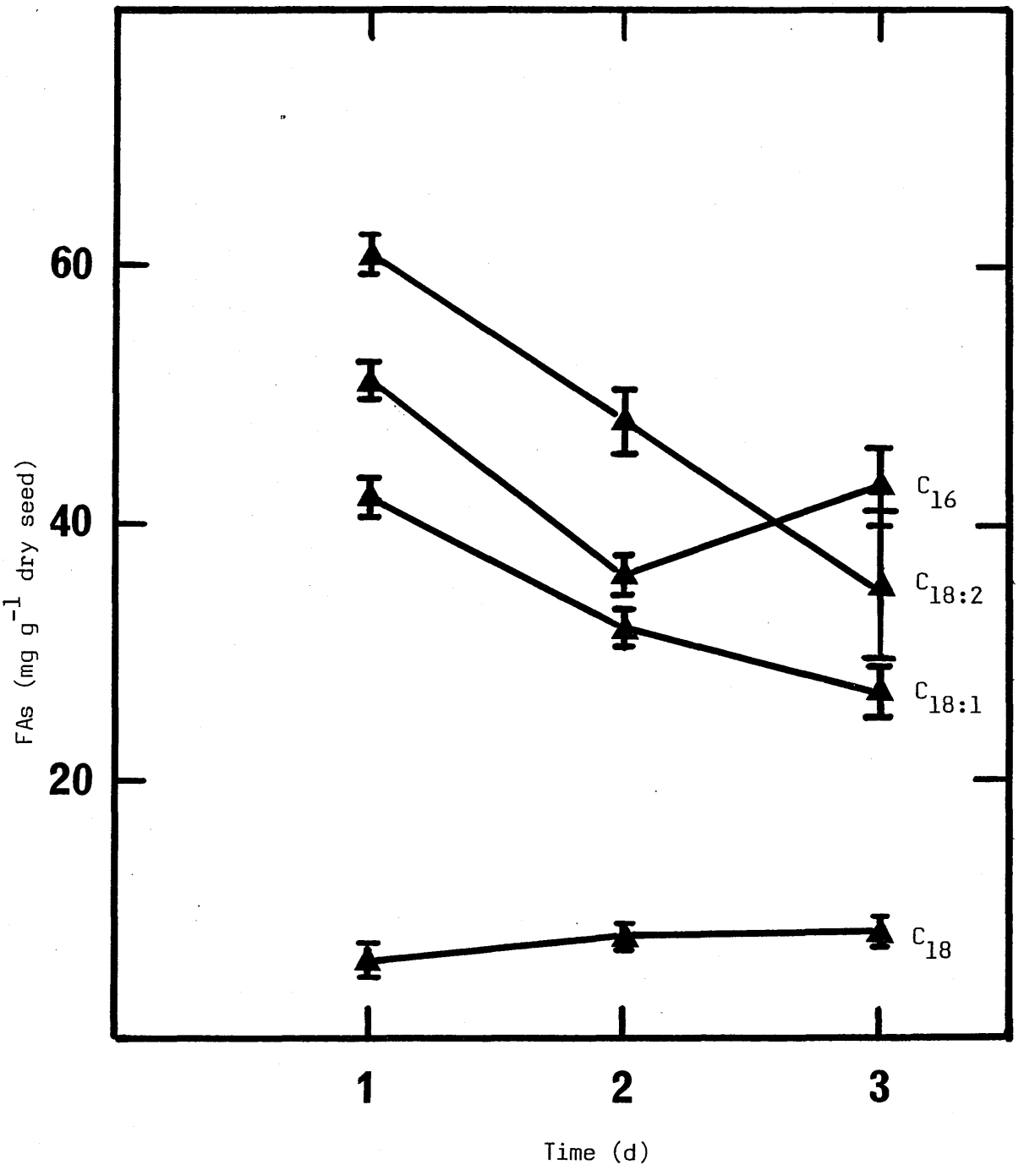


Figure 25 LCFAME of cotyledonary neutral lipid during germination of Gossypium.

The C_{16}, C_{18}, $C_{18:1}$ and $C_{18:2}$ contents of phospholipids extracted from the cotyledons of Gossypium did not vary over the three-day germination period - C_{16} (average 4.8 mg g^{-1} dry seed with relative values from 47 to 44 between 1 d and 3 d), C_{18} (average 0.4 mg g^{-1} dry seed, with relative value 3), $C_{18:1}$ (average 4.4 mg g^{-1} dry seed, with relative values 45 to 39) and $C_{18:2}$ (average 10.4 mg g^{-1} dry seed). $C_{18:3}$ (average 0.23 mg g^{-1} dry seed) appeared at 2 and 3 d with relative value 2. There were no changes in the contents of the fatty acids of phospholipids from the radicles. The values relative to $C_{18:2}$ slightly increased for C_{16} (from 70 - 73), C_{18} (from 5 - 7) and slightly decreased for $C_{18:1}$ (from 30 - 27) during the germination period (Tables 38 - 39 and Figure 26).

The three fatty acids C_{16}, $C_{18:1}$ and $C_{18:2}$ of glycolipids extracted from the cotyledons appeared during the three sampling times: C_{16} increased from 0.8 - 1.6 mg g^{-1} dry seed, with relative values 25 to 38, $C_{18:1}$ from 1.2 to 1.9 mg g^{-1} dry seed, with relative values 39 to 48, and $C_{18:2}$ changed slightly from 3.2 to 3.9 mg g^{-1} dry seed. In glycolipids extracted from Gossypium radicles, the fatty acid levels were low, and C_{16} and $C_{18:2}$ varied from 0.1 - 0.4 mg g^{-1} dry seed but $C_{18:1}$ and $C_{18:3}$ were at levels less than 0.1 mg g^{-1} dry seed and the relative values of C_{16} showed an increase from 90 to 119 after 3 d (Tables 40 - 42).

Direct comparisons of the data obtained from extracts of intact seeds/seedlings and lipid classes are possible (Tables 43 - 58), and it can be seen that there is reasonably close

Table 38. Long-chain fatty acids of phospholipids extracted from Gossypium cotyledons during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	47.30	—	3.26	44.97	100	—
2	47.77	—	3.53	41.54	100	2.08
3	43.88	—	3.22	39.28	100	2.48

Table 39. Long-chain fatty acids of phospholipids extracted from Gossypium radicles during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	69.90	—	4.85	30.10	100	21.36
2	66.67	—	5.56	29.91	100	21.36
3	72.79	—	6.80	27.09	100	18.37

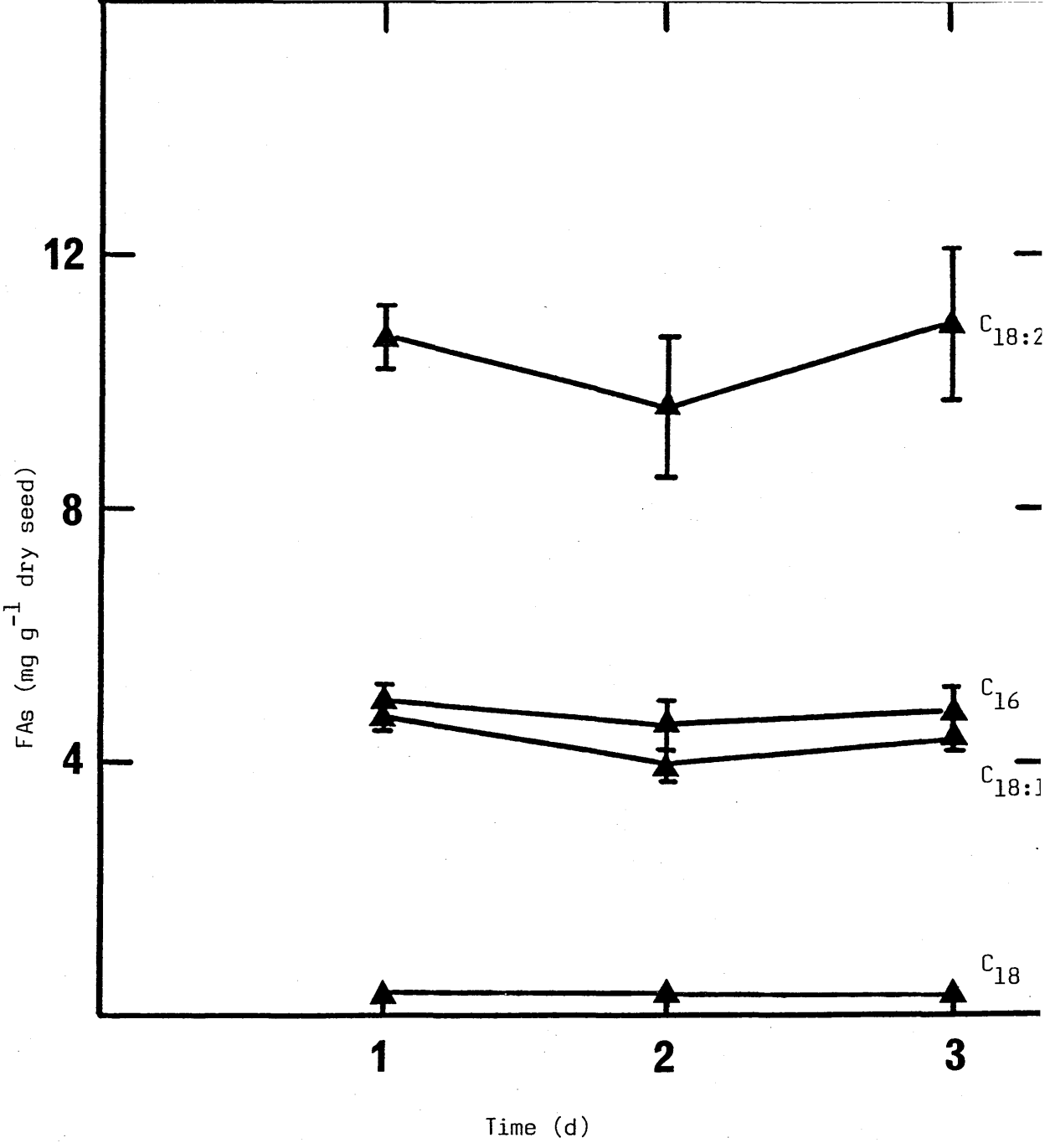


Figure 26 LCFAME of cotyledonary phospholipid during germination of Gossypium.

Table 40. Long-chain fatty acids of glycolipids extracted from Gossypium cotyledons and radicles during germination.
Values are means ± SE. ND - not detected.

Germinated seed tissues (d)	Fatty acids (mg g^{-1} dry seed)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1 d						
Cotyledons	0.78 ± 0.2	ND	ND	1.24 ± 0.3	3.17 ± 0.4	ND
Radicle	0.18 ± 0.03	ND	ND	0.07 ± 0.02	0.20 ± 0.04	0.03 ± 0.01
2 d						
Cotyledons	0.91 ± 0.3	ND	ND	1.42 ± 0.33	3.16 ± 0.6	ND
Radicle	0.29 ± 0.05	ND	ND	0.09 ± 0.02	0.26 ± 0.03	0.03 ± 0.01
3 d						
Cotyledons	1.63 ± 0.4	ND	ND	1.86 ± 0.4	3.85 ± 0.7	ND
Radicle	0.38 ± 0.06	ND	ND	0.09 ± 0.03	0.32 ± 0.03	0.04 ± 0.01

Table 41. Long-chain fatty acids of glycolipids extracted from Gossypium cotyledons during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	24.61	—	—	39.12	100	—
2	28.80	—	—	44.93	100	—
3	37.66	—	—	48.31	100	—

Table 42. Long-chain fatty acids of glycolipids extracted from Gossypium radicles during germination.

Germination time (d)	Fatty acids relative to $C_{18:2}$ (100)					
	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
1	90.00	—	—	35.00	100	15.00
2	113.46	—	—	34.62	100	11.54
3	118.75	—	—	28.13	100	12.50

Table 43. C_{16} of Helianthus cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	32.2	18.3	12.2	0.6	31.1
2	19.3	14.1	5.1	0.4	19.6
3	22.7	18.3	4.3	0.3	22.9

Table 44. C_{16} of Helianthus radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	4.6	4.1	0.3	0.2	4.6
2	3.2	2.4	0.7	0.1	3.2
3	4.5	3.1	1.1	0.1	4.3

Table 45. C_{18} of *Helianthus* cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	19.7	13.9	5.1	0.7	19.7
2	13.8	12.4	1.1	0.3	13.8
3	15.1	13.7	1.2	0.1	15.0

Table 46. C_{18} of *Helianthus* radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	2.6	2.1	0.1	0.4	2.6
2	1.8	1.1	0.5	0.1	1.7
3	2.0	1.4	0.4	0.1	1.9

Table 47. $C_{18:1}$ of Helianthus cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	69.7	58.8	9.8	1.1	69.7
2	59.6	51.7	7.7	0.3	59.7
3	52.1	44.1	7.7	1.1	52.9

Table 48. $C_{18:1}$ of Helianthus radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	4.7	3.8	0.7	0.2	4.7
2	5.8	4.3	1.1	0.4	5.8
3	4.7	3.5	0.7	0.2	4.4

Table 49. $C_{18:2}$ of Helianthus cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	120.8	105.4	11.8	2.1	119.3
2	123.8	111.7	8.4	0.4	120.5
3	111	96.8	8.8	2.8	108.4

Table 50. $C_{18:2}$ of Helianthus radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	2.8	1.8	0.8	0.2	2.8
2	6.8	5.4	0.7	0.6	6.7
3	3.4	3.1	0.7	0.1	3.9

Table 51. C_{16} of Gossypium cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	63.2	51.3	5.1	0.8	57.2
2	43.3	35.8	4.6	0.9	41.3
3	53.5	42.9	4.8	1.6	49.3

Table 52. C_{16} of Gossypium radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	4.6	4.3	0.7	0.2	5.2
2	7.4	4.4	0.8	0.3	5.7
3	4.4	3.6	1.1	0.4	5.1

Table 53. C_{18} of Gossypium cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	7.2	6.2	0.4	0.0	6.6
2	9.3	7.6	0.3	0.0	7.9
3	8.8	8.3	0.4	0.0	8.7

Table 54. C_{18} of Gossypium radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	0.5	0.5	0.05	0.0	0.55
2	0.8	0.6	0.07	0.0	0.67
3	0.6	0.6	0.10	0.0	0.70

Table 55. $C_{18:1}$ of Gossypium cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	45.5	41.7	4.8	1.2	47.7
2	41.3	31.6	4.0	1.4	37.0
3	40.5	27	4.4	1.9	33.3

Table 56. $C_{18:1}$ of Gossypium radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	3.4	2.7	0.3	0.07	3.07
2	2.5	2.8	0.3	0.09	3.19
3	3.0	2.3	0.4	0.09	2.79

Table 57. $C_{18:2}$ of Gossypium cotyledons

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	60.5	60.5	10.7	3.2	74.4
2	64.9	47.5	9.6	3.2	60.3
3	41	34.9	10.9	3.9	49.7

Table 58. $C_{18:2}$ of Gossypium radicles

Germination time (d)	FA (mg g^{-1} dry seed)				
	Total FA	Lipid classes			
		NL	PL	GL	Total
1	7.2	5.2	1.0	0.2	6.4
2	5.3	5.1	1.2	0.3	6.6
3	6.6	5.2	1.4	0.3	6.9

correspondence betwen the results. When the data for quantification of fatty acids for the three lipid fractions are considered in terms of mg g^{-1} lipid, then the overall pattern for the individual fatty acids does not alter, although the values differ (e.g. C_{18} in the neutral lipids of Helianthus cotyledons was 14 mg g^{-1} dry seed after 1 d, 12 mg g^{-1} dry seed after 2 d, and 14 mg g^{-1} dry seed after 3 d, compared with 43 mg g^{-1} lipid after 1 d, 36 mg g^{-1} lipid after 2 d, and 44 mg g^{-1} lipid after 3 d.

In a study of polar lipids from cottonseeds (G. hirsutum), Karshiev et al. (1981) extracted polar lipids from two varieties of cotton, 108-F and 5904-I, using solvent system chloroform-methanol (2:1, v/v). The fatty acids of these polar lipids extracted from two varieties presented in Table 59 compare with polar lipids of G. barbadense in this thesis.

For neutral lipids, Yoshida (1984) reported that the triglycerides of soybean declined and became minor components by 12 d germination, but quantitative estimations were not carried out. Khor and Cheang, 1984, studied the changes of fatty acids for 56 d so-called 'germination' of palm seed; C_{16} decreased from 18 - 11% of the total lipid after 35 d, $C_{18:1}$ decreased from 16 - 14% after 21 d but increased to 25% after 56 d, and $C_{18:2}$ decreased from 28 - 7% of the total lipid during 56 d. They also found that neutral lipids and glycolipids increased slightly, but phospholipids decreased rapidly during the germination period.

Table 59. Comparison of polar lipids in Gossypium.

ND = not detected nd = not determined

FA	Polar lipids extracted from Gossypium (% of total PL)				
	G. barbadense variety: Giza 66 Cotyledons + Radicle during germination			G. hirsutum Karshiev et al. (1981) Dry whole seeds Two varieties	
	1 d	2 d	3 d	108-F	5904-I
C_{10}	nd	nd	nd	1.2	nd
C_{12}	nd	nd	nd	2.7	3.2
C_{14}	nd	nd	nd	2.2	4.0
C_{16}	23.7	21.8	22.7	26.4	20.7
$C_{16:1}$	ND	ND	ND	1.4	3.0
C_{18}	2.2	2.3	2.2	1.7	4.0
$C_{18:1}$	19.6	18.2	19.6	17.5	21.1
$C_{18:2}$	45.7	45.5	47.8	46.9	31.9

4. Short-chain fatty acid methyl esters (SCFAME)

Short-chain fatty acids were extracted by co-distilling in steam from ground dry seeds, cotyledons, radicles, pericarp and testa of both Helianthus and Gossypium at intervals of 16, 24, 40, 48, 64 and 72 h during germination. These SCFA were methylated by diazomethane and determined by GC-FID and GC-MS (See Appendix). The major SCFAME in both species were caprylic (C_8), pelargonic (C_9), capric (C_{10}) and lauric (C_{12}).

Quantification of SCFAME in extracts may be complicated by the nature of the extraction system. Losses of these volatile components can occur and any losses may have been erratic. The data are presented as both μg g^{-1} extract, which is arguably less reliable than μg g^{-1} dry seed, and as relative to C_{12} (100).

In Tables 60 and 61 the values of the two or three replicate extracts at each of the six sampling times for both Helianthus and Gossypium are given as mg g^{-1} seed. There was considerable variation in the values, confirming the decision to use μg SCFAME g^{-1} extract.

Helianthus - whole seed

In dry seed of Helianthus C_8 increased markedly at 64 h from an average of 352 μg g^{-1} extract during 16 - 64 h to 2100 μg g^{-1} extract at 72 h. The values relative to C_{12}, however, showed a peak (600) at 24 h. After rising to 800 μg g^{-1} extract at 24 h, C_9 content declined to about 160 μg g^{-1} extract at 64 h but increased to 500 μg g^{-1} extract at 72 h. There was a large peak (800) in relative

Table 60. Raw data of co-distilling in steam from ground dry Helianthus seeds (2 - 3 replicates).

Germination time (h)	Total extract (mg g^{-1} seed)	SCFA ug g^{-1} extract				SCFA ug g^{-1} seed			
		C_8	C_9	C_{10}	C_{12}	C_8	C_9	C_{10}	C_{12}
16	430	384	512	67	430	165	220	29	185
	224	316	287	36	169	70	64	8	38
	306	350	402	48	302	167	123	15	92
24	294	618	870	654	115	181	256	192	34
	493	565	660	492	70	278	325	243	35
40	406	190	299	908	552	77	121	369	224
	242	146	178	716	399	35	43	173	399
48	385	298	232	1090	4388	114	89	420	1689
	522	335	327	1235	4738	174	171	645	2473
	202	267	161	975	4074	54	33	197	823
64	389	397	210	794	1320	154	82	309	513
	202	322	135	653	1140	65	27	132	230
72	392	2086	489	252	4182	818	192	99	1639
	522	2339	657	292	4554	1221	343	152	2377
	228	1875	355	236	3864	428	81	54	881

Table 61. Raw data of co-distilling in steam from ground dry Gossypium seeds (2 - 3 replicates).

ND = not detected.

Germination time (h)	Total extract (mg g^{-1} seed)	SCFA ug g^{-1} extract				SCFA ug g^{-1} seed			
		C_8	C_9	C_{10}	C_{12}	C_8	C_9	C_{10}	C_{12}
16	352	1244	1358	449	1731	438	478	158	609
	185	966	1054	361	1401	179	195	67	259
	241	1091	1188	391	1548	263	286	94	373
24	244	119	829	1374	1464	29	202	335	357
	255	91	636	1114	1218	23	162	284	311
40	332	1061	743	1249	1278	352	246	415	424
	176	878	615	1102	1044	155	108	194	184
48	333	110	195	479	724	37	65	160	241
	198	85	153	410	538				
64	351	100	177	452	657	35	62	159	231
	204	80	147	395	486	16	30	81	99
72	356	ND	78	70	178	ND	28	25	63
	224	ND	44	42	117	ND	10	9	26
	171	ND	28	37	94	ND	5	6	16

value at 24 h. For C_{10}, there was a peak at 48 h (1100 µg g^{-1} extract), but relative to C_{12}, there was a peak (600) at 24 h. Two peaks were noted for C_{12}, at 48 h (4400 µg g^{-1} extract) and 72 h (4200 µg g^{-1} extract).

Gossypium - whole seed

In dry seed of Gossypium C_8 showed two peaks at 16 h (1100 µg g^{-1} extract, relative value of 71) and 40 h (1000 µg g^{-1} extract, relative value of 83), and C_8 was not detected at 72 h. The highest peak of C_9 was 1200 µg g^{-1} extract (relative value of 77) at 16 h, decreased up to 50 µg g^{-1} extract (relative value of 39) at 72 h, but the lowest peak (10) in relative value at 48 h. After rising to 1200 µg g^{-1} extract (relative value of 100) at 40 h, C_{10} declined to 50 µg g^{-1} extract (relative value 39) at 72 h. Two peaks were noted for C_{12}, at 16 h (1560 µg g^{-1} extract) and 48 (3100 µg g^{-1} extract). Likewise C_8, C_9 and C_{10}, the lowest C_{12} peak (130 µg g^{-1} extract) at 72 h (Tables 62 - 63 and Figure 27).

I. Helianthus - seed tissues

SCFA C_8 in the cotyledons increased at 48 h (1600 µg g^{-1} extract, relative value of 42) and 72 h (1900 µg g^{-1} extract, relative value of 158). There was a large peak (219) in relative value at 16 h. C_8 was not detected at 16 h in the radicles, the levels were low from an average of 144 µg g^{-1} extract during five sampling times. After rising to 1000 µg g^{-1} extract (relative value 45) at 48 h in the pericarp and testa, C_8 declined to 150 µg g^{-1} extract (relative value 23) at 64 h, but increased to 1500 µg g^{-1} extract (large peak with relative

Table 62. Short-chain fatty acid contents during germination of Gossypium and Helianthus.

Values are means ± SE. ND = not detected

Germinated whole seed (h)	Fatty acids ($\mu g \cdot g^{-1}$ extract)			
	C_8	C_9	C_{10}	C_{12}
16 h				
Cotton	1100 ± 13	1200 ± 15	400 ± 4	1560 ± 16
Sunflower	350 ± 14	400 ± 12	50 ± 6	300 ± 13
24 h				
Cotton	100 ± 3	700 ± 12	1200 ± 16	1300 ± 15
Sunflower	600 ± 17	800 ± 10	600 ± 14	100 ± 6
40 h				
Cotton	1000 ± 13	700 ± 9	1200 ± 16	1200 ± 16
Sunflower	160 ± 1.5	200 ± 3	780 ± 11	450 ± 9
48 h				
Cotton	600 ± 7	300 ± 4	400 ± 5	3100 ± 32
Sunflower	300 ± 3	240 ± 3	1100 ± 13	4400 ± 33
64 h				
Cotton	93 ± 1.5	167 ± 2.4	433 ± 4.2	600 ± 9
Sunflower	350 ± 4	160 ± 4	700 ± 8	1200 ± 11
72 h				
Cotton	ND	50 ± 2.5	50 ± 4.5	130 ± 2
Sunflower	2100 ± 23	500 ± 5	260 ± 2	4200 ± 34

Table 63. Short-chain fatty acid contents during germination of Gossypium and Helianthus.

Germinated whole seed (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16 h				
Cotton	70.5	76.9	25.6	100
Sunflower	116.7	133.3	16.7	100
24 h				
Cotton	7.7	53.8	92.3	100
Sunflower	600.0	800.0	600.0	100
40 h				
Cotton	83.3	58.3	100.0	100
Sunflower	35.6	44.4	173.3	100
48 h				
Cotton	19.4	9.7	12.9	100
Sunflower	6.8	5.5	25.0	100
64 h				
Cotton	15.5	27.8	72.2	100
Sunflower	29.2	13.3	58.3	100
72 h				
Cotton	—	38.5	38.5	100
Sunflower	50.0	11.9	6.2	100

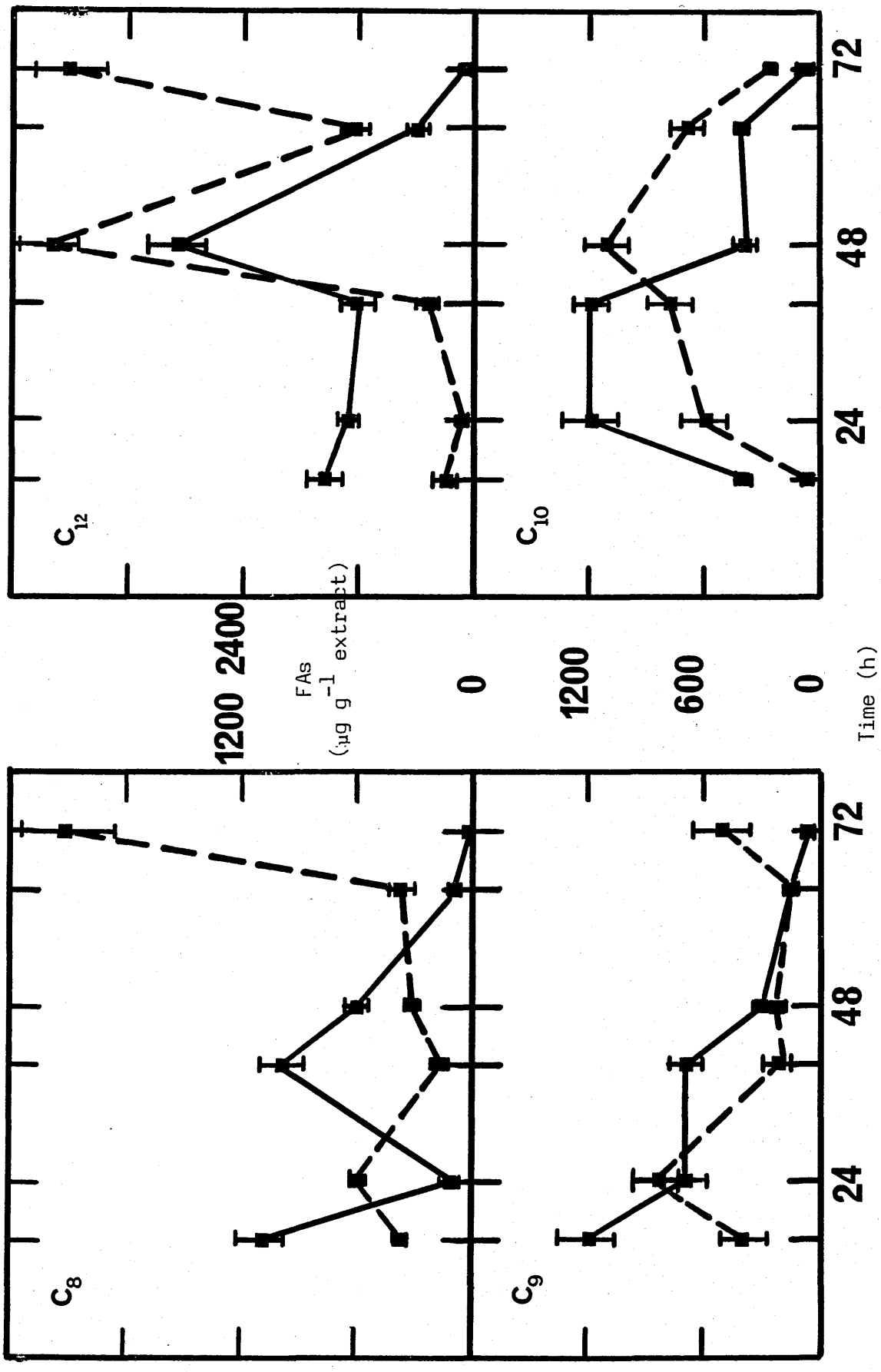


Figure 27 SCFAME during germination of Helianthus (broken line) and Gossypium (solid line) seeds.

value 143) at 72 h (Figure 28).

In the cotyledons of Helianthus, the absolute levels of C_9 was highest at 48 h (1200 µg g^{-1} extract) and varied from 240 - 700 µg g^{-1} extract during other sampling times. There was a large peak (188) in relative value at 16 h. The highest content of C_9 in the radicles was at 24 h (1200 µg g^{-1} extract, relative value of 94) with an average of 380 µg g^{-1} extract at 16, 48, 64 and 72 h but there was peak (200) in relative value at 64 h. The highest two peaks of C_9 in the pericarp and testa were at 48 h (1200 µg g^{-1} extract, relative value 55) and 72 h (1500 µg g^{-1} extract, relative value 142); the lowest content was 160 µg g^{-1} extract at 64 h (Figure 29).

In the cotyledons of Helianthus C_{10} contents increased from 200 µg g^{-1} extract at 16 h to the highest level (2000 µg g^{-1} extract) at 48 h. There were also two peaks at 64 h (1800 µg g^{-1} extract, relative value 95) and 72 h (1500 µg g^{-1} extract, relative value 125). The levels of C_{10} varied from 150 µg g^{-1} extract at 64 h to 400 µg g^{-1} extract at 48 h in the radicles. There was a peak (75) in relative value at 64 h. In the pericarp and testa, the highest C_{10} contents were at 24 h (600 µg g^{-1} extract, relative value 30) and 48 h (700 µg g^{-1} extract, relative value 32), and there was a peak (52) in relative value at 72 h (Figure 30).

C_{12} levels increased in the cotyledons from 320 µg g^{-1} extract at 16 h to 3800 µg g^{-1} extract at 48 h, and declined thereafter to 1200 µg g^{-1} extract at 72 h (Figure 31). The highest content of C_{12} in the radicles was at 24 h (1280 µg g^{-1} extract) and the lowest content at 64 h (200 µg g^{-1} extract).

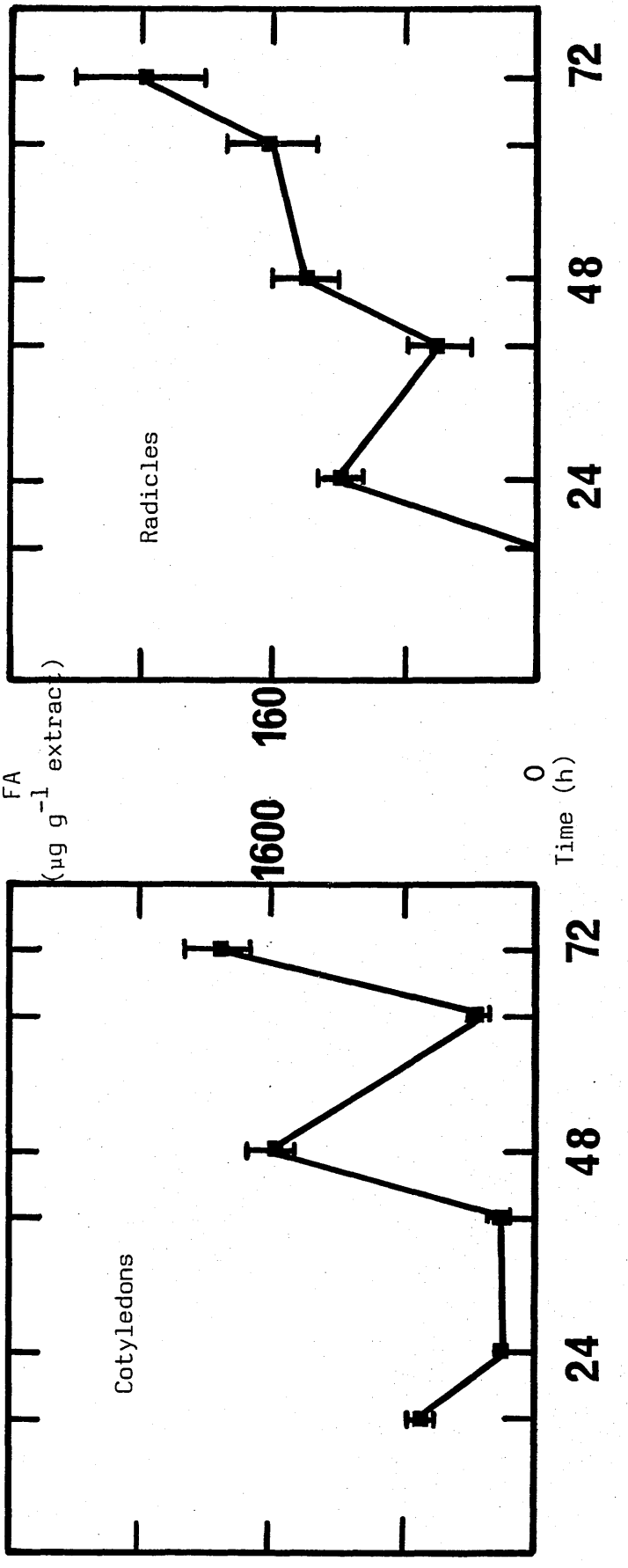


Figure 28 FAME C_8 during germination of Helianthus.

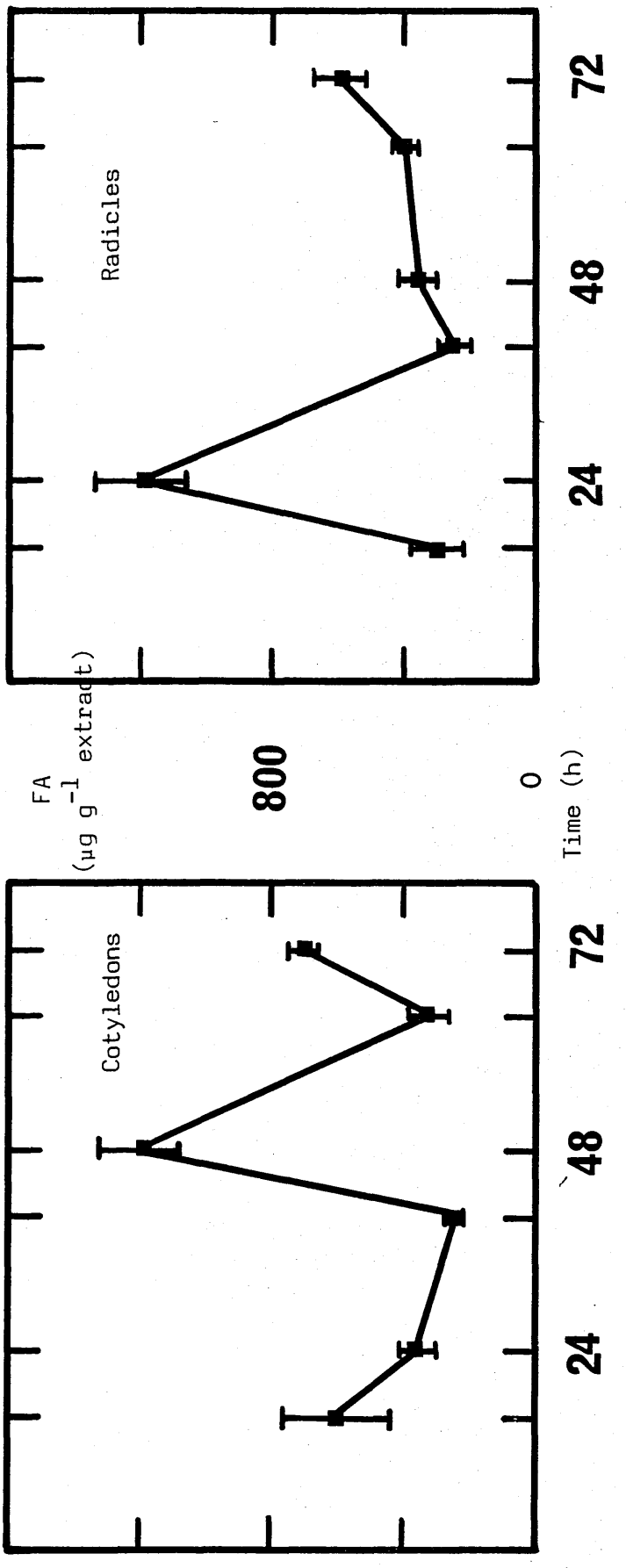


Figure 29 FAME C_9 during germination of Helianthus.

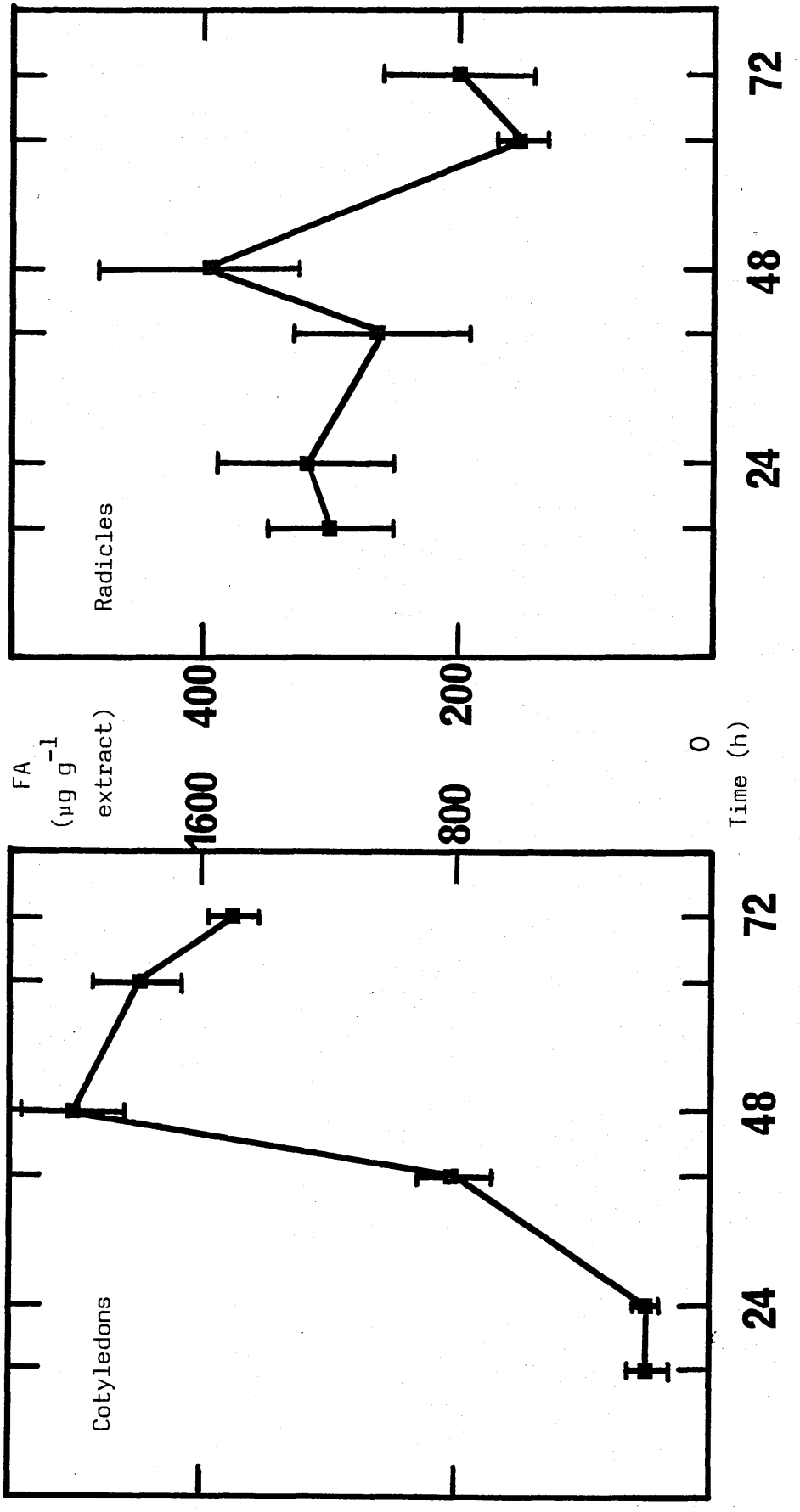


Figure 30 FAME C_{10} during germination of Helianthus.

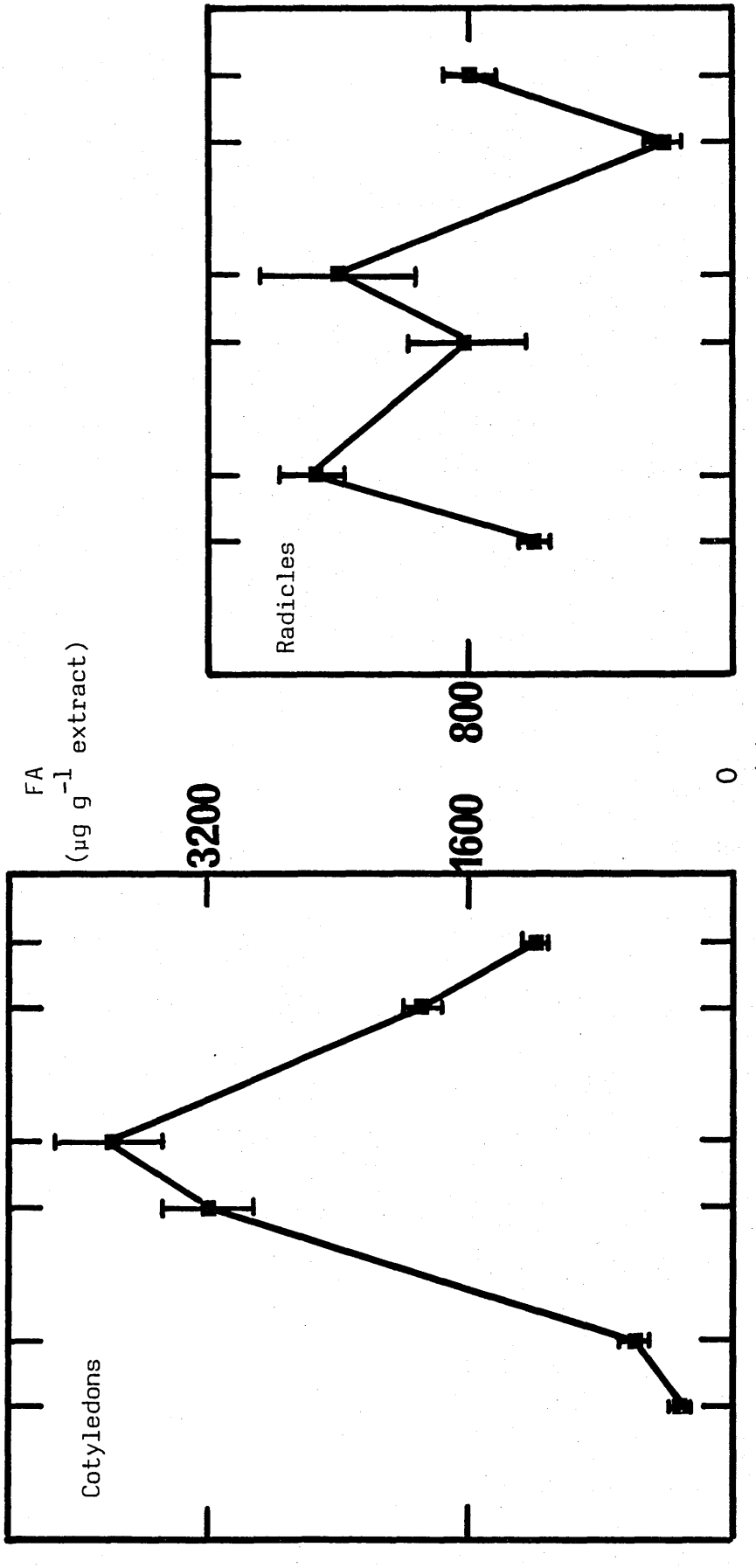


Figure 31 FAME C_{12} during germination of Helianthus.

Table 64. Short-chain fatty acids extracted from Helianthus cotyledons during germination.

Germination time (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16	218.75	187.50	62.50	100
24	33.33	60.00	33.33	100
40	6.25	7.50	25.00	100
48	42.11	31.58	52.63	100
64	16.84	16.84	94.74	100
72	158.33	58.33	125.00	100

Table 65. Short-chain fatty acids extracted from Helianthus radicles during germination.

Germination time (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16	0.00	50.00	50.00	100
24	9.38	93.75	25.00	100
40	7.50	30.00	32.50	100
48	11.67	30.00	33.33	100
64	80.00	200.00	75.00	100
72	30.00	75.00	25.00	100

Table 66. Short-chain fatty acids extracted from Helianthus pericarp and testa during germination.

Germination time (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16	12.50	25.00	16.67	100
24	40.00	40.00	30.00	100
40	27.78	11.11	9.72	100
48	45.45	54.55	31.82	100
64	23.08	24.62	10.77	100
72	142.86	142.86	52.38	100

The average content of C_{12} in the pericarp and testa was 2370 µg g^{-1} extract during the first 48 h, but decreased to 650 µg g^{-1} extract at 64 h (Tables 64 - 66 and Figures 28 - 31). These results showed that highest contents of SCFA C_8, C_9, C_{10} and C_{12} in the cotyledons and C_{10} and C_{12} in the radicles occurred at 48 h.

II. Gossypium - seed tissues

SCFA C_8 content in the cotyledons varied from 60 µg g^{-1} extract at 24 h to 420 µg g^{-1} extract at 48 h. There was a large peak (100) in relative value at 40 h. In the radicles, C_8 was at its highest content at 16 h (600 µg g^{-1} extract, relative value 43) with an average of 150 µg g^{-1} extract at 24, 40, 48 and 64 h, but C_8 was not detected at 72 h. The highest content of C_8 in the testa was 417 µg g^{-1} extract (relative value 69) at 16 h, with an average of 111 µg g^{-1} extract at 40, 48, 64 and 72 h, but C_8 was not detected at 24 h (Figure 32).

In the cotyledons of Gossypium the absolute level of C_9 ranged from 350 to 700 µg g^{-1} extract during 48 h but decreased to 160 µg g^{-1} extract at 72 h. There were two peaks relative to C_{12} at 24 h (139) and 64 h (200). After rising to 1200 µg g^{-1} extract at 40 h, C_9 declined in the radicles to 250 µg g^{-1} extract at 72 h. There was a peak (200) in relative value at 64 h. The highest C_9 content in the testa was 800 µg g^{-1} extract (relative value 42) at 40 h, but C_9 was not detected at 16 h (Figure 33).

In the cotyledons, C_{10} contents were highest at 24 h

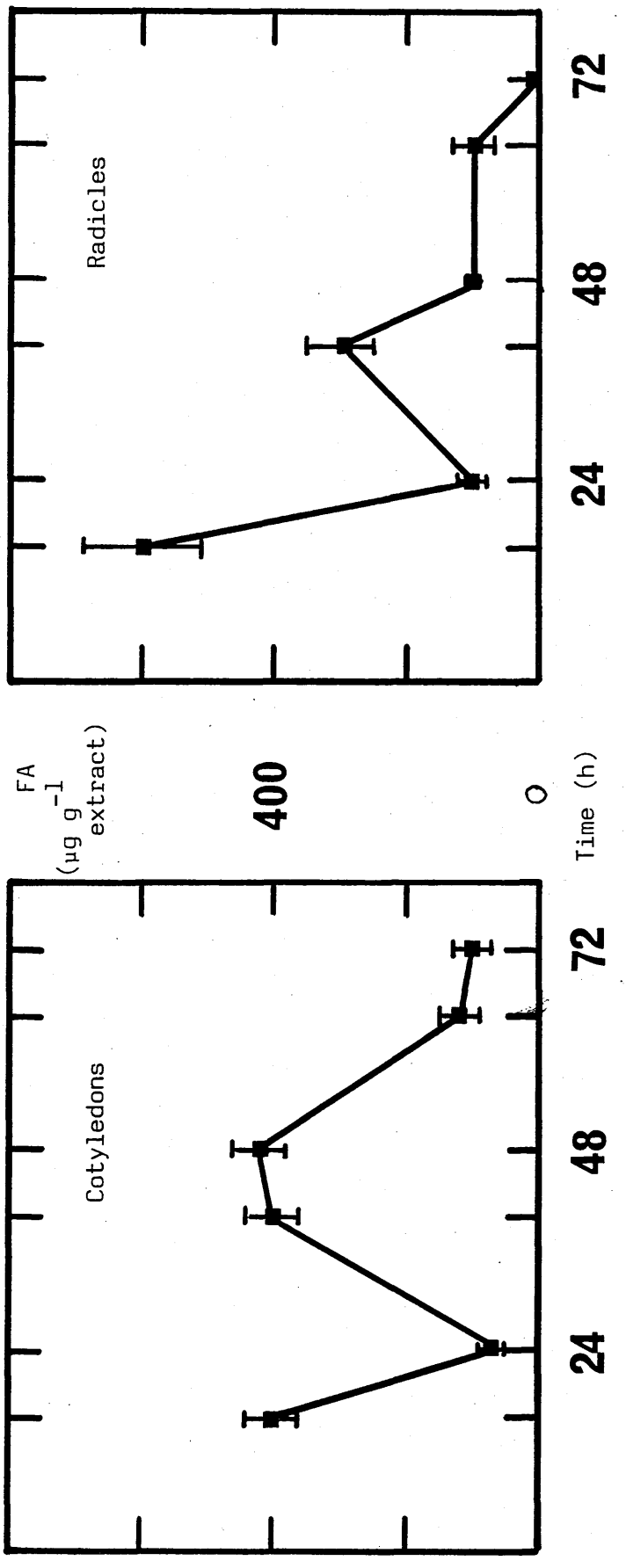


Figure 32 FAME C_8 during germination of Gossypium.

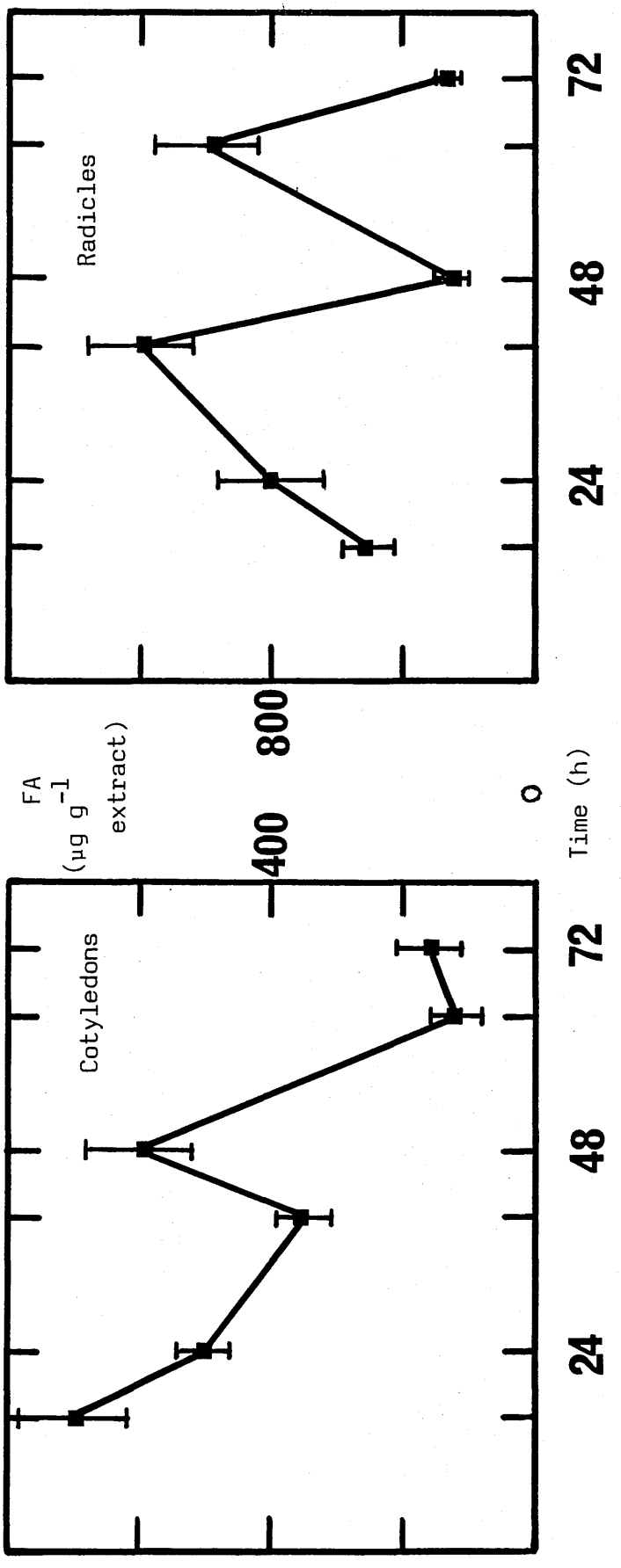


Figure 33 FAME C_9 during germination of Gossypium.

(1000 μg g^{-1} extract, relative value 278) and at 72 h (800 μg g^{-1} extract, relative value 114), but varied from 110 to 320 μg g^{-1} extract at other sampling times. In the radicles, the absolute level of C_{10} was 600 μg g^{-1} extract at 40 h and 72 h, varying from 200 to 360 μg g^{-1} extract at other times. There was a peak (72) in relative value at 64 h. In the testa, C_{10} levels varied from 50 μg g^{-1} extract at 48 h to 200 μg g^{-1} extract at 24, 40 and 72 h (Figure 34).

The highest level of C_{12} in the cotyledons of Gossypium was 4200 μg g^{-1} extract at 48 h, and the lowest content 360 μg g^{-1} extract at 24 h (Figure 35). The highest content of C_{12} in the radicles was 1960 μg g^{-1} extract at 40 h and the lowest 500 μg g^{-1} extract at 64 h, but in the testa the highest content was 2400 μg g^{-1} extract at 24 and 72 h and the lowest content was 300 μg g^{-1} extract at 64 h. These results showed that the highest contents of C_8 and C_{12} occurred in the cotyledons at 48 h, the highest content of C_9 and C_{12} occurred in the radicles at 40 h and in the testa the levels of C_{10} and C_{12} occurred at 72 h (Tables 67 - 69 and Figures 32 - 35).

Very few studies of SCFA during germination have been reported. Sreenivasan (1968) showed that dry cottonseed contained C_{10} (0.5%) and C_{12} (0.4% of the total fatty acids). Cheang and Stumpf (1983) reported that kernel of germinating oil palm seedling contained C_6 and C_8 (3.5%), C_{10} (3.8%) and C_{12} (48.3% of the total fatty acids).

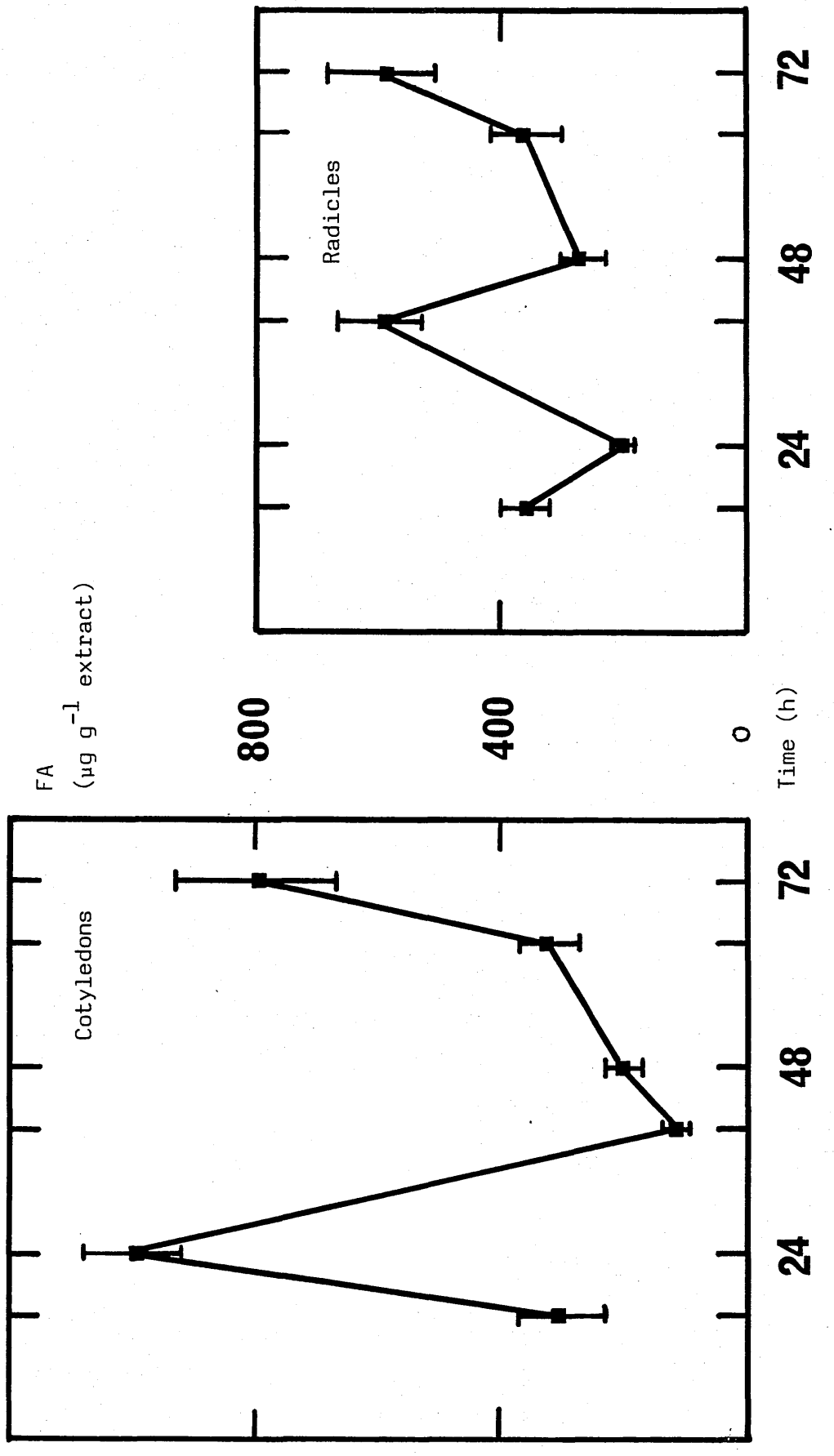


Figure 34 FAME C_{10} during germination of Gossypium.

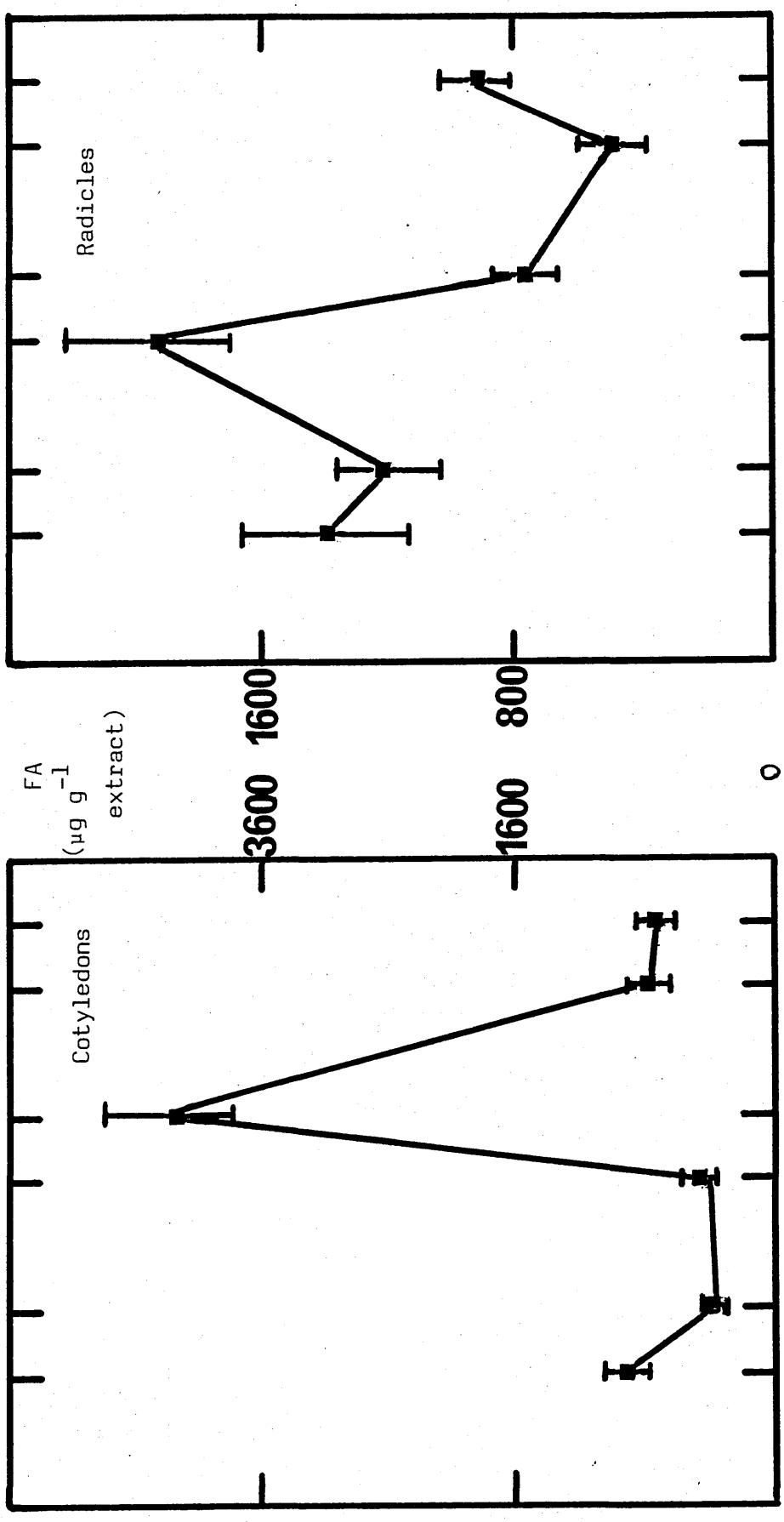


Figure 35 FAME C_{12} during germination of Gossypium.

Table 67. Short-chain fatty acids extracted from Gossypium cotyledons during germination.

Germination time (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16	44.44	77.78	33.33	100
24	16.67	138.89	277.78	100
40	100.00	87.50	27.50	100
48	10.00	14.29	4.76	100
64	16.67	200.00	72.00	100
72	14.29	22.86	114.29	100

Table 68. Short-chain fatty acids extracted from Gossypium radicles during germination.

Germination time (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16	42.86	35.71	25.71	100
24	8.33	66.67	16.67	100
40	15.31	61.22	30.61	100
48	13.64	31.79	36.43	100
64	20.00	200.00	72.00	100
72	——	26.32	63.16	100

Table 69. Short-chain fatty acids extracted from Gossypium testa during germination.

Germination time (h)	Fatty acids relative to C_{12} (100)			
	C_8	C_9	C_{10}	C_{12}
16	68.93	——	21.98	100
24	——	8.33	8.33	100
40	12.63	42.11	30.61	100
48	25.00	25.00	12.00	100
64	21.67	33.33	26.67	100
72	1.67	25.00	8.33	100

5. Lipid storage organelles

This section concentrates on spherosomes (oleosomes), but related studies were carried out for comparative purposes on mitochondria and endoplasmic reticulum in order to establish the lipid and fatty acid composition of these organelles.

5.1 Characterisation of spherosomes (oleosomes)

Spherosomes were isolated from the cotyledons of dry seeds and seedlings of both Helianthus and Gossypium after 1, 2 and 3 d germination. Isolated spherosomes were studied morphologically by electron microscopy and chemically by analysis of lipids and fatty acids using GC-FID and GC-MS.

5.1.1 Morphological studies

Isolated spherosomes were fixed in glutaraldehyde; in some experiments the fixed spherosomes were post-fixed in osmium tetroxide (Materials and Methods, 3.7.4.2.).

I. Helianthus

Inspection of electron micrographs revealed that the cotyledonary cells of 3 d-old sunflower seedlings possessed spherosomes around 0.5 - 1 μm in diameter (Figures 36 - 38).

These spherosomal preparations were surrounded by microbodies (Figure 37a and b) and bounded by a half-unit membrane (Figure 37b), and became darkened after contact with osmium tetroxide and lost their classical spherical shape (Figure 38) owing to dehydration by osmium (Frey-Wyssling et al., 1963). This ultrastructural appearance of the spherosome preparation from Helianthus cotyledons is

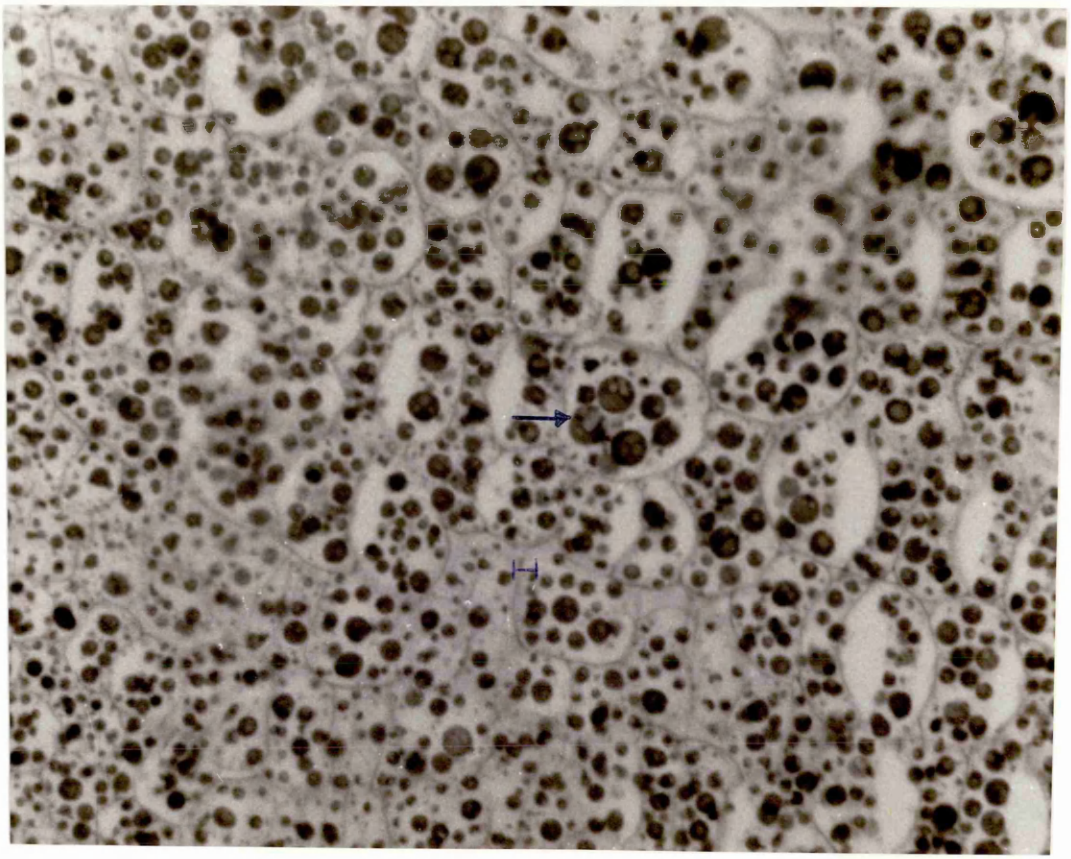


Figure 36 Electron micrograph of Helianthus cotyledons showing spherosomes.

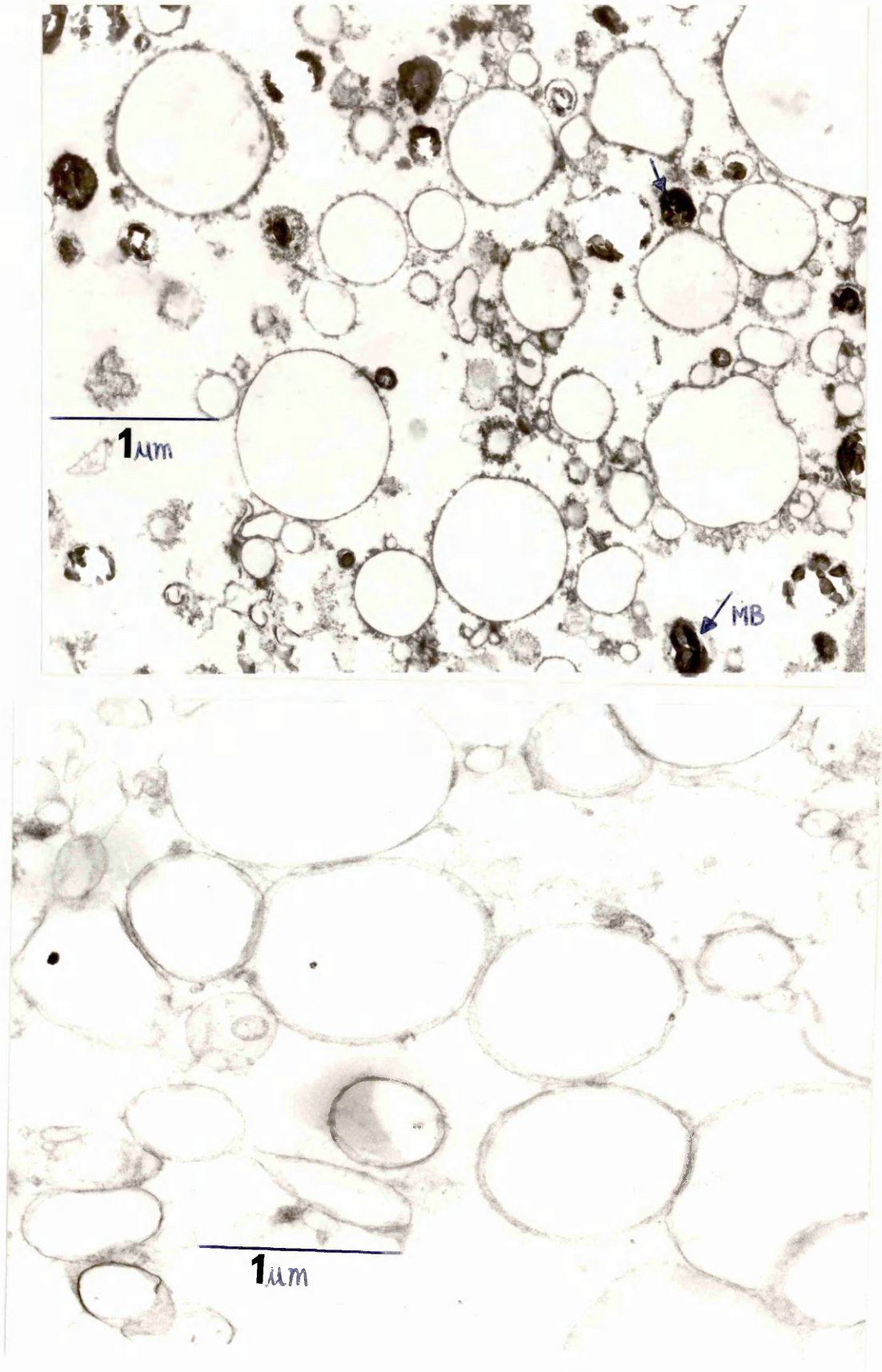


Figure 37 Electron micrograph of isolated spherosomes from Helianthus cotyledons fixed in glutaraldehyde. Note a single layer (SL) and microbodies (MB).

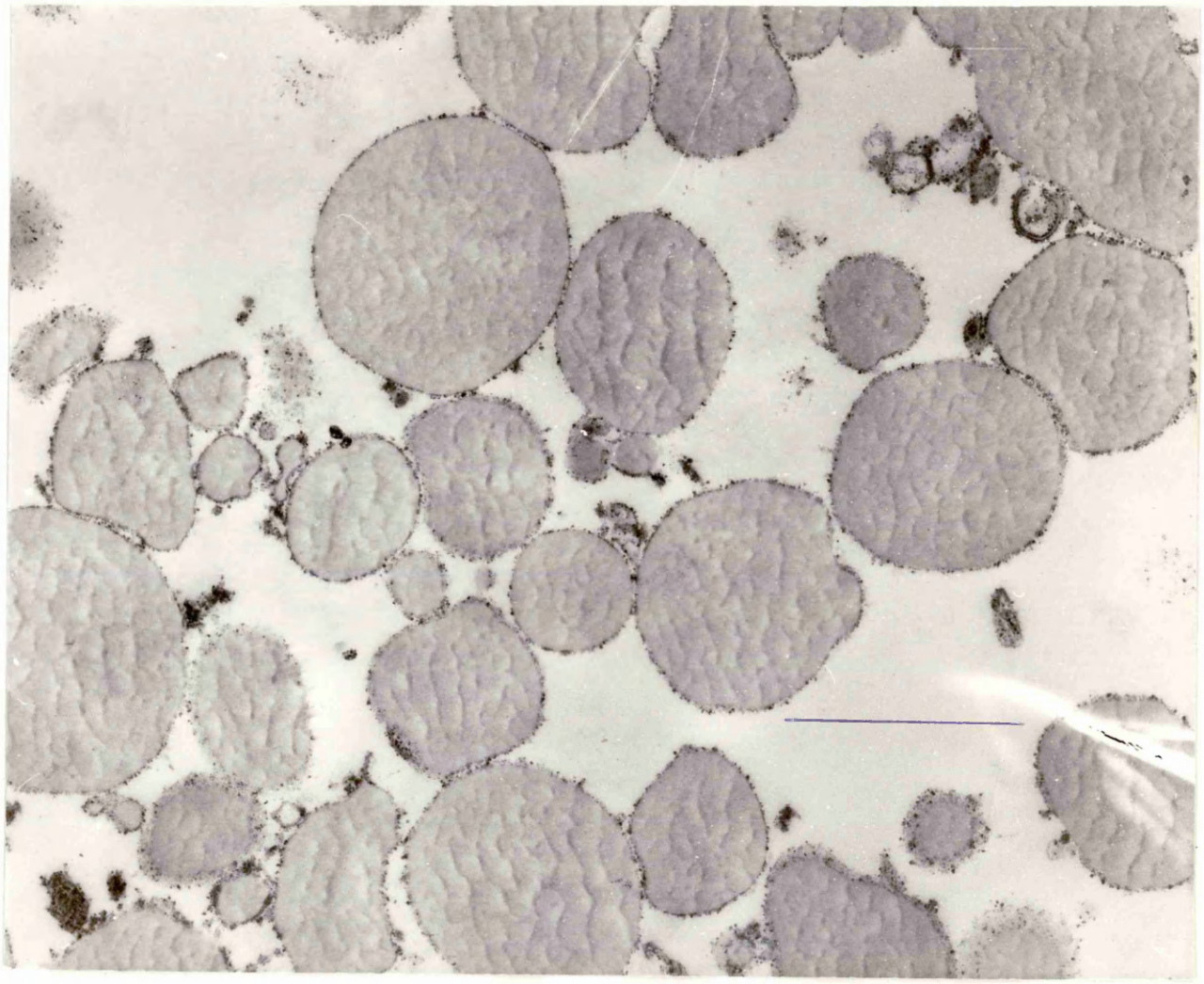


Figure 38 Electron micrograph of isolated spherosomes from Helianthus cotyledons fixed in glutaraldehyde and post-fixed in osmium tetroxide.

in agreement with Sorokin (1967) who found that spherosomes ranged from 0.8 to 1.0 μm in diameter, and the results are also similar to those of Wanner and Theimer (1978) who showed that spherosomes from cotyledons of 4 d-old sunflower were around 0.5 to 2 μm in diameter, surrounded by microbodies (the so-called glyoxysomes), and that they are bounded by a half-unit membrane.

II. Gossypium

The cotyledonary cells of 3 d-old cotton seedlings contain spherosomes (Figure 40 - 41) which were similar in appearance to those observed in intact seed (Figure 39). These spherosomes range in size from 0.3 to 2 μm and are bounded by a half-unit membrane. Figure 41 shows the spherosome areas to be electron-opaque after fixation with glutaraldehyde and post-fixation in osmium tetroxide. This is caused by the high content of unsaturated fatty acids in the spherosomes of cottonseed and osmium reacts with double bonds of these unsaturated lipids (Yatsu, 1965). The matrix of spherosomes, after osmium-tetroxide fixation, is uniformally electron-dense. The presence of the osmium-stained lipids within the membrane confirms that the fat pad obtained by centrifuging a homogenate of cotton cotyledons is indeed a concentrated form of these fat-storage organelles (spherosomes). The ultrastructural appearance of the spherosome preparation agrees with the report of Yatsu (1965) who found that spherosomes of cottonseeds ranged from 0.3 to 3 μm in diameter and he noted that these spherosomes were electron-opaque after osmium fixation. The results also

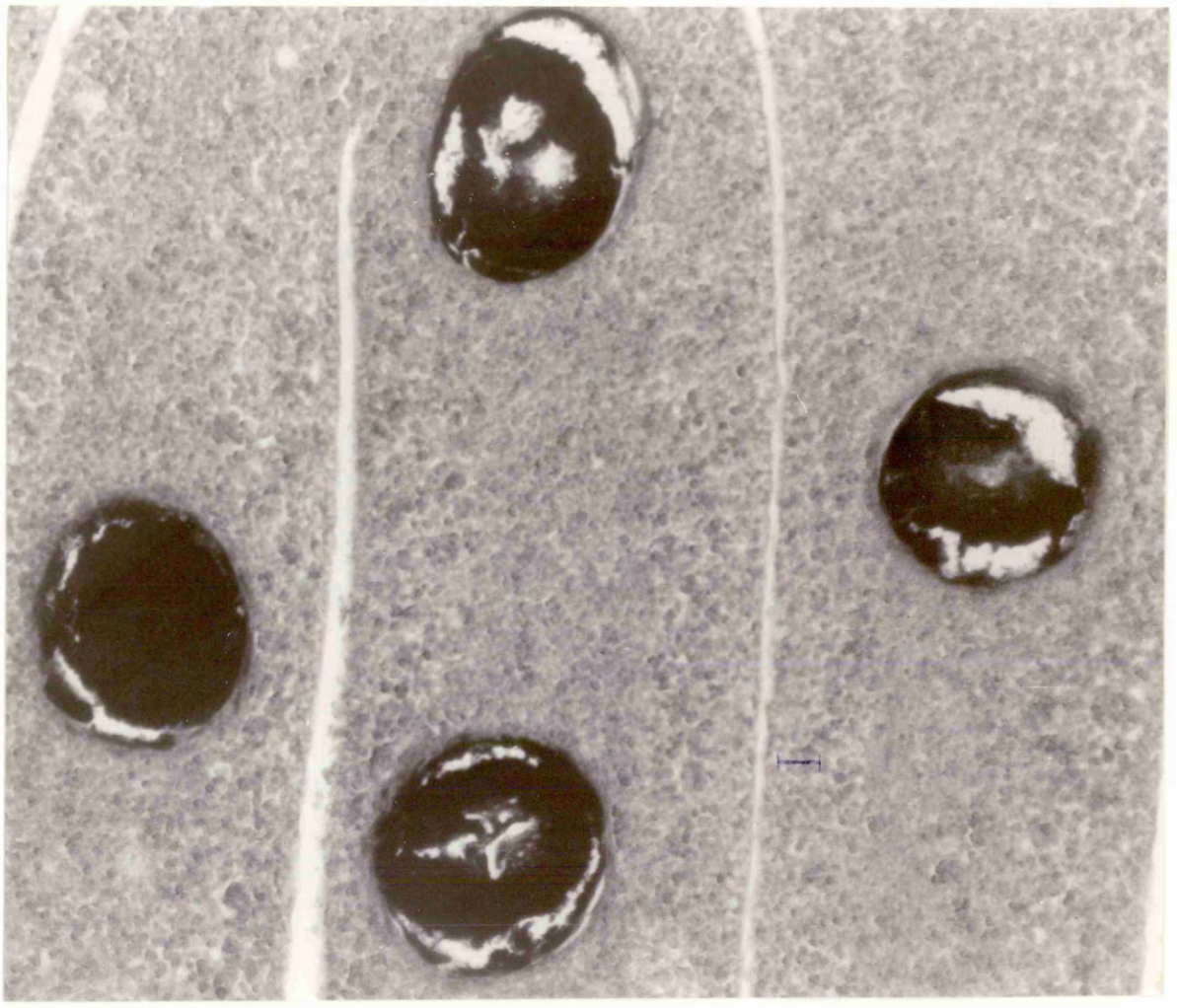


Figure 39 Electron micrograph of Gossypium cotyledons showing spherosomes.

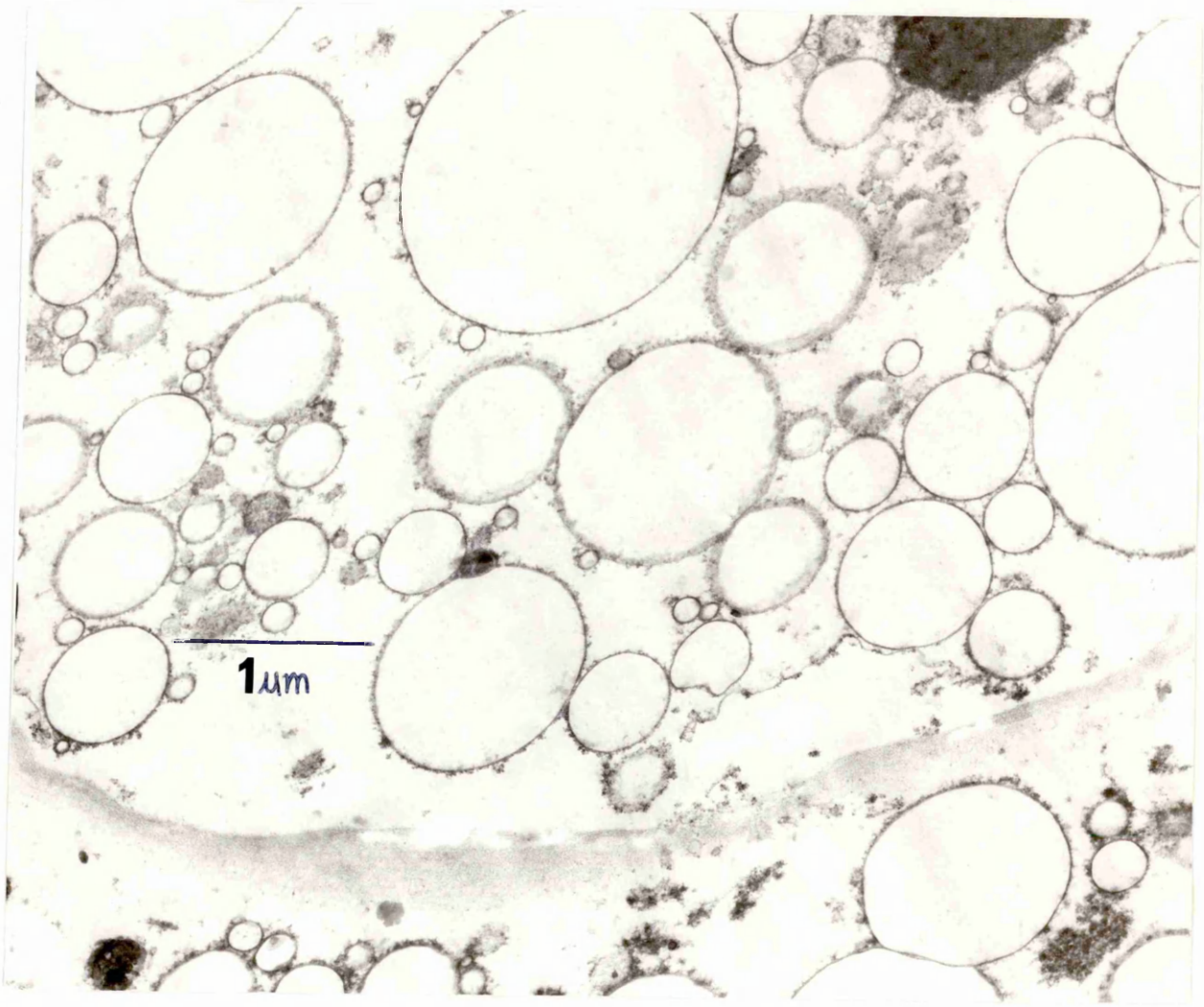


Figure 40 Electron micrograph of isolated spherosomes from Gossypium cotyledons fixed in glutaraldehyde.

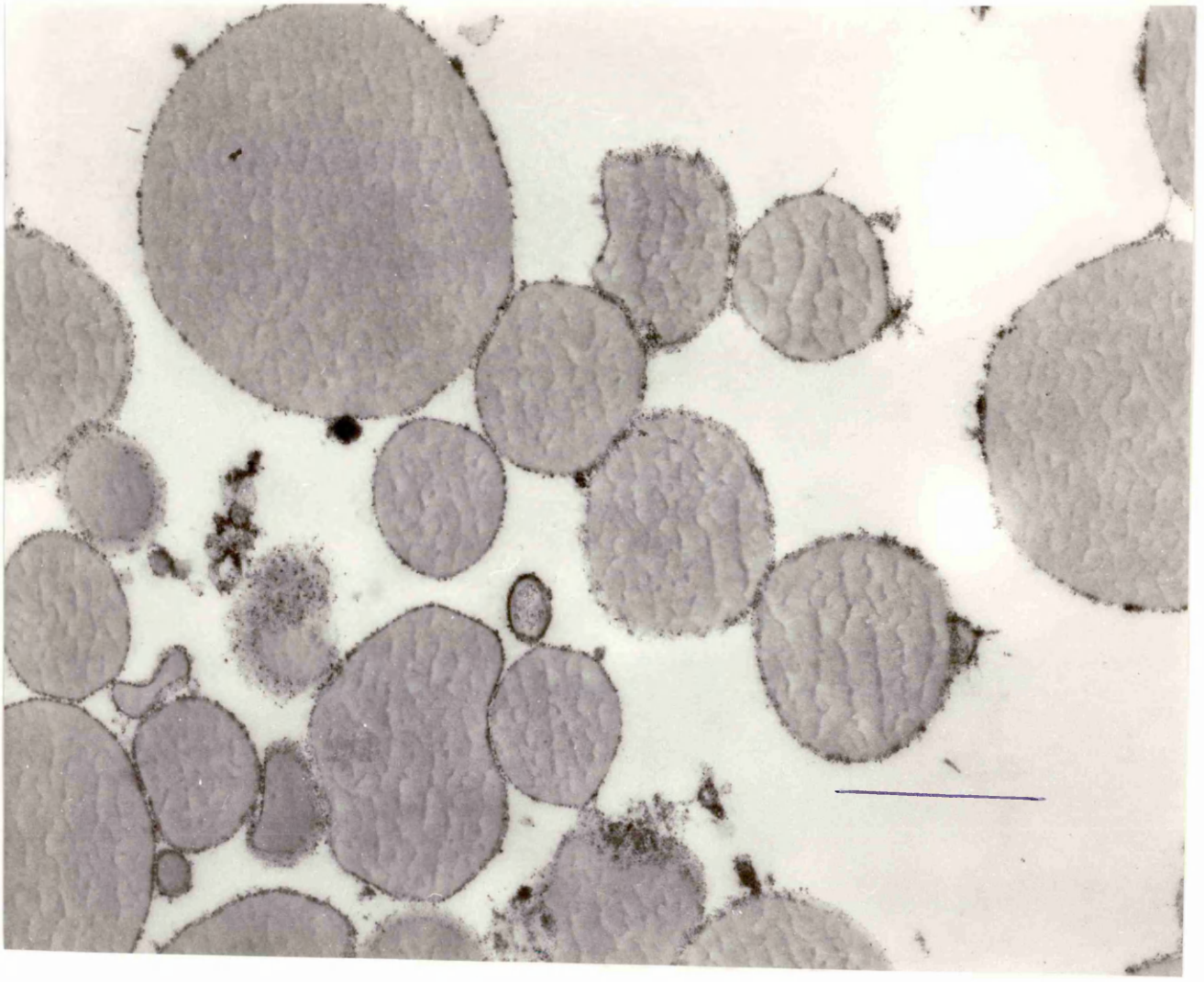


Figure 41 Electron micrograph of isolated spherosomes from Gossypium cotyledons fixed in glutaraldehyde and post-fixed in osmium tetroxide.

agree with those of Ory et al. (1968) who noticed very dense, precipitated lead soaps surrounding the spherosomes. They also reported channelling or small breaks in the lipid matrix which could be an effect of lipase (Figure 41). Also the appearance of the spherosomes agrees with that reported by Yatsu et al. (1971) who showed that spherosomes were bounded by a single, fine line, the so-called "half-unit membrane".

5.1.2 Lipid content and fatty acid composition of spherosomes

Spherosomal fractions from both Helianthus and Gossypium were prepared for chemical analysis by washing with distilled water several times and freeze-drying to constant weight; the lipids were extracted and long-chain fatty acids were determined during three days germination.

Losses of spherosomes undoubtedly occurred during isolation, and this is evidenced by the high standard errors when lipid was calculated as mg g^{-1} fresh seed or mg g^{-1} fresh cotyledons (Tables 70 and 78); therefore the data are also presented as mg g^{-1} dry spherosomal fraction, as a percentage of the total lipid and as relative to $C_{18:2}$ (100).

The identity of LCFAME was confirmed by GC-MS (see Appendix I.

Helianthus

Total lipid content of spherosomal preparations from dry Helianthus cotyledons constituted approximately 900 mg g^{-1} dry spherosomal fraction, a figure which decreased during 3 d germination to about 635 mg g^{-1} dry spherosomal fraction. Hall et al. (1974) reported that spherosomes of castor bean seeds decreased in number during the germination processes,

Table 70 Spherosomes and total lipid contents during *Helianthus* germination. Values are means ± SE.

Germination time (d)	Spherosomes content (dry weight) of		Spherosomal lipids (total) as related to		
	seed	cotyledons	fresh seed	fresh cotyledons	dry spherosomes
	(mg g^{-1} fresh wt)		(mg g^{-1})		
Dry	95.0 ± 71.0	176.0 ± 121.2	85.1 ± 72.1	157.7 ± 151.2	896.2 ± 26.3
1	93.2 ± 69.4	174.5 ± 119.1	73.3 ± 58.1	132.3 ± 113.3	760.8 ± 24.6
2	45.7 ± 35.6	86.6 ± 70.3	33.6 ± 32.1	63.7 ± 61.8	735.4 ± 45.2
3	64.1 ± 56.8	119.8 ± 87.9	40.4 ± 34.0	75.9 ± 66.1	633.4 ± 38.8

confirming the decrease in total lipid content of spherosomal seeds and cotyledons.

In the study of fatty acid composition from total lipids of spherosomes, the major FAswere C_{16}, C_{18}, $C_{18:1}$ and $C_{18:2}$. Saturated C_{16} acid decreased sharply during the imbibition period from 179 mg g^{-1} dry spherosomal fraction (relative value 47) in dry cotyledons to 101 mg g^{-1} dry spherosomal fraction (relative value 27) at 1 d, and to 61 mg g^{-1} dry spherosomal fraction (relative value 19) at 3 d. C_{18} increased from 36 mg g^{-1} dry spherosomal fraction (relative value 10) in dry cotyledons to 66 mg g^{-1} dry spherosomal fraction (relative value 17) at 1 d, but decreased again to 35 mg g^{-1} dry spherosomal fraction at 3 d. $C_{18:1}$ decreased during the imbibition period to 208 mg g^{-1} dry spherosomal fraction at 1 d and to 171 mg g^{-1} dry spherosomal fraction (relative value 52) at 3 d. $C_{18:2}$ levels were unchanged during germination with an average of 364 mg g^{-1} dry spherosomal fraction (Table 71 and Figure 42).

These results of the total fatty acid composition of the spherosomal fraction did not differ from those of Helianthus cotyledons as shown in Table 72.

In the study of lipid classes of spherosomal preparation from dry Helianthus cotyledons, neutral lipid constituted 786 mg g^{-1} dry spherosomal fraction (87.95% of the total lipid), polar lipid (phospholipid) constituted 83 mg g^{-1} dry spherosomal fraction (9.25%), glycolipid and other constituted 25 mg g^{-1} dry spherosomal fraction (2.80%). These results are in close agreement with those of Sorokin (1967), who found that

Table 71 Long-chain fatty acid of spherosomes from Helianthus cotyledons during germination.
Values are means ± SE. ND = not detected

Germination time (d)	Fatty acids - mg g^{-1} dry spherosomal fraction (% of total lipid)				
	C_{14}	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	11.9 ± 1.6	178.6 ± 30.3	35.9 ± 11.8	290.9 ± 41.5	376.4 ± 58.8
	(1.34)	(19.98)	(4.03)	(31.44)	(41.10)
1	ND	101.4 ± 12.2	65.8 ± 13.8	208.5 ± 24.4	378.4 ± 81.2
	-	(13.44)	(8.72)	(27.65)	(50.18)
2	ND	78.4 ± 21.3	48.7 ± 13.6	193.8 ± 21.3	374.0 ± 61.1
	-	(11.28)	(7.01)	(27.88)	(53.82)
3	ND	61.2 ± 11.4	35.4 ± 8.4	170.8 ± 29.2	328.2 ± 54.2
	-	(10.27)	(5.94)	(28.68)	(55.10)
	Fatty acids relative to $C_{18:2}$ (100)				
Dry	3.18	47.4	9.5	77.2	100
1	-	26.8	17.3	55.1	100
2	-	20.9	13.0	51.8	100
3	-	18.6	10.7	52.0	100

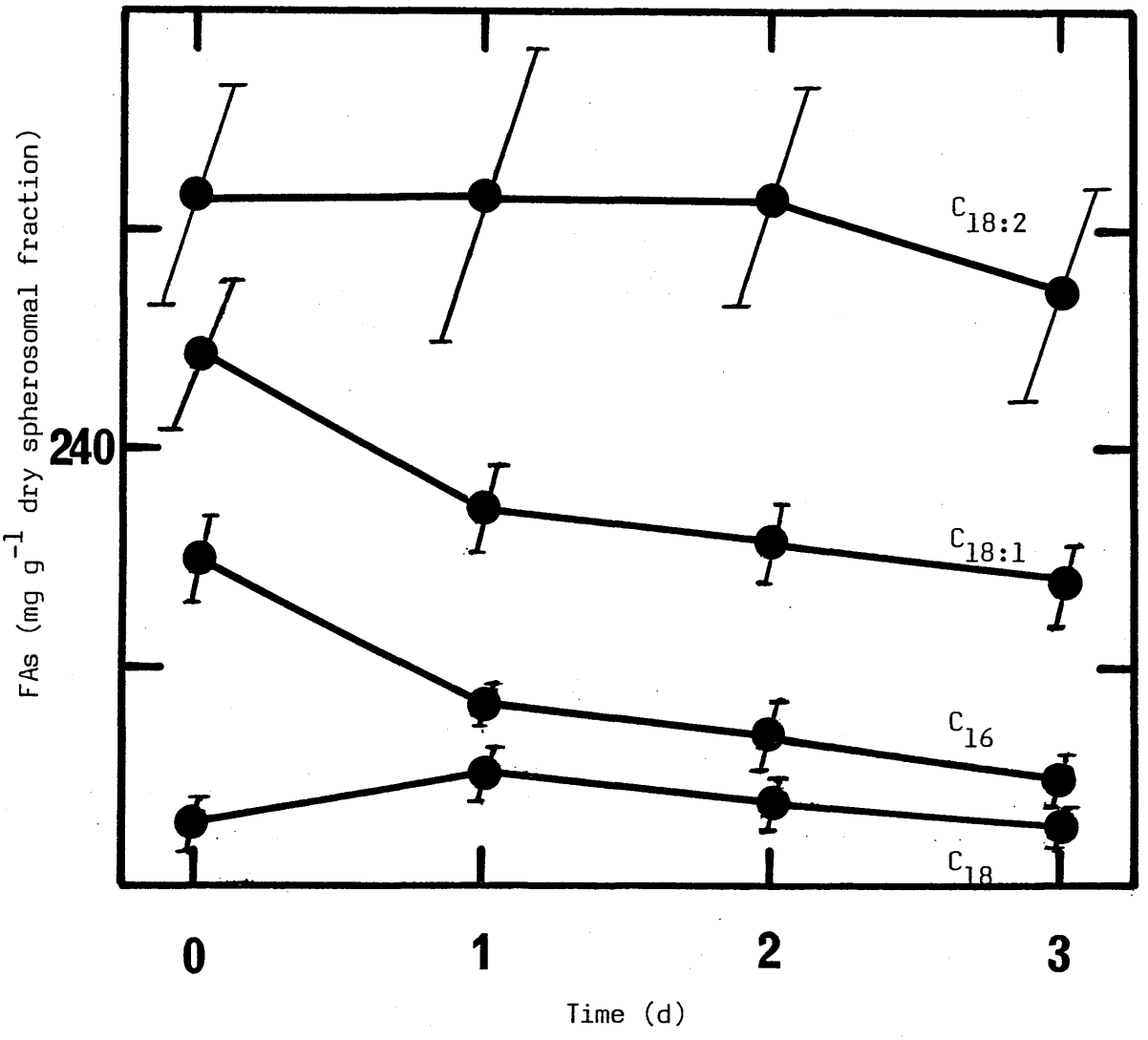


Figure 42 LCFAME of spherosomes extracted from cotyledons during germination of Helianthus.

Table 72 LCFA in the spherosomal fraction of Helianthus cotyledons.

Fatty acids	Spherosomal fraction isolated from cotyledons			Direct extraction of cotyledons from intact seeds		
	Germination time (d)			Germination time (d)		
	1	2	3	1	2	3
	(% of total lipid)					
C_{16}	13.44	11.28	10.27	13.27	8.92	11.32
C_{18}	8.72	7.01	5.94	8.12	6.31	7.49
$C_{18:1}$	27.65	27.88	28.68	28.76	27.52	25.94
$C_{18:2}$	50.18	53.82	55.10	49.85	57.25	55.24

triglycerides of sunflower constituted 85.9% of the total lipid, and of Gurr et al. (1974) who found that oil bodies of sunflower contained 89.3% of the total lipid. Neutral lipids of spherosomes decreased sharply during the imbibition period to 628 mg g^{-1} dry spherosomal fraction at 1 d, and to 551 mg g^{-1} dry spherosomal fraction at 3 d; similarly, glycolipid decreased to 9 mg g^{-1} dry spherosomal fraction at 3 d, but phospholipid levels changed only slightly during 3 d germination (Table 73 and Figure 43).

In the spherosomal neutral lipid subfraction, C_{16} increased sharply from 16 mg g^{-1} dry spherosomal fraction (relative value 3) in dry cotyledons to 61 mg g^{-1} spherosomal fraction (relative value 18) at 1 d; similarly, C_{18} increased from 13 mg g^{-1} dry spherosomal fraction (relative value 3) in dry cotyledons to 40 mg g^{-1} dry spherosomal fraction (relative value 13) at 3 d. $C_{18:1}$ decreased from 266 mg g^{-1} dry spherosomal fraction (relative value 54) to 137 mg g^{-1} dry spherosomal fraction (relative value 44) at 3 d; likewise, $C_{18:2}$ decreased from 490 mg g^{-1} dry spherosomal fraction in dry cotyledons to 314 mg g^{-1} dry spherosomal fraction at 3 d (Table 74 and Figure 44).

These results on the analysis of fatty acids in the spherosomal neutral lipid subfraction are similar to those obtained with cotyledons in the same species, as presented in Table 75.

In the spherosomal phospholipid subfraction, C_{16} and C_{18} increased during the imbibition period to 28 mg g^{-1} dry spherosomal fraction (relative value 77) and to 12 mg g^{-1} dry

Table 73 Lipid classes of spherosomes from Helianthus cotyledons during germination.

Values are means ± SE.

Germination time (d)	mg g^{-1} dry spherosomal fraction (% of total lipid)		
	Neutral lipid	Phospholipid	Glycolipid + others
Dry	786.2 ± 61.6 (87.95)	82.7 ± 20.5 (9.26)	24.9 ± 10.2 (2.79)
1	628.4 ± 80.7 (84.01)	101.6 ± 36.6 (13.59)	17.9 ± 9.1 (2.40)
2	651.4 ± 120.9 (89.70)	60.1 ± 23.3 (8.28)	14.7 ± 7.4 (2.02)
3	551.0 ± 92.1 (89.43)	56.4 ± 27.7 (9.16)	8.6 ± 4.1 (1.41)

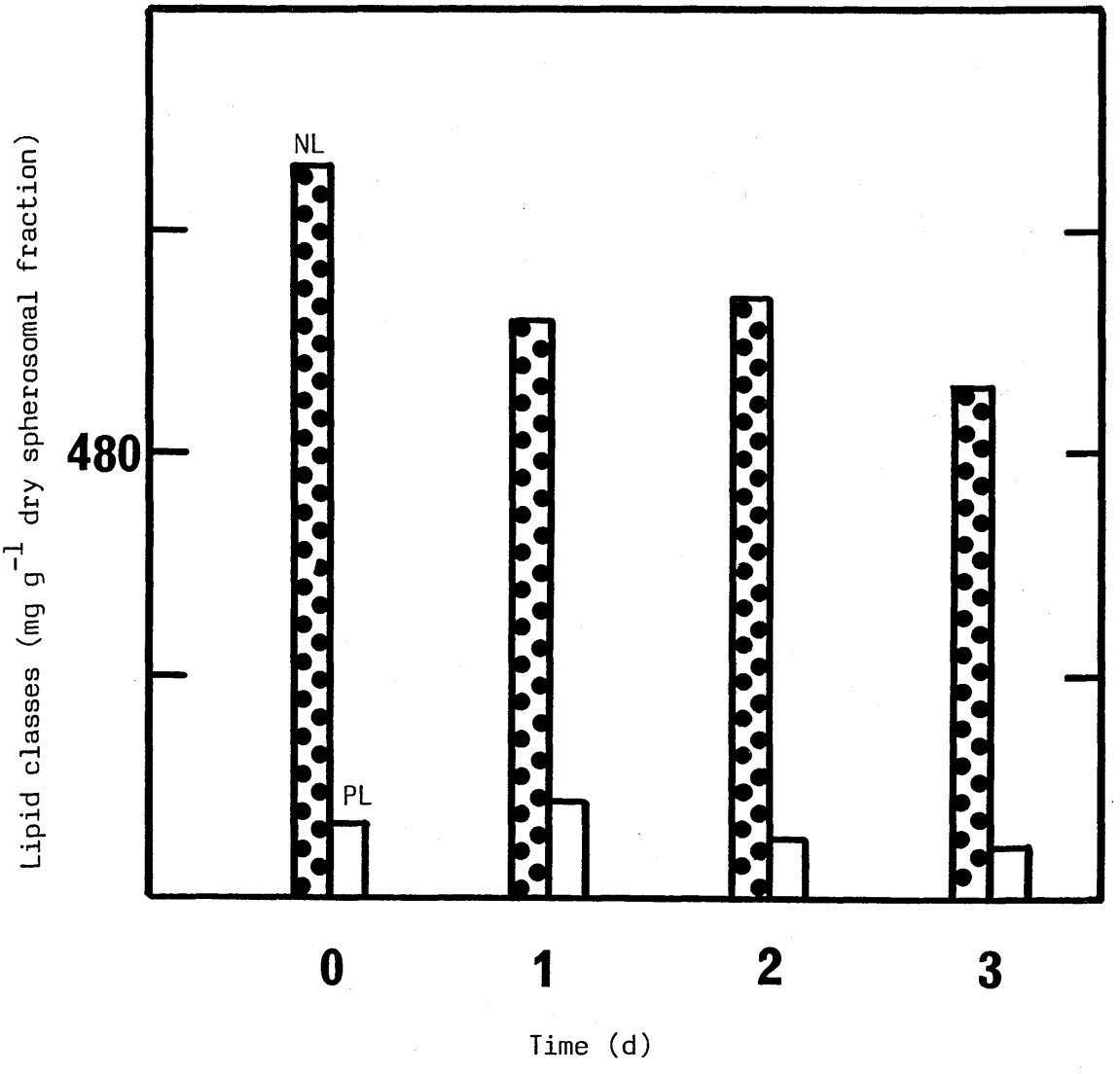


Figure 43 Neutral (NL) and phospholipid (PL) of spherosomes extracted from cotyledons during germination of Helianthus.

Table 74 Long-chain fatty acids of spherosomal neutral lipid subfraction from Helianthus cotyledons during germination. Values are means ± SE.

Germination time (d)	Fatty acids - mg g^{-1} dry spherosomal fraction (% of NL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	15.9 ± 4.9	12.9 ± 5.3	266.1 ± 34.4	490.1 ± 71.8
	(2.03)	(1.65)	(33.89)	(62.42)
1	61.4 ± 14.2	40.8 ± 20.1	177.1 ± 51.3	343.1 ± 62.2
	(9.87)	(6.55)	(28.45)	(55.12)
2	48.9 ± 21.3	39.2 ± 13.2	185.6 ± 59.8	375.2 ± 102.2
	(7.54)	(6.05)	(28.59)	(57.81)
3	58.1 ± 27.8	40.1 ± 23.1	137.2 ± 47.6	313.8 ± 96.4
	(10.58)	(7.30)	(24.97)	(57.13)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	3.2	2.6	54.3	100
1	17.9	11.8	51.6	100
2	13.0	10.4	49.4	100
3	18.5	12.7	43.7	100

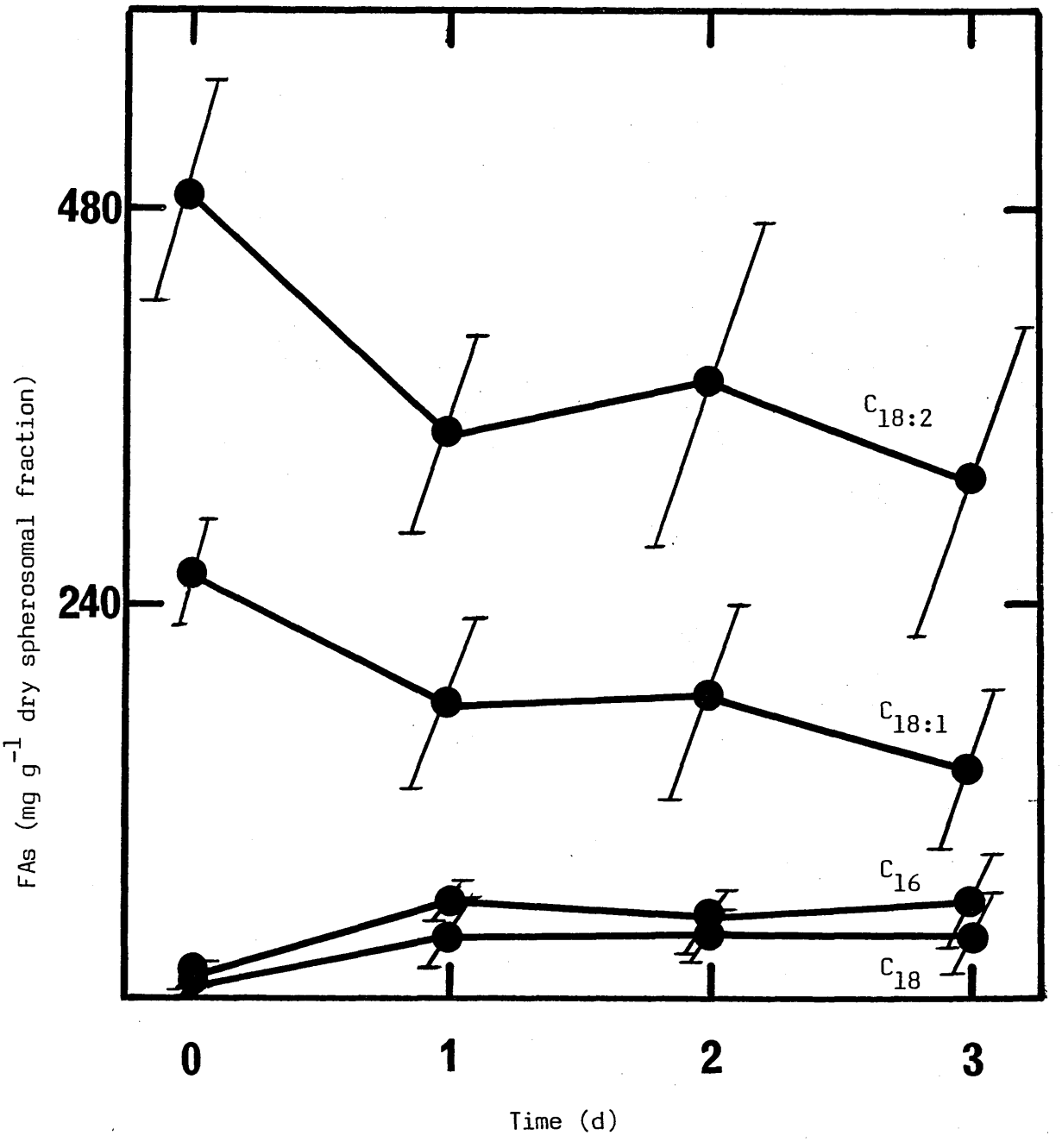


Figure 44 LCFAME of spherosomal neutral lipid extracted from cotyledons during germination of Helianthus.

Table 75 LCFA composition in the spherosomal neutral lipid subfraction of Helianthus cotyledons.

Fatty acids	Spherosomal neutral lipid subfraction isolated from cotyledons			Direct extraction of cotyledons from intact seed		
	Germination time (d)			Germination time (d)		
	1	2	3	1	2	3
	(% of total NL)					
C_{16}	9.87	7.54	10.58	9.11	7.42	10.58
C_{18}	6.55	6.05	7.30	6.94	6.54	7.92
$C_{18:1}$	28.45	28.59	24.97	29.35	27.22	25.59
$C_{18:2}$	55.12	57.81	57.13	54.58	58.80	55.89

spherosomal fraction (relative value 34) respectively at 1 d. $C_{18:1}$ decreased from 29 mg g^{-1} to 20 mg g^{-1} dry spherosomal fraction at 3 d, and $C_{18:2}$ decreased from 44 mg g^{-1} to 22 mg g^{-1} dry spherosomal fraction at 3 d (Table 76 and Figure 45). These results are similar to those describing the fatty acids from intact Helianthus cotyledons (Table 77).

II. Gossypium

Total lipid content of spherosomal preparation from dry Gossypium cotyledons was estimated to be 786 mg g^{-1} dry spherosomal fraction, a figure which decreased during germination to 318 mg g^{-1} dry spherosomes at 3 d (Table 78).

In the study of fatty acid composition of total lipids of spherosomes, the major fatty acids were found to be C_{14}, C_{16}, $C_{16:1}$, C_{18}, $C_{18:1}$ and $C_{18:2}$. Little change was noted in C_{14} which was present at an average of 6 mg g^{-1} dry spherosomal fraction in dry cotyledons and during germination. C_{16}, however, increased during the imbibition period from 156 mg g^{-1} dry spherosomal fraction (relative value 48) in dry cotyledons to 202 mg g^{-1} dry spherosomal fraction (relative value 93) at 1 d, declining thereafter to 106 mg g^{-1} dry spherosomal fraction at 3 d, yet at this time the relative value was 118. C_{18} increased from 8 mg g^{-1} dry spherosomal fraction (relative value 2) to 24 mg g^{-1} dry spherosomal fraction (relative value 14) at 2 d. Unsaturated $C_{18:1}$ decreased sharply from 256 mg g^{-1} dry spherosomal fraction (relative value 79) to 81 mg g^{-1} dry spherosomal fraction at 3 d but the value relative to $C_{18:2}$ showed a peak (91) at the same time. $C_{18:2}$ decreased from

Table 76 Long-chain fatty acids of the spherosomal phospholipid subfraction from Helianthus cotyledons during germination.
Values are means ± SE.

Germination time (d)	Fatty acids - mg g^{-1} dry spherosomal fraction (% of PL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	7.2 ± 2.2	2.1 ± 1.1	29.0 ± 9.1	44.0 ± 11.2
	(8.81)	(2.54)	(35.21)	(53.43)
1	27.5 ± 3.4	12.1 ± 1.1	25.4 ± 8.8	35.8 ± 21.4
	(27.26)	(12.06)	(25.18)	(35.49)
2	13.3 ± 2.3	3.0 ± 1.2	20.2 ± 8.6	22.6 ± 8.2
	(22.57)	(5.06)	(34.13)	(38.22)
3	11.1 ± 6.1	2.5 ± 1.4	19.7 ± 12.2	22.3 ± 6.8
	(19.97)	(4.48)	(35.45)	(40.08)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	16.5	4.7	65.9	100
1	76.8	33.9	70.9	100
2	59.0	13.3	89.3	100
3	49.8	11.2	88.4	100

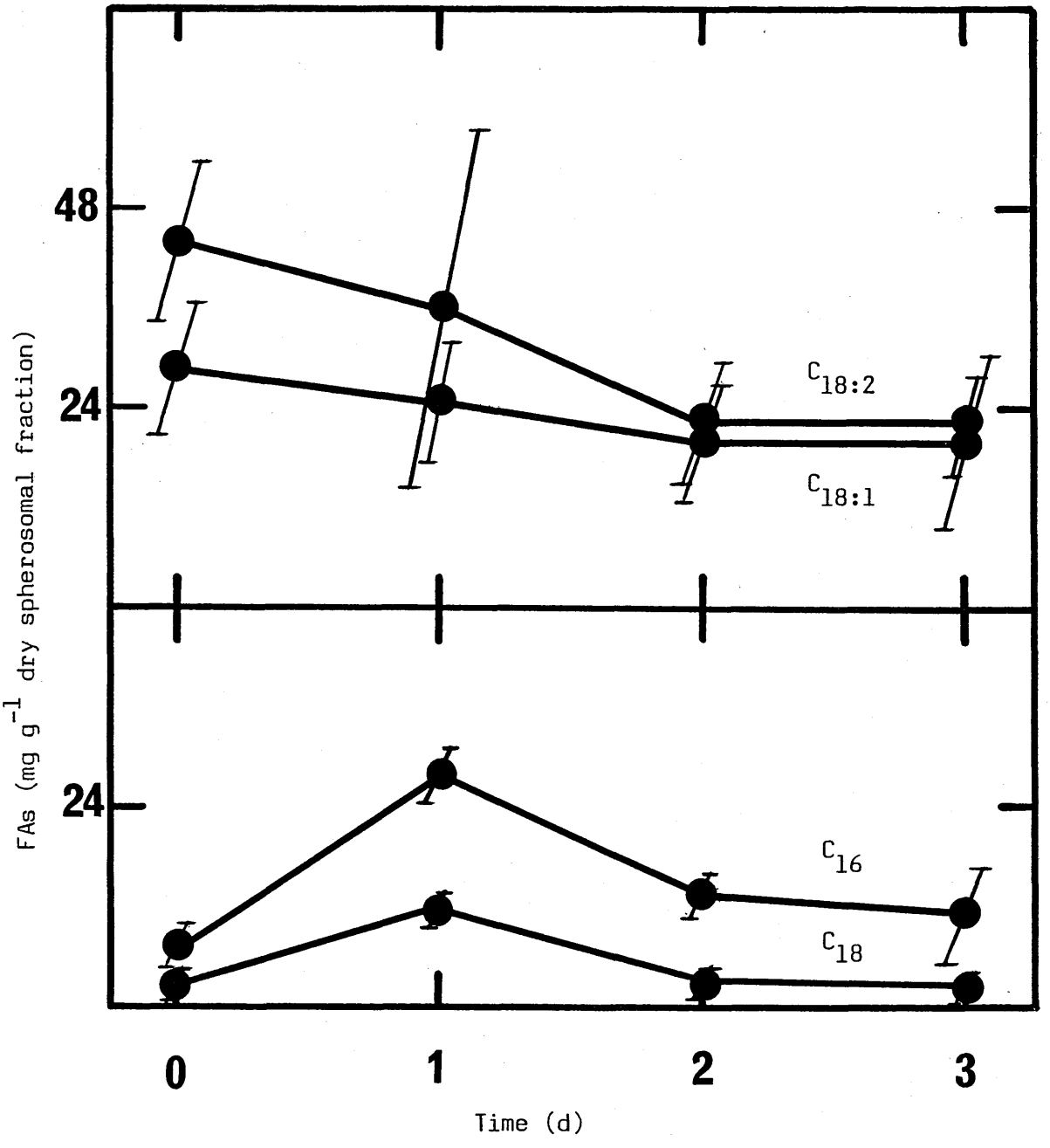


Figure 45 LCFAME of spherosomal phospholipid extracted from cotyledons during germination of Helianthus.

Table 77 LCFA composition in the spherosomal phospholipid subfraction of Helianthus cotyledons.

Fatty acids	Spherosomal phospholipid subfraction isolated from cotyledons			Direct extraction of cotyledons from intact seed		
	Germination time (d)			Germination time (d)		
	1	2	3	1	2	3
	(% of PL)					
C_{16}	27.26	22.57	19.97	30.46	23.01	19.29
C_{18}	12.06	5.06	4.48	12.82	4.71	5.50
$C_{18:1}$	25.18	34.13	33.45	24.62	34.67	35.39
$C_{18:2}$	35.49	38.22	40.08	32.09	37.59	39.81

Table 78 Spherosomes and total lipid contents during Gossypium germination. Values are means ± SE.

Germination time (d)	Spherosomes content (dry weight) of		Spherosomal lipids (total) as related to		
	seed	cotyledons	fresh seed	fresh cotyledons	dry spherosomes
	(mg g^{-1} fresh wt)		(mg g^{-1})		
Dry	74.0 ± 58.1	134.9 ± 113.5	58.2 ± 46.2	106.1 ± 82.7	785.7 ± 31.2
1	68.1 ± 56.2	122.2 ± 112.3	44.5 ± 37.1	79.7 ± 70.2	652.8 ± 28.3
2	52.2 ± 37.5	98.2 ± 61.2	25.7 ± 23.2	48.3 ± 38.3	492.6 ± 26.4
3	44.2 ± 35.8	83.3 ± 71.3	14.0 ± 11.3	26.4 ± 24.7	317.8 ± 21.5

325 mg g^{-1} dry spherosomal fraction in dry cotyledons to 90 mg g^{-1} dry spherosomal fraction at 3 d (Table 79 and Figure 46). These results of the analyses of total fatty acids are similar to those obtained by direct extraction of Gossypium cotyledons (Table 80).

Analysis of lipid classes of spherosomal preparations from dry Gossypium cotyledons showed that the netural lipid subfraction was approximately 754 mg g^{-1} dry spherosomal fraction (96.1% of the total lipid), phospholipid subfraction 26 mg g^{-1} dry spherosomal fraction (3.3%) and the glycolipid subfraction 5 mg g^{-1} dry spherosomal fraction (0.6%). These results of spherosomal neutral lipid are quite similar to those of Yatsu et al. (1971) who found that spherosomes isolated from cottonseeds contained 98.8% of the total lipid triglycerides. In addition Gurr et al. (1974) found that oil bodies of cottonseeds contained 98.4% of the total lipid triglycerides.

The neutral lipid subfraction of spherosomes decreased sharply during germination from 754 mg g^{-1} to 227 mg g^{-1} dry spherosomal fraction. Polar lipid (phospholipid subfraction) on the other hand, increased during the imbibition period from 26 mg g^{-1} to 86 mg g^{-1} dry spherosomal fraction at 1 d with an average of 75 mg g^{-1} dry spherosomal fraction. Similarly, glycolipid subfraction increased to 15 mg g^{-1} dry spherosomal fraction (Table 81 and Figure 47).

In the spherosomal neutral lipid subfraction, C_{16} increased sharply from 62 mg g^{-1} dry spherosomal fraction

Table 79 Long-chain fatty acid of spherosomes from Gossypium cotyledons during germination.

Values are means ± SE. ND - not detected

Germination time (d)	Fatty acids - mg g^{-1} dry spherosomal fraction (% of total lipid)						
	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Dry	6.4 ± 3.6	155.8 ± 28.6	ND	7.6 ± 5.6	256.2 ± 41.4	325.1 ± 42.8	ND
	(0.86)	(20.80)	-	(1.02)	(34.20)	(43.38)	-
1	5.0 ± 3.3	201.5 ± 29.2	5.6 ± 4.1	21.4 ± 7.3	150.8 ± 30.2	216.9 ± 41.6	30.4 ± 11.1
	(0.80)	(31.92)	(0.88)	(3.40)	(23.88)	(34.35)	(4.70)
2	10.1 ± 4.1	142.0 ± 42.4	13.1 ± 3.7	24.1 ± 6.2	120.7 ± 21.3	173.8 ± 26.4	ND
	(2.09)	(29.35)	(2.69)	(4.98)	(24.94)	(35.93)	-
3	2.0 ± 1.1	106.3 ± 29.2	13.9 ± 6.1	14.4 ± 6.3	81.4 ± 23.2	89.9 ± 32.4	ND
	(0.67)	(35.50)	(4.54)	(4.67)	(26.42)	(29.19)	-
	Fatty acids relative to $C_{18:2}$ (100)						
Dry	1.9	47.9	-	2.3	78.8	100	-
1	2.3	92.9	2.5	9.8	69.5	100	2.17
2	5.8	81.7	7.5	13.8	69.4	100	-
3	2.3	118.1	15.5	16.0	90.5	100	-

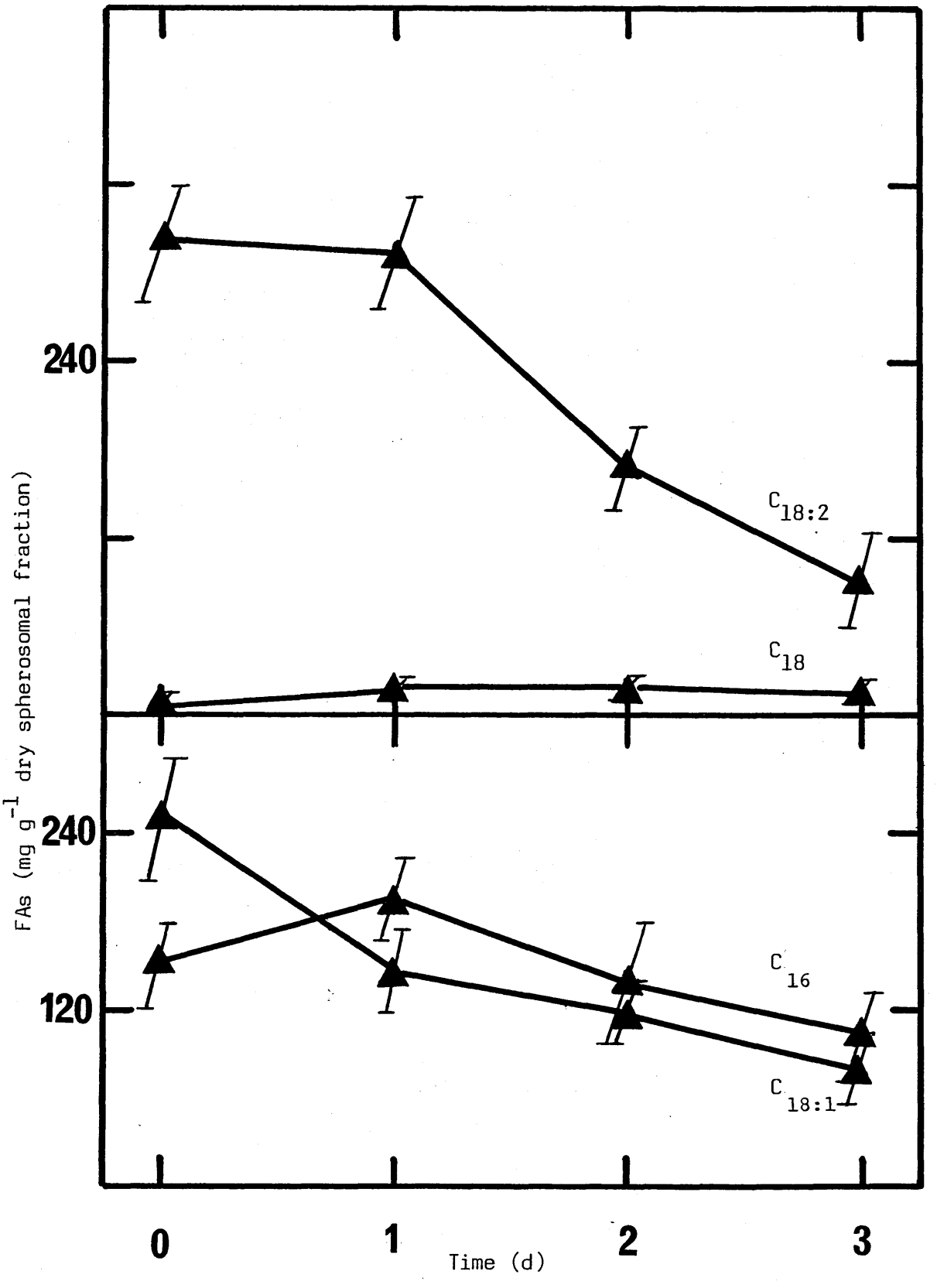


Figure 46 LCFAME of spherosomes extracted from cotyledons during germination of Gossypium.

Table 80 LCFA in the spherosomal fraction of Gossypium cotyledons. ND = not detected

Fatty acids	Spherosomes isolated from cotyledons			Direct extraction of cotyledons from intact seeds		
	Germination time (d)			Germination time (d)		
	1	2	3	1	2	3
	(% of total lipid)					
C_{14}	0.80	2.09	0.67	ND	1.15	ND
C_{16}	31.92	29.33	35.50	34.17	26.22	36.09
$C_{16:1}$	0.88	2.69	4.54	0.90	2.66	2.96
C_{18}	3.40	4.98	4.67	3.89	5.63	5.93
$C_{18:1}$	23.88	24.94	26.42	24.61	25.01	27.32
$C_{18:2}$	34.33	35.93	29.19	32.72	39.31	27.66
$C_{18:3}$	4.70	ND	ND	3.73	ND	ND

Table 81 Lipid classes of spherosomes from Gossypium cotyledons during germination.

Values are means ± SE.

Germination time (d)	mg g^{-1} dry spherosomal fraction (% of total lipid)		
	Neutral lipid	Phospholipid	Glycolipid + other components
Dry	754.0 ± 53.1 (96.07)	26.1 ± 6.8 (3.33)	4.6 ± 2.3 (0.60)
1	551.7 ± 51.3 (85.14)	86.3 ± 33.5 (13.33)	9.8 ± 4.1 (1.53)
2	405.6 ± 42.3 (81.69)	78.5 ± 31.4 (15.82)	12.3 ± 4.7 (2.48)
3	226.6 ± 71.3 (72.16)	72.8 ± 28.1 (23.18)	14.6 ± 5.3 (4.66)

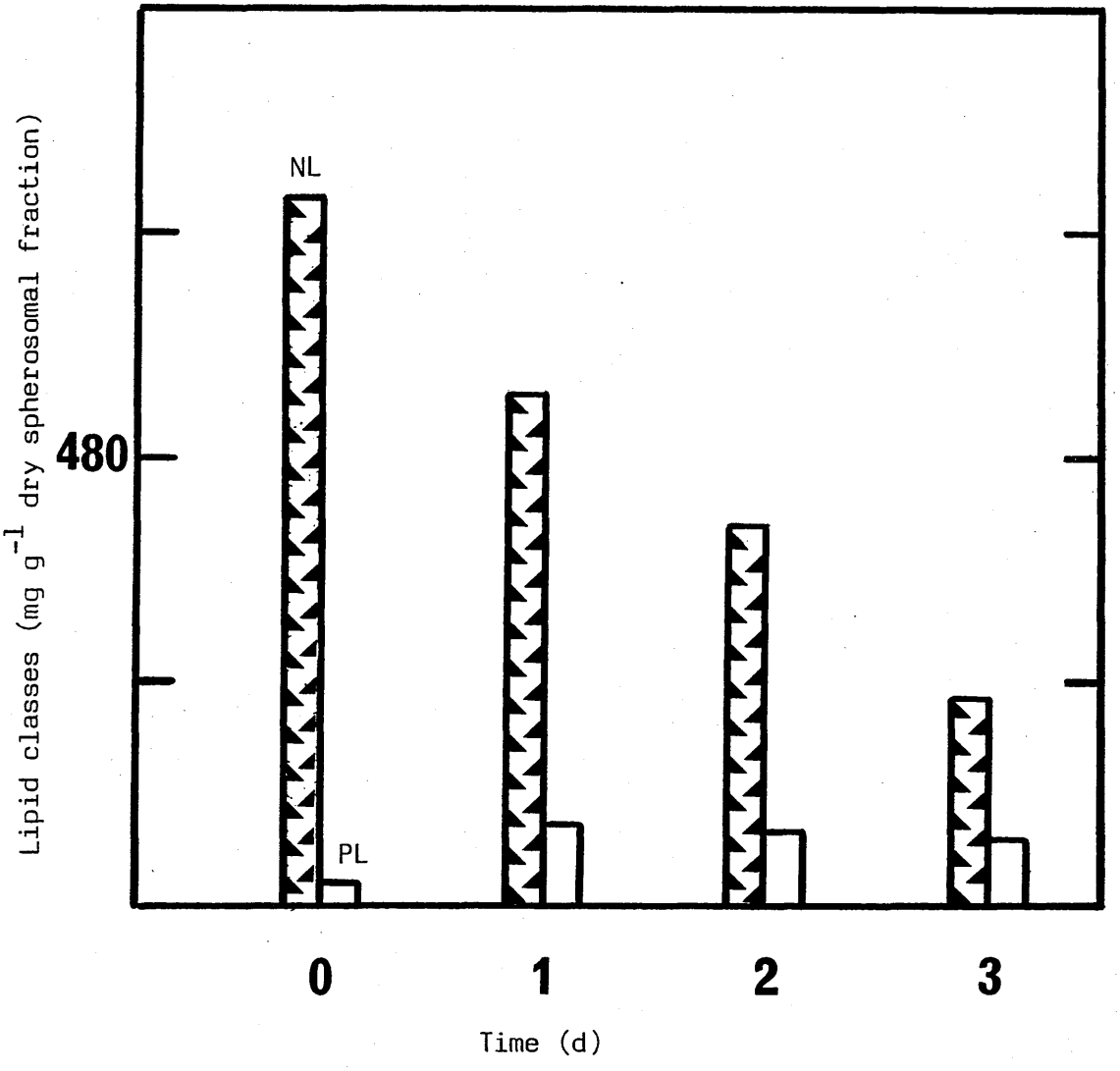


Figure 47 Neutral (NL) and phospholipid (PL) of spherosomes extracted from cotyledons during germination of Gossypium.

(relative value 13) in dry cotyledons to 173 mg g^{-1} dry spherosomal fraction (relative value 84) at 1 d, but the relative value calculation showed a peak (117) at 3 d. C_{18} increased to 30 mg g^{-1} dry spherosomal fraction (relative value 19) at 2 d but declined thereafter. $C_{18:1}$ decreased from 182 mg g^{-1} dry spherosomal fraction (relative value 37) in dry cotyledons to 57 mg g^{-1} dry spherosomal fraction at 3 d but the relative value increased to 82. $C_{18:2}$ decreased sharply from 490 mg g^{-1} dry spherosomal fraction in dry cotyledons to 69 mg g^{-1} dry spherosomal fraction at 3 d (Table 82 and Figure 48).

These results of the fatty acid composition of the spherosomal neutral lipid subfraction are similar to those obtained by direct extraction of Gossypium cotyledons (Table 83).

In the spherosomal phospholipid subfraction, saturated and unsaturated fatty acids increased during the 3 d germination period. Thus C_{16} increased from 8 mg g^{-1} dry spherosomal fraction (relative value 66) in dry cotyledons to average 19 mg g^{-1} dry spherosomal fraction, but its relative value decreased (44) at 1 d; C_{18} was not detected in dry cotyledons and averaged 2 mg g^{-1} dry spherosomal fraction during 3 d germination. $C_{18:1}$ increased from 7 mg g^{-1} dry spherosomal fraction (relative value 58) in dry cotyledons to average 17 mg g^{-1} dry spherosomal fraction but the relative value decreased (40) at 1 d. $C_{18:2}$ increased from 12 mg g^{-1} dry spherosomal fraction to average 41 mg g^{-1} dry spherosomal fraction (Table 84 and Figure 49). These results compare

Table 82 Long-chain fatty acids of the spherosomal neutral lipid subfraction from Gossypium cotyledons during germination.
Values are means ± SE.

Germination time (d)	Fatty acids - mg g^{-1} dry spherosomal fraction (% of NL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	62.3 ± 13.3	14.6 ± 4.6	182.0 ± 41.4	490.3 ± 81.2
	(8.32)	(1.95)	(24.28)	(65.43)
1	173.1 ± 35.2	20.5 ± 13.1	148.6 ± 43.2	205.8 ± 72.4
	(31.59)	(3.74)	(27.11)	(37.55)
2	117.8 ± 21.4	30.1 ± 9.2	101.1 ± 32.1	155.3 ± 44.4
	(29.18)	(7.42)	(24.93)	(38.46)
3	81.1 ± 23.1	15.1 ± 5.8	56.8 ± 21.2	69.1 ± 31.5
	(36.49)	(6.76)	(25.62)	(31.13)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	12.7	2.9	37.1	100
1	84.1	9.9	72.2	100
2	75.8	19.3	65.1	100
3	117.3	21.8	82.3	100

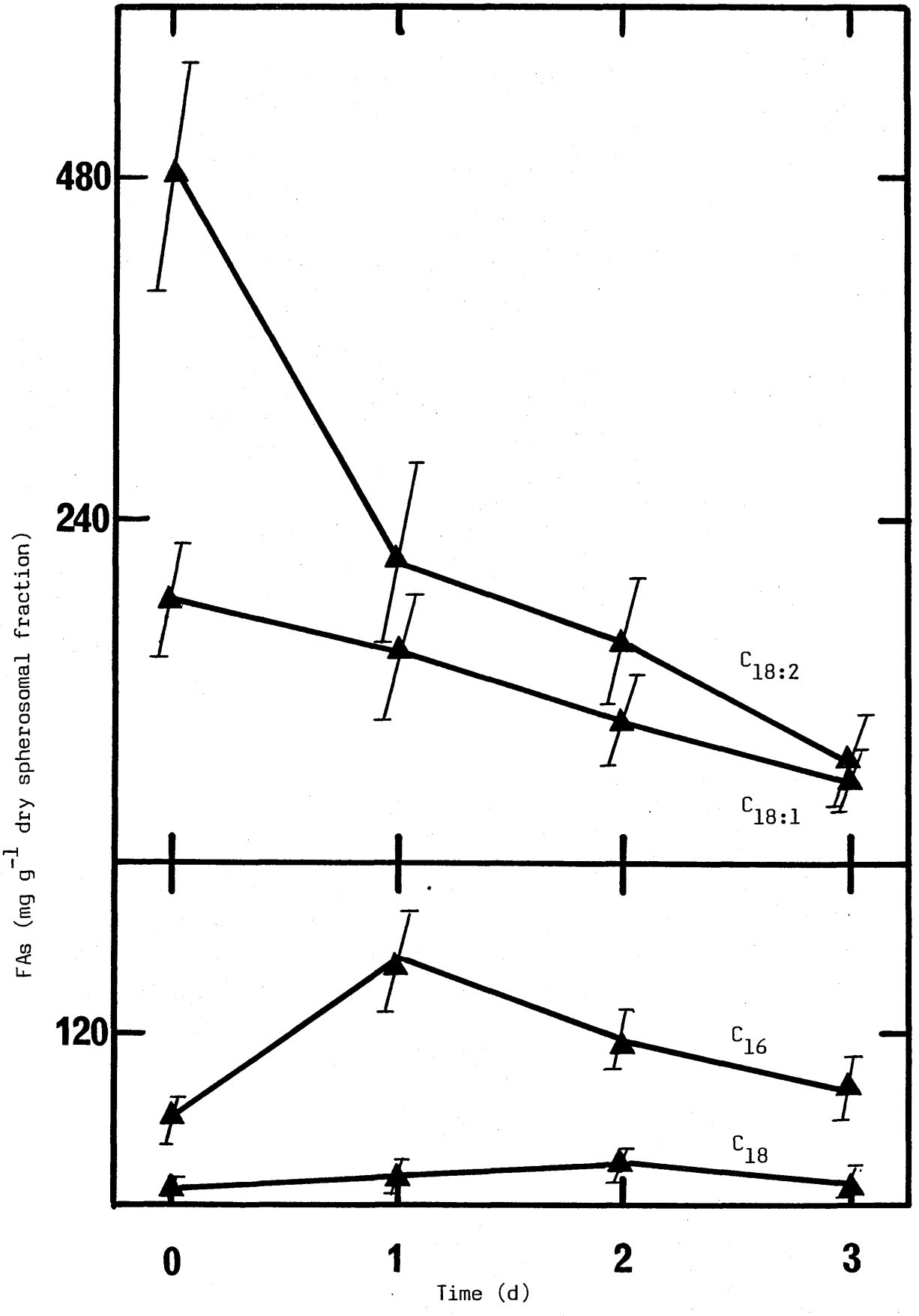


Figure 48 LCFAME of spherosomal neutral lipid extracted from cotyledons during germination of Gossypium.

Table 83 LCFA composition in the spherosomal neutral lipid subfraction of Gossypium cotyledons.

Fatty acids	Spherosomal neutral lipid subfraction isolated from cotyledons — Germination time (d)			Direct extraction of cotyledons from intact seed — Germination time (d)		
	1	2	3	1	2	3
	(% of total NL)					
C_{16}	31.59	29.18	36.49	32.12	29.19	37.90
C_{18}	3.74	7.42	6.76	3.88	6.19	7.30
$C_{18:1}$	27.11	24.93	25.62	26.11	25.79	23.89
$C_{18:2}$	37.55	38.46	31.13	37.88	38.82	30.89

Table 84 Long-chain fatty acids of the spherosomal phospholipid subfraction from Gossypium cotyledons during germination.
Values are means ± SE. ND = not detected

Germination time (d)	Fatty acids - mg g^{-1} dry spherosomal fraction (% of PL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	7.6 ± 3.6	ND	6.7 ± 3.1	11.5 ± 8.3
	(29.53)	-	(25.86)	(44.59)
1	19.5 ± 5.6	1.7 ± 0.8	17.9 ± 5.1	44.6 ± 11.3
	(23.32)	(2.07)	(21.41)	(53.19)
2	18.7 ± 11.6	2.1 ± 1.1	18.0 ± 3.1	38.9 ± 12.4
	(24.09)	(2.69)	(23.20)	(50.00)
3	16.8 ± 15.8	2.1 ± 1.4	15.9 ± 2.8	37.8 ± 10.8
	(23.19)	(2.75)	(21.92)	(52.12)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	66.2	-	58.0	100
1	43.8	3.9	40.2	100
2	48.1	5.3	46.4	100
3	44.5	5.5	42.0	100

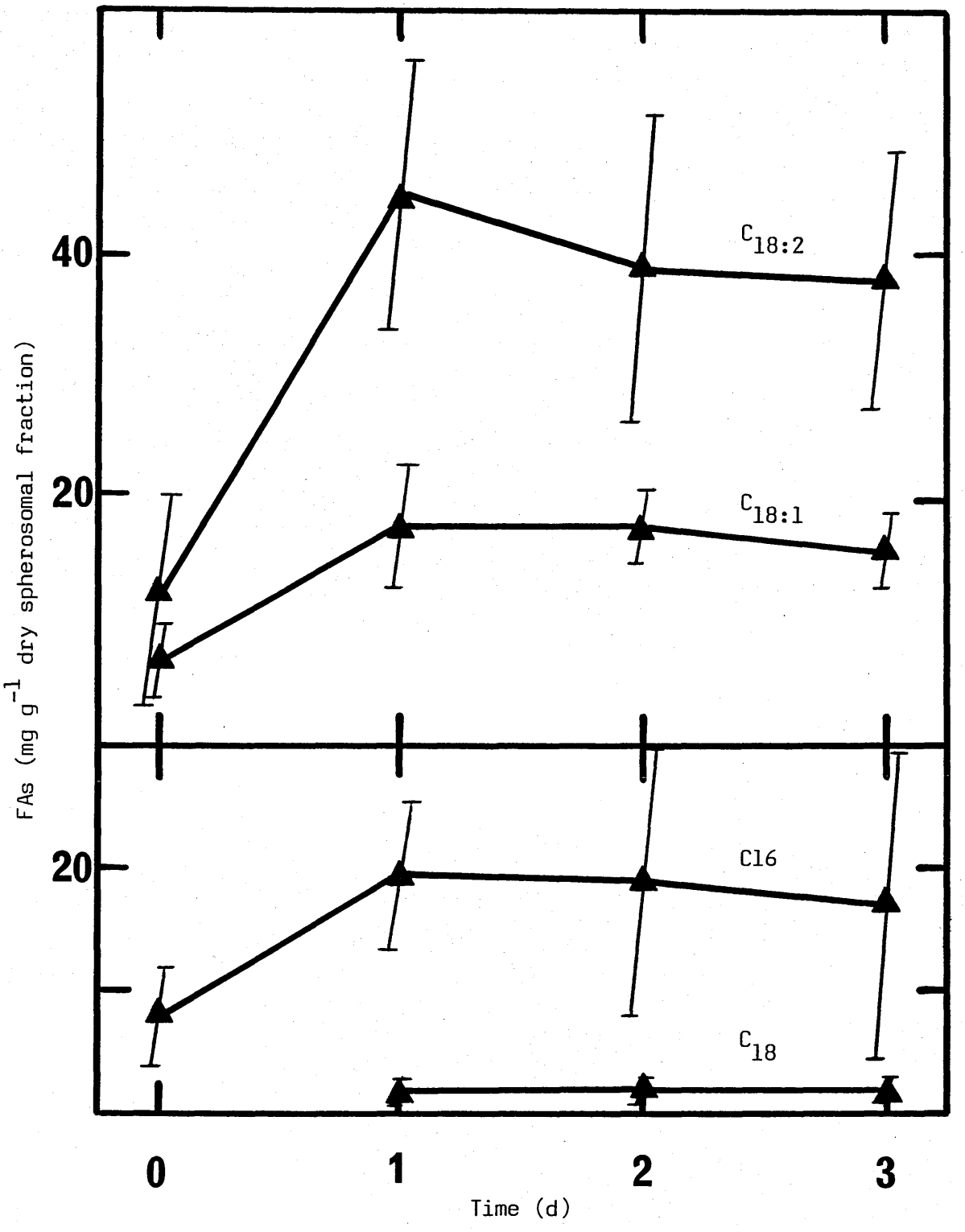


Figure 49 LCFAME of spherosomal phospholipid extracted from cotyledons during germination of Gossypium.

closely with those obtained from analysing fatty acids of phospholipids from Gossypium cotyledons of intact seeds (Table 85).

There are problems in defining the terms of spherosomes, oleosomes, oil bodies, oil droplets, lipid vesicles, lipid storage bodies. These may all be the same and may include microsomes or other microbodies. Patently, the nomenclature and classification of these storage bodies need clarification, but this would necessitate detailed electron microscopy with parallel microchemical analysis. It is possible that there are several different types of storage body, which contain a specific type of lipid and/or related enzymes. For this investigation, however, it is assumed that there is one type of storage body.

Table 85 LCFA composition in the spherosomal phospholipid subfraction in Gossypium cotyledons.

ND = not detected

Fatty acids	Spherosomal phospholipid subfraction isolated from cotyledons			Direct extraction of cotyledons from intact seed		
	Germination time (d)			Germination time (d)		
	1	2	3	1	2	3
	(% of total PL)					
C_{16}	23.32	24.09	23.19	24.19	24.51	23.13
C_{18}	2.07	2.69	2.75	1.66	1.81	1.69
$C_{18:1}$	21.41	23.20	21.92	23.00	21.31	21.14
$C_{18:2}$	53.19	50.00	52.12	51.14	51.31	52.72
$C_{18:3}$	ND	ND	ND	ND	1.06	1.30

5.2 Characterisation of Mitochondria

5.2.1 Isolation of mitochondrial fraction

Mitochondria-rich fractions from the cotyledons of both Helianthus and Gossypium were prepared by centrifugation (15000 g). This method follows closely that of Leonard and Van Der Woude (1976), and Klaus and Kindle (1979) who isolated mitochondrial fractions by a low-speed centrifugation step (13000 g).

Electron micrographs of the mitochondria-rich fraction reveal variable shaped structures (Figures 50 and 51) possibly showing the mitochondrial outer and inner membranes (Figure 50). Tribe and Whillaker (1972) noted that mitochondria range in morphology from spherical bodies to very elongate and even cup-shaped structures.

The mitochondria-rich fractions showed enrichment in the enzyme marker succinate dehydrogenase (Tables 86-87). These tables also show that fat pad (spherosome) fractions were free from mitochondria. This observation matches other observations on plant tissues (e.g. Williamson et al., 1975, Janiszowska et al., 1979 and Baydoun, 1980).

5.2.2 Mitochondrial lipids and fatty acid composition

Mitochondria-rich fractions were prepared for chemical analysis by washing with distilled water several times, freeze-drying to constant weight, then the lipids were extracted using chloroform-methanol (2:1 by volume). The lipid classes were classified by column chromatography using silicic acid and long-chain fatty acid methyl esters of neutral lipids and

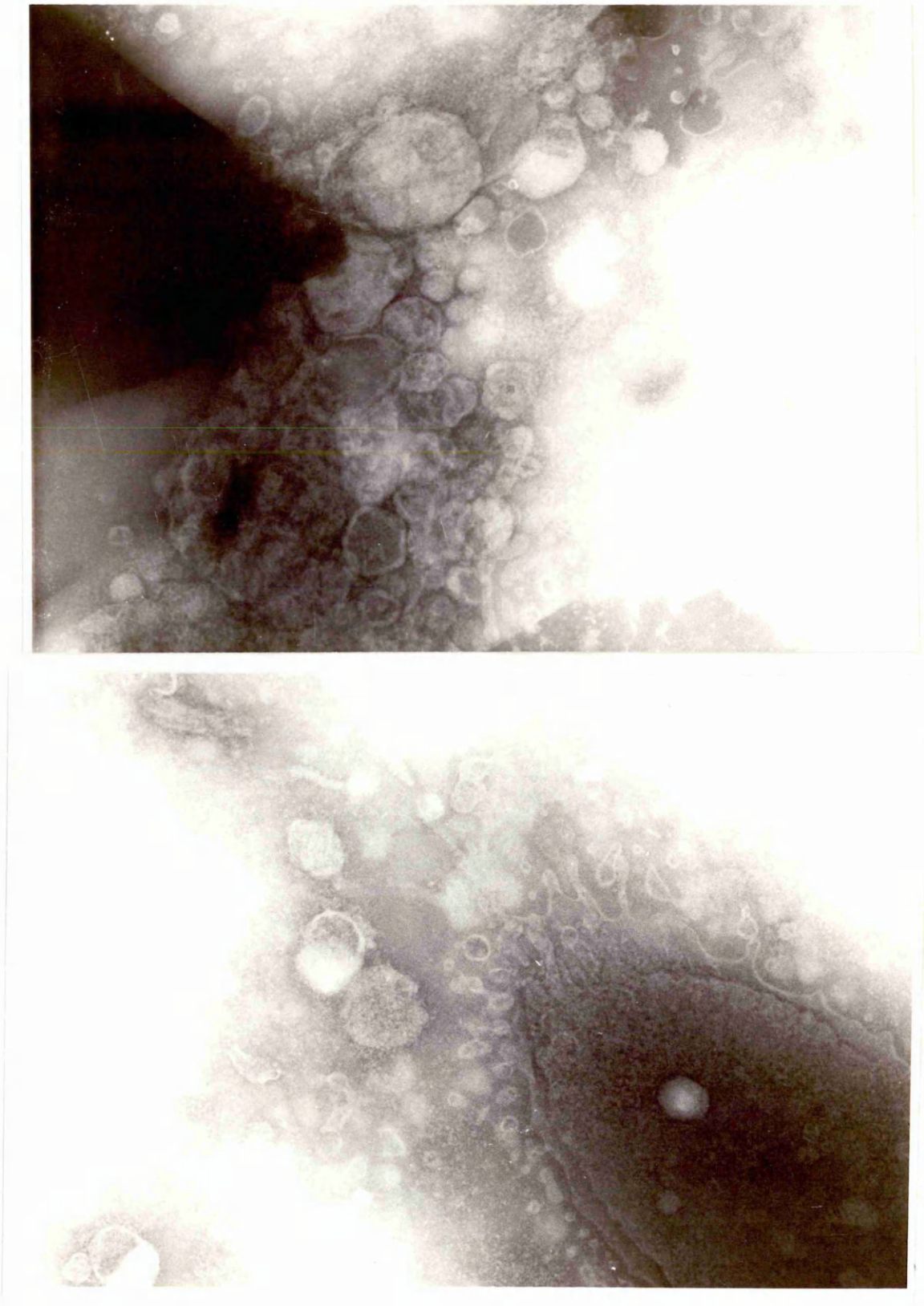


Figure 50 Electron micrograph of isolated mitochondria from Helianthus cotyledons negatively stained with 1% methylamine tungstate.

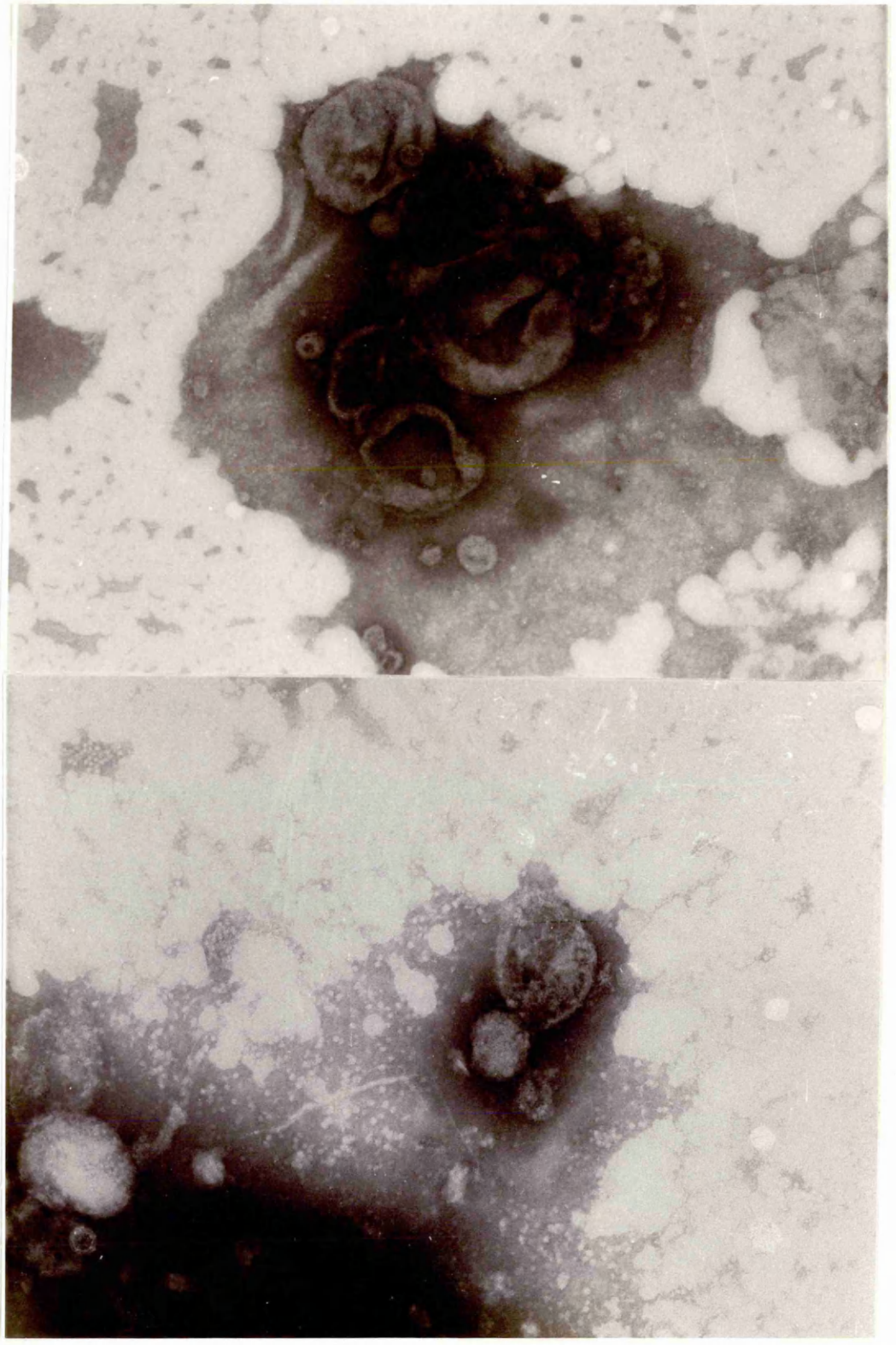


Figure 51 Electron micrograph of isolated mitochondria from Gossypium cotyledons negatively stained with 1% methylamine tungstate.

Table 86 Distribution of lipid and total activity of succinate dehydrogenase in Helianthus.

	Lipid		Succinate dehydrogenase			
			Total activity		Specific activity	Relative sp. activity
	mg g^{-1} fresh cotyledons	% relative to homogenate	n mol min^{-1}	%	n mol min^{-1} mg^{-1} lipid	
Homogenate	205.20	100	728.20	100	3.50	1.00
Pellet (800g)	21.40	10.4	22.20	3.0	1.0	0.29
Supernatant (Fat pad)	157.70	76.9	0	0	0	0
Pellet (1500g)	14.80	7.2	686.40	94.3	46.4	13.26
Recovery		94.5		97.3		

Table 87 Distribution of lipid and total activity of succinate dehydrogenase in Gossypium.

	Lipid		Succinate dehydrogenase			
			Total activity		Specific activity	Relative sp. activity
	mg g^{-1} fresh cotyledons	% relative to homogenate	n mol min^{-1}	%	n mol min^{-1} mg^{-1} lipid	
Homogenate	133.40	100	682.10	100	5.1	1.00
Pellet (800g)	13.20	9.9	19.60	2.9	1.5	0.29
Supernatant (Fat pad)	106.10	79.5	0	0	0	0
Pellet (15000g)	7.90	5.9	631.80	92.6	80.0	15.7
Recovery		95.3		95.5		

phospholipids were determined during the 3 d germination period using GC-FID and GC-MS. The data are presented as mg g^{-1} dry mitochondrial fraction, as a percentage of total lipid and as relative to $C_{18:2}$ (100).

I. Helianthus

The total lipid content of the mitochondria-rich fraction isolated from dry Helianthus cotyledons was 254 mg g^{-1} dry fraction (25.4% of total dry weight fraction), a result in agreement with Van Deenen (1965) who stated that in plant tissue the whole mitochondrial organelle fraction contained between 20 - 30% lipid of their dry weight, and Jacks et al. (1967) who estimated total lipid to be 35% of the dry weight recovered from the mitochondrial fraction from peanut.

In Helianthus, the total lipid contents increased during imbibition, averaging 327 mg g^{-1} dry mitochondrial fraction during 3 d germination (Table 88). A few brief reports are available on the change in mitochondrial components during the earliest stages of germination (Cherry, 1963, and Bain and Mercer 1966a and b).

In the study of lipid classes of the mitochondrial fraction from dry Helianthus cotyledons, the phospholipid subfraction was present in the highest content (188 mg g^{-1} dry mitochondrial fraction; 74.6% of the total lipid). Neutral lipid subfraction was 62 mg g^{-1} dry fraction (25% of the total lipid) but glycolipid subfraction was present at less than 1%. These results are similar to those of Munn (1974), Harrison and Lunt (1975) and Mazliak (1977) who found that the phospholipids of mitochondria from plant tissues were between 70 - 75% of the

Table 88 Mitochondria and total lipid contents during *Helianthus* germination.

Values are means ± SE.

Germination time (d)	Mitochondria (dry wt) content of		Mitochondrial lipids (total) as related to		
	seed	cotyledons ($mg\ g^{-1}$ fresh wt)	fresh seed	fresh cotyledons ($mg\ g^{-1}$)	dry mitochondria
Dry	31.3 ± 14.8	58.3 ± 30.2	7.9 ± 4.1	14.8 ± 9.2	253.8 ± 25.6
1	30.7 ± 16.2	62.4 ± 32.5	10.5 ± 6.3	21.4 ± 13.5	343.1 ± 41.2
2	28.5 ± 11.1	59.9 ± 41.2	9.2 ± 5.3	19.3 ± 17.2	323.2 ± 39.8
3	26.8 ± 12.1	57.5 ± 38.5	8.5 ± 6.1	18.2 ± 13.7	316.5 ± 55.6

total mitochondrial lipid; these phospholipids were phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidic acid (PA) and phosphatidylinositol (PI). Nevertheless Harwood and Russell (1984) mentioned that the total lipids of mitochondria contain a high proportion (98%) of phospholipids. The phospholipid content of the Helianthus mitochondria increased during the imbibition period - 255 mg g^{-1} mitochondrial fraction at 1 d, averaging 244 mg g^{-1} dry fraction at 3 d. Neutral lipid increased slightly to 82 mg g^{-1} dry fraction at 1 d, and averaged 78 mg g^{-1} dry fraction during germination (Table 89). When the results are presented as a percentage of total lipid, no changes were noted in both phospholipid and neutral lipid subfractions during germination. This apparent confusion is attributable to an increase in the total lipid of mitochondria during germination. Nawa and Asahi (1971) previously noted that mitochondrial protein and phospholipids increased in the cotyledon fraction during imbibition of pea seeds.

In the study of mitochondrial neutral lipid subfraction, the major fatty acids were C_{16}, C_{18}, $C_{18:1}$ and $C_{18:2}$. The levels of these fatty acids did not change during the three-days germination period. C_{16} was present at an average of 7 mg g^{-1} dry mitochondrial fraction, C_{18} averaged 3 mg g^{-1} dry mitochondrial fraction, $C_{18:1}$ 21 mg g^{-1} dry mitochondrial fraction and $C_{18:2}$ 39 mg g^{-1} dry mitochondrial fraction (Table 90).

The mitochondrial phospholipid subfraction was also analysed. C_{16} increased during imbibition period to more than double its original value, averaging 23 mg g^{-1} dry mitochondrial

Table 89 Lipid classes of mitochondrial fraction from *Helianthus* cotyledons during germination.

Values are means ± SE. tr = trace (less than 1)

Germination time (d)	mg g^{-1} dry mitochondrial fraction (% of total lipid)		
	Neutral lipid	Phospholipid	Glycolipid + others
Dry	61.8 ± 6.2 (24.51)	188.2 ± 16.8 (74.62)	2.2 ± 1.2 (0.87)
1	82.7 ± 11.3 (24.27)	254.9 ± 21.1 (74.78)	3.2 ± 1.8 (0.95)
2	75.5 ± 10.4 (23.85)	241.3 ± 23.7 (75.34)	tr
3	76.2 ± 13.8 (24.35)	236.7 ± 28.2 (74.94)	tr

Table 90 Long-chain fatty acids of the mitochondrial neutral lipid subfraction from Helianthus cotyledons during germination.
Values are means ± SE.

Germination time (d)	Fatty acids - mg g^{-1} dry mitochondrial fraction (% of NL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	4.8 ± 1.2	2.2 ± 0.8	18.3 ± 9.1	37.1 ± 12.1
	(7.68)	(3.52)	(29.37)	(59.43)
1	6.5 ± 2.5	3.6 ± 1.2	22.4 ± 6.6	39.7 ± 13.2
	(9.01)	(5.03)	(31.02)	(54.94)
2	7.9 ± 4.2	3.7 ± 1.3	21.7 ± 7.3	37.6 ± 11.4
	(11.22)	(5.31)	(30.56)	(52.92)
3	9.2 ± 3.6	3.2 ± 1.4	20.6 ± 5.4	42.7 ± 10.2
	(12.19)	(4.30)	(27.21)	(56.30)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	12.9	5.9	49.4	100
1	16.4	9.1	56.4	100
2	21.2	10.0	57.7	100
3	21.6	7.6	48.3	100

fraction during germination, with a peak (21) relative to $C_{18:2}$ at 3 d. C_{18} content increased to 38 mg g^{-1} dry mitochondrial fraction (relative value 36) at 3 d. Unsaturated $C_{18:1}$ decreased to 56 mg g^{-1} dry mitochondrial fraction (relative value 52) at 3 d but $C_{18:2}$ changed only slightly in value during germination (Table 91).

II. Gossypium

The total lipid content of the mitochondria-rich fraction isolated from dry Gossypium cotyledons was calculated to be 206 mg g^{-1} dry mitochondrial fraction (20.6% of the total dry wt fraction). This result is in the same range of total lipid from mitochondrial fraction as given in an early publication by Van Deenen (1965). The total lipid contents were noted to increase during the imbibition period from 206 mg g^{-1} to 251 mg g^{-1} mitochondrial fraction at 1 d averaging 232 mg g^{-1} dry mitochondrial fraction at 2 d and 3 d germination (Table 92).

In the study of lipid classes of the mitochondria-rich fraction from dry Gossypium cotyledons, phospholipid was present in the highest levels (166 mg g^{-1} dry mitochondrial fraction; 80% of the total lipid), neutral lipid (40 mg g^{-1} dry mitochondrial fraction 19%), but glycolipid was present at less than 1%. The results show that the mitochondrial phospholipid content from Gossypium is higher than the equivalent subfraction from Helianthus. The mitochondrial phospholipid of Gossypium averaged 196 mg g^{-1} dry mitochondrial fraction during germination, and the neutral lipid averaged 41 mg g^{-1} dry fraction (Table 93).

Table 91 Long-chain fatty acids of mitochondrial phospholipid subfraction from Helianthus cotyledons during germination.
Values are means ± SE.

Germination time (d)	Fatty acid - mg g^{-1} dry mitochondrial fraction (% of PL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	8.8 ± 5.2	12.2 ± 5.8	85.2 ± 37.8	109.8 ± 59.5
	(4.10)	(5.65)	(39.36)	(50.74)
1	21.2 ± 4.8	19.5 ± 5.4	69.0 ± 22.1	130.7 ± 32.2
	(8.81)	(8.12)	(28.72)	(54.35)
2	24.6 ± 8.2	26.4 ± 6.6	62.6 ± 13.4	126.8 ± 44.2
	(10.24)	(10.98)	(26.03)	(52.75)
3	22.8 ± 10.2	38.1 ± 7.6	56.2 ± 18.8	107.5 ± 36.5
	(10.18)	(16.98)	(25.00)	(47.84)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	8.0	11.1	77.5	100
1	16.2	14.9	52.8	100
2	19.4	20.8	49.3	100
3	21.2	35.5	52.2	100

Table 92 Mitochondria and total lipid contents during Gossypium germination. Values are means ± SE.

Germination time (d)	Mitochondria (dry wt) ($mg\ g^{-1}$ fresh)		Total lipid in mitochondria ($mg\ g^{-1}$)		
	seed	cotyledons	fresh seed	fresh cotyledons	dry mitochondria
Dry	19.9 ± 11.5	35.4 ± 12.2	4.1 ± 2.1	7.2 ± 3.8	206.1 ± 24.5
1	23.5 ± 12.1	41.7 ± 17.7	5.9 ± 2.2	10.4 ± 5.5	251.1 ± 22.1
2	21.2 ± 11.8	35.1 ± 15.5	4.7 ± 2.3	7.8 ± 2.6	223.3 ± 31.2
3	34.2 ± 13.2	38.5 ± 18.5	5.8 ± 3.2	9.3 ± 3.7	241.7 ± 27.5

Table 93 Lipid classes of mitochondrial fraction from *Gossypium* cotyledons during germination.

Values are means ± SE. tr = trace (less than 1)

Germination time (d)	mg g^{-1} dry mitochondrial fraction (% of total lipid)		
	Neutral lipid	Phospholipid	Glycolipid + others
Dry	40.2 ± 14.8 (19.52)	165.8 ± 26.2 (80.10)	tr
1	45.4 ± 16.5 (18.38)	202.7 ± 23.2 (81.62)	tr
2	34.4 ± 18.5 (15.36)	190.5 ± 29.5 (84.17)	tr
3	43.8 ± 14.2 (18.25)	196.2 ± 32.8 (81.15)	tr

In the study of the mitochondrial neutral lipid subfraction, the major fatty acids were C_{16}, $C_{18:1}$ and $C_{18:2}$. C_{16} decreased from 7 mg g^{-1} dry fraction (relative value 28) in dry cotyledons to 2 mg g^{-1} dry fraction (relative value 10) at 3 d, C_{18} appeared only in dry cotyledons (1.4 mg g^{-1} dry fraction), $C_{18:1}$ increased from 7 mg g^{-1} dry fraction (relative value 30) in dry cotyledons to 18 mg g^{-1} dry fraction (relative value 83) at 3 d, but $C_{18:2}$ levels did not change, averaging 23 mg g^{-1} dry fraction during germination (Table 94).

The fatty acids in the mitochondrial phospholipid subfraction did not change during germination. C_{16} averaged 35 mg g^{-1} dry fraction, C_{18} decreased after 2 d to 2 mg g^{-1} dry fraction (relative value 2) at 3 d, $C_{18:1}$ averaged 39 mg g^{-1} dry fraction and $C_{18:2}$ 107 mg g^{-1} dry fraction during germination (Table 95).

Previously, Mudd (1980) showed that $C_{18:2}$ was the most abundant FA in mitochondrial phospholipids from castor bean, constituting 52% in PC, 58% in PE and 36% in PI. On the other hand, Mazliak and Kader (1980) who determined fatty acids of the mitochondrial fraction from potato tubers presented figures of C_{16} 4.7%, C_{18} 2.7%, $C_{18:1}$ 1.4%, $C_{18:2}$ 63.4% and $C_{18:3}$ 16.9%. The results of the fatty acids analyses of mitochondrial fractions from both Helianthus and Gossypium are in general agreement with these publications especially in respect of the content of $C_{18:2}$ being the most abundant.

Table 94 Long-chain fatty acids of mitochondrial neutral lipid subfraction from *Gossypium* cotyledons during germination.

Values are means ± SE. ND = not detected

Germination time (d)	Fatty acids - mg g^{-1} dry mitochondrial fraction (% of NL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	6.8 ± 2.2 (17.16)	1.4 ± 0.5 (3.51)	7.3 ± 2.4 (18.46)	24.2 ± 8.3 (60.87)
1	3.1 ± 2.1 (7.28)	ND -	13.4 ± 5.6 (31.10)	26.5 ± 9.5 (61.62)
2	2.1 ± 1.2 (5.82)	ND -	15.2 ± 5.3 (41.37)	19.5 ± 7.5 (52.81)
3	2.1 ± 1.1 (4.95)	ND -	18.3 ± 8.4 (43.11)	22.1 ± 9.8 (51.94)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	28.1	5.7	30.3	100
1	11.2	-	50.4	100
2	11.0	-	78.3	100
3	9.5	-	83.0	100

Table 95 Long-chain fatty acids of mitochondrial phospholipid subfraction from the Gossypium cotyledons during germination. Values are means ± SE.

Germination time (d)	Fatty acids - mg g^{-1} dry mitochondrial fraction (% of PL)			
	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$
Dry	36.1 ± 12.6 (22.27)	7.1 ± 2.5 (3.37)	40.5 ± 7.5 (25.00)	81.4 ± 14.6 (50.24)
1	33.3 ± 11.4 (16.65)	9.5 ± 6.5 (4.76)	48.8 ± 11.2 (24.37)	108.6 ± 26.2 (54.22)
2	39.4 ± 12.6 (20.66)	6.2 ± 3.7 (3.28)	30.5 ± 9.4 (15.99)	114.5 ± 32.8 (60.07)
3	31.1 ± 13.8 (16.27)	2.1 ± 1.8 (1.12)	36.9 ± 12.1 (19.29)	121.2 ± 36.6 (63.32)
	Fatty acids relative to $C_{18:2}$ (100)			
Dry	44.3	5.0	49.8	100
1	30.7	8.7	44.9	100
2	34.4	5.4	26.6	100
3	25.6	1.7	30.4	100

5.3 Isolation of endoplasmic reticulum (ER)

The relationship between the spherosomes and the ER was investigated by isolating ER from both species.

Fresh tissues were first chopped with a razor blade or scissors, washed with homogenisation medium, and then treated in a pestle and mortar using a squashing action. This is considered to be superior to grinding because the shearing forces on the tissues are lessened (Nagahashi and Beevers, 1978; Lehle et al., 1978). The homogenisation medium used in this experiment (Material and Methods 3.7.2) is similar to that used by Bowles and Kauss (1976), Baydoun (1980) and Waldron (1984) on other plant tissues. Centrifugation was carried out at 100000 g after the supernatant was layered on a discontinuous sucrose gradient (50 - 10% sucrose); the boundaries between the four conspicuous layers became less clear after centrifugation. This experiment was repeated several times during a six-month period. Sometimes after centrifugation, two fractions were detected, one from the top (20% sucrose) and one from the medium (on 40% sucrose) of the tube were collected and assayed by enzyme markers, NADH-cytochrome c reductase or succinate dehydrogenase. Most of these top fractions showed enrichment in NADH-cytochrome c reductase (total activity more than 4000 n mol min^{-1}). This result is in agreement with those of Baydoun (1980). Unfortunately, these amounts of fraction were not adequate for chemical analysis.

GENERAL DISCUSSION AND CONCLUSIONS

The results of this investigation provide some information on fatty acid content and composition in Helianthus and Gossypium seeds and seedlings.

In the first section of the Results, a study of germination behaviour showed that the optimal temperatures and amounts of water for the germination of 20 seeds in a 9 cm plastic Petri dish with a single sheet of seed test paper in darkness were 25°C and 5 cm^3 distilled water for Helianthus, and 30°C and 5 cm^3 distilled water for Gossypium. The germination percentages obtained under these conditions closely matched the seed viability data as tested by the tetrazolium assay. In general, the results in this section were broadly similar to published work on both species. Such germination tests must be regarded as straightforward laboratory assessments and only give an indirect measurement of germination and seedling establishment in the field where other environmental factors (gaseous, allelochemicals etc.) may markedly influence these developmental processes.

In the second section, experiments on determining the lipid contents of both species revealed that total lipids decreased during a 72 h germination period. In pregerminating Helianthus seeds, the lipid content was 329 mg g^{-1} dry seed (about 33% of the dry weight), decreasing by 72 h to 232 mg g^{-1} dry seed (about 23%). These gross measurements mask the fact that although lipids decreased to 205 mg g^{-1} in the cotyledons, which constitute a major part of the seed,

there was an approximately two-fold increase to about 18 mg g^{-1} dry seed in the radicles and there was no change in the lipid content of the pericarp and testa during the 72 h germination period. The total lipids of Helianthus were composed of neutral lipid (86% of the total lipid) and polar lipid (phospholipid and glycolipid; 14%). As expected these lipid classes decreased in the cotyledons, and there was no change in the polar lipid of the cotyledons and radicles during the germination period.

In ungerminated Gossypium seeds, the total lipid content was 244 mg g^{-1} dry seed (24% of the dry weight) but by 72 h germination this level decreased to 172 mg g^{-1} dry seed (17%). There was a marked decrease in the cotyledons. There was no change in the lipid contents of both radicles (unlike Helianthus) and testa (like Helianthus) during germination. The ratio of neutral to polar lipid was similar to that of Helianthus. Neutral lipid decreased in the cotyledons but polar lipid increased in both cotyledons and radicles during germination. Many previous studies have reported that in oilseeds the lipid content decreases during germination (e.g. Yamada, 1957; Boatman and Crombie, 1958; Beevers, 1961; Zimmerman and Klosterman, 1965; Bhatia et al., 1978; Doman et al., 1982; Noggle and Fritz, 1983 and Knowles and Kennedy, 1984). One of these earlier studies (Beevers, 1961) showed that in castor bean total fat (i.e. lipid) content of the seedling fell to about 13% of the original weight in dry seeds, while the carbohydrate level increased 15-fold compared with dry seeds after a protracted germination period of 8 days.

In spite of the fact that sugars are used extensively in the growth and respiration of the seedling proper, Beevers' studies showed the production of more than 1 gm of sugar (principally sucrose) for each gm of fat consumed. Likewise, Noggle and Fritz (1983) recently showed conversion of fat to sucrose in the endosperm of castor bean seed and this conversion began with the hydrolysis of triglycerides to glycerol and fatty acids through the action of lipase. It would be interesting to analyse the carbohydrates of Helianthus and Gossypium seeds to investigate whether or not all oilseeds share a similar lipid metabolism pattern. Such studies would need to monitor the incorporation of some form of labelling (e.g. ^{14}C radio-label) from the lipids to any newly synthesised carbohydrate. Parallel studies would need to be carried out on the rates of metabolism and the utilisation of the carbohydrate - possibly in lipid metabolism.

The most abundant long-chain fatty acid in both species (Section three of Results) was $C_{18:2}$, and other major LCFAs were C_{16}, C_{18}, $C_{18:1}$; the minor fatty acids were C_{14}, $C_{16:1}$, $C_{18:3}$ and C_{20}. In Helianthus seeds, saturated fatty acids constituted 11% and unsaturated constituted 89% of the total lipid. A change in the total fatty acid composition was detected in the cotyledons: C_{16} and $C_{18:1}$ decreased to about half their original content whereas $C_{18:}$ and $C_{18:2}$ did not change during germination. The contents of these fatty acids from the total lipids changed only slightly in the radicles, pericarp and testa. When analysed individually in the three lipid classes C_{16}, C_{18},

and $C_{18:2}$ of the neutral lipid fraction did not vary to any marked extent in the cotyledons and radicles during germination. The LCFAsof the phospholipid and glycolipid subfraction of the cotyledons decreased during germination. The fatty acids of phospholipid (C_{16} and C_{18}) increased slightly in the radicles but there were no changes in $C_{18:1}$ and $C_{18:2}$.

In Gossypium seeds, saturated fatty acids constituted 29% and unsaturated 71% of the total lipid. As in Helianthus very limited changes of the total fatty acid composition were noted in the cotyledons. C_{16}, $C_{18:1}$ and $C_{18:2}$ decreased only slightly and C_{18} did not apparently change. The amounts of these fatty acids changed slightly in the radicles and testa during the germination period. $C_{18:1}$ and $C_{18:2}$ of the neutral lipid fraction from cotyledons decreased gradually, C_{16} decreased slightly and C_{18} increased slightly but there were no detectable changes in these fatty acids in the radicles. All the analysed fatty acids in the phospholipid fractions of cotyledons and radicles did not change. C_{16}, $C_{18:1}$ and $C_{18:2}$ of the glycolipid fraction of cotyledons increased slightly during germination.

It could be argued that where changes in fatty acids were detected, then this could be due to changes in the relative amounts of the lipid classes during germination, or they could be related to conversion of saturated to unsaturated molecules, or even interconversion of unsaturated to different unsaturated molecules.

Stobart and Stymne (1985) established that $C_{18:1}$ entering position 1 of PC from DG is desaturated *in situ* to $C_{18:2}$. Likewise, the reports of Dybing and Craig (1970); Slack *et al.* (1978); Stymne and Appelqvist (1980); Browse and Slack (1981) and Roughan and Slack (1982) on the kinetics of labelling of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ in various lipid classes have led to suggestions that $C_{18:1}$ esterified to PC is the probable substrate and ($C_{18:2}$) PC a possible substrate for the desaturation of $C_{18:1}$ and $C_{18:2}$ respectively, in the cotyledons of oilseeds. This scheme is perhaps operating in *Helianthus* and *Gossypium* seeds studied in this thesis because during the period of observation $C_{18:2}$ remains the most abundant fatty acid in both species.

Molecules in the various fatty acid pools may be undergoing metabolic transformation but measurements of "levels" or "amounts" without concomitant metabolic studies may not be able to indicate the nature and characteristics of any interconversion taking place. Further studies will be needed to ascertain the details of what is occurring.

In section four the major short-chain fatty acids in both species were found to be C_8, C_9, C_{10} and C_{12}. In *Helianthus* seeds, C_8 and C_9 decreased by 72 h to about 10% of their original amounts in dry seed, C_{10} levels varied between 4 and 49% during this time. C_{12} increased sharply to about 60% of the SCFAs after 3 d of germination. Dissection of the seed into cotyledons, radicles and covering tissues allowed the observations that in the cotyledons C_9 decreased to about one-third, and C_{10} and C_{12} increased four-fold by 3 days. In the radicles, C_8 and C_9 were at their highest respective contents at

64 h, C_{10} decreased by about half but C_{12} did not change. In the pericarp and testa, C_8 and C_9 increased two-fold and C_{10} and C_{12} changed slightly during the entire germination period.

Comparable analyses were carried out with Gossypium seeds: C_8 content declined to the extent that it was not detected at 72 h, C_9 decreased to about half content, C_{10} increased to reach a level of about 30% of the total SCFA content and C_{12} increased sharply to be about 60% of SCFA at 72 h. In the cotyledons, C_9 decreased by about half and C_{12} increased sharply to account for 60% of the total SCFA. In the radicles, C_8 decreased sharply and was not detectable at 72 h, C_{10} increased to be about 30% of the total SCFA. C_8, C_9, C_{10} and C_{12} were readily detected in the testa and changes took place during germination.

SCFA appear to vary in level more than LCFA during germination. This could be a reflection of problems of extraction of SCFA, given their relative volatility, but it could also indicate active interconversions and metabolism. Two pressing questions need to be answered by further investigations. Firstly, what are the roles of SCFA in the physiology of germination? Perhaps the SCFA in the testa of Helianthus and Gossypium and pericarp of Helianthus arise simply by endospermal contamination during preparation of these parts of the seed, and SCFA may have no role in seed-coat-controlled germination. Secondly, what are the metabolic inter-relationships between the various SCFA, and SCFA and LCFA? Very little precise work has been carried out

on this intriguing aspect which appears to be amenable to experimentation. Increases in C_{12} during germination in both species could be due to degradation of C_{18} acids by β-oxidation, and decreases in C_9 could be due to oxidation of Δ 9 in C_{18} acids. Several well-known SCFA were not detected in extract of Helianthus and Gossypium; these include C_4 and C_6 (see Appendix). A more general issue is the definition of SCFA and LCFA, and where the division between the categories takes place. In this thesis, SCFA are C_4 - C_{12}, LCFA are C_{14} and above (see Appendix), a classification in general usage.

Electron microscopy is the most convenient method for studying the morphology of lipid-storage bodies (spherosomes) from the cotyledons of both species (Section five of Results). Spherosomes were around 0.5 - 1 μm in diameter in Helianthus, and 0.3 - 2 μm in diameter in Gossypium. Following processing, these spherosomes were bounded by a half-unit or single membrane; the matrix was uniformly electron-dense and surrounded by precipitated lead soaps. These results agree with previous publications reporting that oil bodies or spherosomes appear to have similar diameters in a range of species (Yatsu et al., 1971; Yatsu and Jacks, 1972; Rest and Vaughan, 1972; Smith, 1974; Gurr et al., 1974 and Bergfeld et al., 1978) and that spherosomes are bounded by a half-unit membrane (Yatsu and Jacks, 1972 and Wanner et al., 1981).

Related studies on the lipid and fatty acid composition of the spherosomal preparations from both species revealed that in Helianthus cotyledons, the spherosomal fraction

contained 900 mg lipid g^{-1} dry spherosomal fraction, decreasing to 650 mg lipid g^{-1} spherosomal fraction after 3 d germination. About 88% of this total lipid was neutral lipid (786 mg neutral lipid g^{-1} dry spherosomal fraction), 9% was phospholipid (83 mg phospholipid g^{-1} dry spherosomal fraction), 3% glycolipid and other (25 mg glycolipid g^{-1} dry spherosomal fraction). Decreases in the levels of these lipid fractions take place during germination.

In Gossypium cotyledons, the spherosomal fraction contained 780 mg lipid g^{-1} dry spherosomal fraction, decreasing to 320 mg lipid g^{-1} dry spherosomal fraction after 3 d germination. About 96% of this total lipid was neutral lipid (754 mg neutral lipid g^{-1} dry spherosomal fraction), 3% was phospholipid (26 mg phospholipid g^{-1} dry spherosomal fraction), 1% glycolipid and other (5 mg glycolipid g^{-1} dry spherosomal fraction). The neutral lipid decreased but phospholipid and glycolipid increased during germination period.

Considerable variation in the results were reported for spherosomes (oleosomes or oil bodies) from several species. Mature peanut and cotton cotyledons (Yatsu et al., 1971) contained 99% and 98% neutral lipid, 0.1% and 0.4% phospholipid and 0.2% and 0.6% protein respectively. Sunflower oil bodies contained about 89% lipid, 4% protein and 7% other, and crambe oil bodies contained 64% neutral lipid, 11% phospholipid, 19% protein and 6% water (Gurr et al., 1974). Recently, oil bodies were isolated from linseed and safflower (Slack et al., 1980); these had very similar composition, contained 96.8% neutral lipid, 0.7% phospholipid and 2.5% protein.

As yet, it is not possible to use enzyme markers to measure the purity of spherosomal preparations, since the enzyme complement of this organelle is not known and hence different criteria of purity have been adopted. There are undoubtedly difficulties in preparing spherosomes free from other cell constituents, but by using SDS-gel electrophoresis to monitor polypeptides present during spherosome purification, it was found that oil body or spherosomal preparations can be isolated that contain little, if any, of the proteins found in other cell fractions (Bergfeld et al., 1978; Slack et al., 1980 and Slack and Roughan, 1980). Therefore, the decrease of spherosomal lipid content during germination in both Helianthus and Gossypium cotyledons noted in this thesis may be connected with an increase of protein or other contaminants, or could be the result of lipid hydrolysis by the action of lipase. This is possible because Huang and Moreau (1978) found that spherosomal preparations from Helianthus cotyledons contained only alkaline lipase activity which increased dramatically during germination. Likewise, in the spherosomes and glyoxysomes of castor bean endosperm (Moreau et al., 1980; Maeshima and Beevers, 1985) acid and alkaline lipases were present in high activity initiating the breakdown of the stored lipid in the endosperm of the young growing seedling.

The major fatty acids of spherosomal preparation in both species cotyledons were C_{16}, C_{18}, $C_{18:1}$ and $C_{18:2}$. The levels of these acids changed during germination. In Helianthus cotyledons, analysis of the total fatty acids of spherosomal preparations showed that C_{16} decreased to one-third, $C_{18:1}$

decreased to half, C_{18} increased about two-fold during imbibition, and there was no change in $C_{18:2}$ content compared with the original levels. Of the fatty acids of spherosomal neutral lipid subfraction, C_{16} and C_{18} increased about three-fold, and $C_{18:1}$ and $C_{18:2}$ decreased to about half content.

In Gossypium cotyledons, C_{16} and C_{18} of the spherosomal preparation increased slightly during imbibition, but $C_{18:1}$ and $C_{18:2}$ decreased to one-third of their original weight by day three. C_{16} and C_{18} in spherosomal neutral lipid subfraction increased about two-fold, $C_{18:1}$ decreased to less than half content and $C_{18:2}$ decreased sharply during germination. All fatty acids in the spherosomal phospholipid subfraction increased two to three-fold except for C_{18} which was present only in trace amounts.

Neutral lipid was the most abundant lipid class in spherosomal preparations from both species. Triglycerides may be the most abundant component of the neutral lipids and they may be synthesised in spherosomes (Shine et al., 1976).

Studies of mitochondria from the cotyledons of both species included analyses of lipid content and fatty acid composition during germination. The mitochondrial fractions of both species showed enrichment in the enzyme marker succinate dehydrogenase.

In Helianthus cotyledons, the mitochondria-rich fraction contained 254 mg lipid g^{-1} dry mitochondrial fraction, increasing to 327 mg lipid g^{-1} dry mitochondrial fraction during day three of germination. Phospholipid constituted 75%, neutral lipid 24%, and glycolipid less than 1% of the total lipid. C_{16}

increased about two-fold in the mitochondrial neutral lipid subfraction, but there were no changes in C_{18}, $C_{18:1}$ and $C_{18:2}$ during germination. C_{16} and C_{18} increased about three-fold in the mitochondrial phospholipid subfraction, $C_{18:1}$ decreased by about one-third but $C_{18:2}$ did not change during germination.

In Gossypium cotyledons, the mitochondria-rich fraction contained 206 mg lipid g^{-1} dry mitochondrial fraction, increasing to 236 mg lipid g^{-1} dry mitochondrial fraction during day three of germination. Phospholipid constituted 80%, neutral lipid 19%, and glycolipid less than 1% of the total lipid. C_{16} decreased to less than half in the mitochondrial neutral lipid subfraction, $C_{18:1}$ increased about two-fold and there was no change in $C_{18:2}$. C_{18} decreased to less than half in the mitochondrial phospholipid subfraction, $C_{18:1}$ and $C_{18:2}$ increased slightly during germination.

Phospholipids have been recognised as a major component of biological membranes. Earlier studies on the chemical composition of mitochondria from silver beet (Martin and Morton, 1956) showed that 38.7% of the dry mitochondrial fraction was lipid, 70% of which was phospholipid. These results are similar to phospholipid contents of Helianthus and Gossypium mitochondria-rich fractions presented in this thesis. Recently, results were reported for lipid classes of mitochondria from the endosperm of castor beans (Donaldson and Beevers, 1977), which contained 17% PI and more PE relative to PC, and were characterised by the presence of cardiolipin, in which 80% of

the fatty acid is linoleate; the predominate fatty acids in each phospholipid class were C_{16}, C_{18}, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$. Phospholipid metabolism has been studied in the endosperm of castor bean (Sparace and Moore, 1979); PG and PC were localised exclusively in the inner mitochondrial membrane fractions while PA synthesis occurred in both the inner and outer mitochondrial membrane. Related work (Moore, 1982) indicated that PA synthesis occurred in the mitochondria and endoplasmic reticulum, PS and PI were distributed between the layers of the outer mitochondrial membranes and PC distribution was restricted to the endoplasmic reticulum and mitochondria.

One of the major problems facing the experimenter when carrying out analyses of a particular chemical component from living tissue is the selection of appropriate techniques. Ideally, rapid systems of high accuracy, sensitivity, selectivity and information content should be employed. This thesis relied on GC and GC-MS, yet there are many other systems such as NMR (Conway and Earle, 1963; and Jambunathan et al., 1985) and HPLC (Erdahl and Privett, 1985) which could also be employed. The statistical reliability of the data requires the analyses of many samples, and this alone poses problems of handling and processing, and raises questions about the critical aspect of cost efficiency per sample. One of the most unsatisfactory aspects of the work must be the isolation of cellular fractions, particularly the ER (DeDuve, 1971; Quail, 1979; Evans, 1982). In the future, considerable effort will need to be spent in devising suitable reliable techniques with purity checks encompassing methods additional to electron microscopy and a

few enzyme markers. Nevertheless, the biochemistry and physiological role of fatty acids in the germination of oilseeds remains an intriguing and important area of research. The newer techniques of gene manipulation may make the task of investigating fatty acids easier, and conversly, fatty acids in seeds may provide a valuable target for the gene engineer.

REFERENCES

Abdel Magid, A.S. and Osman, A.M. (1975). Influence of storage period and temperature on viability and chemical composition of cottonseeeds. Ann. Bot., 39, 237-248.

Abdel Magid, A.S. and Osman, A.M. (1977). On the germination and changes in chemical composition during seedling emergence and development of cottonseeds. Ann. Bot., 41, 697-705.

Achaya, K.T. (1975). Cottonseed chemistry and technology. Publication and Information Directorate, CSIR, Hellside Rd., New Delhi, India.

Afzalpurkar, A.B. and Lakshminarayana, G. (1980). Variation in oil content and fatty acid composition with sunflower head size and shape. JAOCS, 57, 105-108.

American Oil Chemists' Society (1973). Official and Tentative Methods, 35 East Weaker Drive, Chicago.

Ando, S., Isobe, M. and Nagai, Y. (1976). High performance preparative column chromatography of lipids using a new porous silica-separation of molecular species of sphingogly colipids. Biochim. Biophys. Acta, 424, 98-105.

Apon, J.M.B. and Nicolaides, N. (1975). The determination of the position isomers of the methyl branched fatty acid methyl esters by capillary GC-MS. J. Chromat. Sci., 13, 467-475.

Appelqvist, L.A. (1975). Biochemical and structure aspects of storage and membrane lipids in developing oilseeds. In "Recent advances in the chemistry and biochemistry of plant lipid". Edited by T. Galliard and E.I. Mercer. Academic Press, London, 4, 245-286.

Astrit, G. and Rudolf, D. (1971). Physiochemical and morphological characteristics of sunflower seed and its industrialization in Albania. Bu Shken eave Bujgesare, 2, 28-31.

Bailey, A.E. (1948). Cottonseed and cottonseed products. Interscience Publishers Inc., New York.

Bain, J.M. and Mercer, F.V. (1966a). Subcellular organization of the cotyledons in germinating seeds and seedlings of Pisum sativum. J. Biol. Sci., 19, 69-84.

Bain, J.M. and Mercer, F.V. (1966b). The relationship of the axis and the cotyledons in germinating seeds and seedlings of Pisum sativum. J. Biol. Sci., 19, 85-96.

Barton, L. (1965)*. Dormancy in seeds imposed by the seed coat. In "Handbuch der Pflanzenphysiologie". Band XV, Teil 2. Herausgegen von W. Ruhland. Springer-Verlag, Berlin.

Barron, E.J. (1964). Enzymes of fat metabolism. A. Plant lipases. In "Moderne methoden der pflanzenanalyse". Begrundet von K. Paech, M.V. Tracey. Springer-Verlag, Berlin, 7, 448-453.

Baydoun, E.A. (1980). Membranes of the cells of maize root tissues. Ph.D. Thesis. University of Cambridge.

Beard, B.H. and Geng, S. (1982). Interrelationships of morphological and economic characters of sunflower. Crop Sci., 22, 817-822.

Beevers, H. (1961). Metabolic production of sucrose from fat. Nature, 29, 433-436.

Bergeron, J.J.M., Ehrenreich, J.H., Siekevitz, P. and Palade, G.E. (1973). Golgi fractions prepared from rat liver homogenate. II. Biochemical characterisation. J. Cell Biol., 59, 73-88.

Bergfeld, R., Hong, Y.N., Kuhnl, T. and Schopfer, P. (1978). Formation of oleosomes during embryogenesis and their breakdown during seedling development in cotyledons of Sinapsis alba. Planta, 143, 297-307.

Berrie, A.M.M. and Drennan, D.S.H. (1971). The effect of hydration-dehydration on seed germination. New Phytol., 70, 135-142.

Berrie, A.M.M., Don, R., Buller, D., Alam, M. and Parker, W. (1975). The occurrence and function of short chain length fatty acids in plants. Plant Sci. Lett., 6, 163-173.

Berrie, A.M.M. (1977). An introduction to the botany of the major crop plants. Edited by J.R. Hillman. Hyden, London.

Berrie, A.M.M. (1984). Germination and dormancy. In "Advanced plant physiology". Edited by M.B. Wilkins, Pitman Publishing Ltd., London.

Bewley, J.D. and Black, M. (1978). Physiology and biochemistry of seeds in relation to germination. Springer-Verlag, Berlin and New York.

Bhatia, I.S., Arora, K.K. and Sukhija, P.S. (1978). Changes of lipids and lipase activity in the germinating sunflower (Helianthus annuus) seed. Plant Biochem. J., 5, 150-156.

Bhatti, A.S. (1974). Treatment of cottonseeds for germination. Plant Soil, 41, 631-638.

Boatman, S.G. and Crombie, W.M. (1958). Fat metabolism in Citrullus vulgaris. J. Exper. Bot., 9, 52-57.

Bonner, J. and Varner, J.E. (1965). Plant biochemistry. Academic Press, New York and London.

Bowles, D.J. and Kauss, H. (1976). Characterization, enzymatic and lectin properties of isolated membrane from Phaseolus aureus. Biochim. Biophys. Acta, 443, 360-374.

Britannica Year Books (1970-85). Encyclopaedia Britannica Inc., Chicago, London, Paris, Rome and Tokyo.

British Standards Institution (1976). Methods of test for essential oils. BSI Methods.

Browse, J.A. and Slack, C.R. (1981)*. Catalase stimulates linoleate desaturation in microsome preparations from developing linseed cotyledons. FEBS Lett., 131, 111-114.

Campbell, E.J. (1983). Sunflower oil. JAOCS, 60, 387-392.

Canella, M. and Caetana, C. (1982). Protein and oil composition of germinated sunflower seeds. Technol., 15, 15-18.

Carter, J. (1978)*. Sunflower, Science and Technology, 19, American Soc. of Agron. Madison, Wisc., USA.

Carver, B.F. and Wilson, R.F. (1984a). Triacylglycerol metabolism in soybean seed with genetically altered unsaturated fatty acid composition. Crop Sci., 24, 1020-1023.

Carver, B.F. and Wilson, R.F. (1984b). Polar-Glycolipid metabolism in soybean seed with genetically altered unsaturated fatty acid composition. Crop Sci., 24, 1023-1026.

Chapman, D. (1965). The structure of lipids by spectroscopic and X-ray techniques. Methuen and Co. Ltd., London.

Cheang, K. and Stumpf, P.K. (1983). The metabolism of the germinating oil palm (Elaeis guineensis) seedling. Plant Physiol., 73, 1033-1037.

Cherry, J.H. (1963). Nucleic acid, mitochondria, and enzyme changes in cotyledons of peanut seeds during germination. Plant Physiol., 38, 440-446.

Ching, T.M. (1963). Metabolism of germinating seeds. Plant Physiol., 38, 722-729.

Christie, W.W. (1982). Lipid analysis. 2nd edition. Pergamon Press, London.

Clark, Jr. J.M. (1964). Experimental biochemistry. Freeman and Co., San Francisco and London.

Conway, T.F. and Earle, F.R. (1963). Nuclear Magnetic Resonance for determining oil content of seeds. JAOCS, 40, 265-271.

Dalgaron, L. and Birt, L.M. (1963). Free fatty acids in carrot-tissues preparation and their effect on isolated carrot mitochondria. Biochem. J., 87, 586-596.

Dangeard, P.A. (1921)*. Cited in Nomenclature of spherosomes. In "The biochemistry of plants". Edited by P.K. Stumpf and E.E. Conn, 4, 1980. Academic Press, New York and London, 223-226.

Daussant, J., Mossé, J. and Vaughan, J. (1983). Seed proteins, Academic Press, London, 83-98.

De Duve, C. (1971). Tissue fractionation in past and present. J. Cell Biol., 50, 20D-55D.

Doby, G. (1965). Plant biochemistry. Interscience Publishers; John Wiley and Sons Ltd., London and New York, 285-327.

Doman, D.C., Walker, J.C., Trelease, R.N. and Moore, B.D. (1982). Metabolism of carbohydrate and lipid reserves in germinated cottonseeds. Planta, 155, 502-510.

Donaldson, R.P. and Beevers, H. (1977). Lipid composition of organelles from germinating castor bean endosperm. Plant Physiol., 59, 259-263.

Dorrell, D.G. and Whelam, E.D.P. (1978). Chemical and morphological characteristics of seeds of some sunflower species. Crop Sci., 18, 969-970.

Doty, H.O. Jr. (1983). Economics of oilseed production. Econ. Bot., 37, 434-443.

Duffus, C.M. and Slaughter, J.C. (1980). Seeds and their uses. John Wiley and Sons Ltd., New York, 35-65.

Dybing, C.D. and Craig, B.M. (1970). Fatty acid biosynthesis and incorporation into lipid classes in seeds and seed tissues of flax. Lipids, 5, 422-429.

Eckey, E.W. (1954). Vegetable fats and oils. Reinhold, New York.

El-Abyed, M.S. and Saleh, Y.E. (1971). Germination and growth of Egyptian cottonseeds. Trans Brymcol. Soc., 57, 427-437.

El-Kateeb, S.Z. and El-Zeany, B.A. (1983). Changes of cottonseed oil during deep fat frying of foods. Riv. Ital. Sost.Grasse, 60, 73-76.

Erdahl, W.L. and Privett, O.S. (1985). Analysis of lipids by High Performance Liquid Chromatography-chemical ionization mass spectrometry. JAOCS, 62, 786-792.

Esau, K. (1977). Anatomy of seed plants. John Wiley and Sons Ltd., New York and London.

Evans, C.D., McConnell, D.G., List, G.R. and Scholfield, C.R. (1969). Structure of unsaturated vegetable oil glycerides: direct calculation from fatty acid composition. JAOCS, 46, 421-424.

Evans, W.H. (1982). Subcellular membranes and isolated organelles: preparative techniques and criteria for purity. Techniques in Lipid and Membrane Biochemistry, B407 a/1. Elsevier/North Holland Scientific Publishers Ltd., 1-46.

Folch, J., Lees, M. and Stanely, G.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226, 497-511.

Francis, A. and Coolbear, P. (1984). Changes in the membrane phospholipid composition of tomato seeds accompanying loss of germination capacity caused by controlled deterioration. J. Exper. Bot., 35, 1764-1770.

Frey-Wyssling, A., Grieshaber, E. and Muhlethaler, K. (1963). Origin of spherosomes in plant cells. J. Ultrastruct. Res., 8, 506-516.

Friedlander, M. and Neumann, J. (1968). Stimulation of photoreaction of isolated chloroplasts. Plant Physiol., 43, 1249-1254.

Galliard, T. and Phillips, D.R. (1971). Lipoxygenase from potato tubers. Biochem. J., 124, 431-438.

Galliard, T. (1973). Cited in Phospholipase activity. In "Form and function of phospholipids". Edited by G.B. Ansell, J.N. Hawthorne and R.N.C. Dawson. Elsevier, Amsterdam, 253-288.

Galliard, T. (1974a). Techniques for overcoming problems of lipolytic enzymes and lipoxygenases in the preparation of plant organelles. In "Methods in Enzymology". Edited by S.P. Colowick and N.O. Kaplan. Academic Press, New York and London, 31, part A, 520-528.

Galliard, T. (1974b). Unusual fatty acids in plants. In "Metabolism and regulation of secondary plant products". Edited by V.C. Runeckles and E.E. Conn. Academic Press, New York and London, 8, 209-241.

Galliard, T. and Mercer, E.I. (1975). Recent advances in the chemistry and biochemistry of plant lipids. Academic Press, London.

Gardner, H.W. (1968). Preparative isolation of monogalactosyl and diglycerides by TLC. J. Lipid Res., 9, 139-141.

Gill, N.T. and Vear, K.C. (1980). Agricultural Botany; Dicotyledonous crops. Duckworth, London, 227-231.

Goldsworthy, P.R. and Fisher, N.M. (1984). The physiology of tropical field crops. John Wiley and Sons, Chichester and New York, 453-528.

Golowick, S.P. and Kaplan, N.O. (1974). Subcellular fractions derived from plant tissues. In "Methods in Enzymology". Edited by S. Fleischer and L. Packer. Academic Press, London, 13, 489-553.

Goodwin, T.W. and Mercer, E.I. (1983). Introduction of plant biochemistry. 2nd edition. Pergamon Press, London.

Gopalakrishman, N., Kaimal, T.N.B. and Lakshiminarayana, G. (1982). Fatty acid changes in Althaea rosea tissues during growth. Phytochem., 21, 2205-2208.

Grewal, S.S., Sukija, P.S. and Bhatia, I.S. (1978). Polar lipids of sunflower (Helianthus annuus L.) seeds. Plant Foods for Human Nutrition, 3, 211-218.

Gummins, D.G., Marion, J.E., Craigmiles, J.P. and Burns, R.E. (1967). Oil content, fatty acid composition and other agronomic characteristics of sunflower. JAOCS, 44, 581-582.

Gunstone, F.D. and Qureshi, F.B. (1965). Glyceride studies. The component glycerides of maturing sunflower seeds. Chem. Phys. Lipids, 1, 429-433.

Gunstone, F.D. and Inglis, R.P. (1971). Nuclear Magnetic Resonance. In "Topicsin lipid chemistry". Edited by F.D. Gunstone. Logos Press, London, 2, 387-407.

Gurr, M.I., Blades, J., Appleby, S., Smith, C. and Nichols, B.W. (1974). Studies on seed-oil triglycerides. Triglycerides biosynthesis and storage in whole seeds and oil bodies of Crambe abyssinica. Eur. J. Biochem., 43, 281-290.

Gurr, M.I. (1980). The biochemistry of triacylglycerols. In "The biochemistry of plants". Edited by P.K. Stumpf. Academic Press Inc., 4, 205-248.

Hall, J.L., Flowers, T.J. and Roberts, R.M. (1974). Plant cell structure and metabolism. Longman Group Ltd., London.

Hall, J.L. and Roberts, R.M. (1975). Biochemical characteristics of membrane fractions isolated from maize roots. Ann. Bot., 39, 983-993.

Halloin, J.M. (1976). Imbibition of cottonseed germination with abscicic acid and its reversal. Plant Physiol., 57, 454-455.

Harborne, J.B. (1984). Phytochemical methods. 2nd edition. Chapman and Hall, London and New York.

Hardman, E.E. and Crombie, W.M. (1958). Fat metabolism in Citrullus vulgaris. J. Exper. Bot., 9, 239-242.

Harris, H.C., William, J.R. and Masson, W.K. (1978). Influence of temperature on oil content and composition of sunflower seed. Aust. J. Agric. Res., 29, 1203-1212.

Harrison, R. and Lunt, G.G. (1975). Biological membranes. Their structure and function. Blackie, Glasgow and London.

Harwood, J.L. and Stumpf, P.K. (1970). Fat metabolism in higher plants. Plant Physiol. Lancaster, 46, 500-508.

Harwood, J.L. Sodja, A. and Stumpf, P.K. (1971). On the origin of oil droplets in maturing castor bean seeds, Ricinus communis. Lipids, 6, 851-854.

Harwood, J.L. (1975). Fatty acid biosynthesis. In "Recent advances in the chemistry and biochemistry of plant lipids". Edited by T. Galliard and E.I. Mercer. Academic Press, London and New York, 44-94.

Harwood, J.L. (1980). Plant acyl lipids, structure, distribution and analysis. In "The biochemistry of plants". Edited by P.K. Stumpf. Academic Press, London 2-55.

Harwood, J.L. and Russell, N.J. (1984). Lipids in plants and microbes. George Allen and Unwin, London.

Hebblethwaite, P.D. (1980). Seed production. Butterworths, London.

Heiser, C.B. (1976). The sunflower. University of Oklahoma Press, U.S.A.

Heydecker, W. and Chetrman, R.S. (1971). Water relations of beetroot seed germination. 1. Microbial factors with special reference to laboratory germination. Ann. Bot., 35, 17-29.

Hilditch, T.P. and Williams, P.N. (1964). The chemical constitution of natural fats. Chapman and Hall, London.

Hitchcock, C. and Nichols, B.W. (1971). Plant lipid biochemistry. Academic Press, London and New York.

Holman, R.T. (1966). General introduction to polyunsaturated acids. Prog. Chem. Fats and Other lipids, 9, 3-12.

Huang, A.H.C. and Moreau, R.A. (1978). Lipases in the storage tissues of peanut and other oilseeds during germination. Planta, 141, 111-116.

Ikuma, H. (1970). Necessary conditions for isolation of tightly coupled higher plant mitochondria. Plant Physiol., 45, 773-781.

Jacks, T.L., Yatsu, L.Y. and Altschul, A.M. (1967). Isolation and characterization of peanut spherosomes. Plant Physiol., 42, 585-597.

Jambunathan, R., Raju, S.M. and Bade, S.P. (1985). Analysis of oil content of groundnuts by Nuclear Magnetic Resonance Spectrometry, J. Sci. Food Agric., 36, 162-166.

Janiszowska, W., Sobocinska, E. and Kasprzyk, Z. (1979)*. Distribution of different forms of sterols in three cellular subfractions of Calendula officinalis leaves. Phytochem., 18, 427-430

Jelsema, C.L., Morre, D.J., Ruddat, M. and Turner, C. (1977). Isolation and characterization of the lipid reserve bodies, spherosomes, from aleurone layers of wheat. Bot. Gaz., 138, 138-149.

Joshi, A.C., Chopra, B.K., Collins, L.C. and Doctor, V.M. (1974). Distribution of fatty acids during germination of soybean seeds. JAOCS, 50, 282-283.

Karshiev, Kh., Mukamedova, Kh.S. and Akramove, S.T. (1981). Extraction of phospholipid from cottonseeds. Khimiya Prirodnykh Soedinenii, 5, 392-392.

Katayama, M. and Funahashi, S. (1969)*. Distribution of fatty acids in plants. J. Biochem., Tokyo, 66, 479-485.

Kates, M. (1970). Plant phospholipids and glycolipids. Adv. Lipid Res., 8, 225-265.

Kawasaki, S. and Sato, S. (1979)*. Succinate dehydrogenase. Bot. Mag. Tokyo, 92, 305-314.

Khan, G.R. and Scheinmann, F. (1978). NMR spectroscopy in lipid analysis. Prog. Chem. Fats and Other Lipids, 15, 343-348.

Khan, L.M. and Hanna, M.A. (1983). Expression of oil from oilseeds. J. Agric. Eng. Res., 28, 495-503.

Khor, H.T. and Cheang, O.K. (1984). Change in lipid profiles in the developing haustorium of oil palm seeds. Phytochem., 23, 1579-1581.

Kinman, M.L. and Earle, F.R. (1964). Agronomic performance and chemical composition of the seed of sunflower hybrids and introduced varieties. Crop Sci., 4, 417-420.

Klaus, U.F. and Kindl, H. (1979). Plant plasma membrane. Hoppe Seyler's Z. Physiol. Chem., 360, 1517-1523.

Klein, R.A. and Kemp, P. (1977). NMR in lipid analysis. In "Methods in membrane biology". Edited by E.D. Korn. Plenum Press, New York, 8, 51-217.

Kleinig, H., Steinki, C., Kopp, C. and Zaar, K. (1978). Oleosomes (spherosomes) from Daucus carota suspension culture cells. Planta, 140, 233-237.

Knowles, L.O. and Kennedy, R.A. (1984). Lipid biochemistry of Echinochloa crus-galli during anaerobic germination. Phytochem., 23, 529-532.

Knowles, P.F., Temple, S.R. and Stolp, F. (1970)*. Viability in the fatty acid composition of sunflower seed oil. Conf. Proc. 4th Int. Sunflower Conf., Memphis, Tenn.

Labaneiah, M.E. (1970). Studies on cottonseed oil. M.Sc. Thesis. University of Alexandria, Egypt.

Lakon, G. (1942)*. Topographischer Nachweis der Keimfahigkeit der getreidefruchte durch Tetrazoliumsalze. Berichte der Deutscher Botanischem Gesellschaft, 60, 299-305.

Lakon, G. (1949). The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiol., 24, 389-394.

Lehle, L., Bowles, D.J. and Tanner, W. (1978). Subcellular site of mannosyl transfer to dolichyl phosphate in Phaseolus aureus. Plant Sci. Lett., 11, 27-34.

Leonard, R.T. and Van Der Woude, W.J. (1976). Isolation of plasma membranes from corn roots by sucrose density gradient centrifugation. Plant Physiol., 57, 105-114.

Leopold, A.C. and Kriedemann, P.E. (1975). Plant growth and development. Tata McGraw-Hill Publishing Co. Ltd., New York.

Lucas, C.C. and Ridout, J.H. (1967). Fatty livers and lipotropic phenomena. Prog. Chem. Fats and Other Lipids, 10, 1-15.

Luddy, F.E., Barford, R.A., Herb, S.F. and Magidman, P. (1968). A rapid and quantitative procedure for the preparation of methyl esters of butter oil and other fats. JAOCS, 45, 549-552.

Lusas, E.W. (1983). Comparative processing practices of the world major oilseed crops. Econ. Bot., 37, 444-458.

McArthur, J.A., Hesketh, J.D. and Baker, D.N. (1975). Crop Physiology. Cambridge University Press, 297-317.

McCloskey, J.A. (1970). Mass Spectrometry (MS). In "Topics in lipid chemistry". Edited by F.D. Gunstone. Logos Press, London, 1, 369-440.

Macdonald, P.M., Sykes, B.D. and McElhaney, R.N. (1984). Fatty acyl chain structure, orientational order, and the lipid phase transition in Acholeplasma laidlawii B membranes. A review of recent ^{19}F nuclear magnetic resonance studies. Can. J. Biochem. Cell., 62, 1134-1150.

McMahon, V. and Stumpf, P.K. (1966). Fat metabolism in higher plants. Biosynthesis of fatty acids in tissues of developing seeds and germinating seedlings of safflower. Plant Physiol., 41, 148-156.

Maddy, A.H. (1976). Biochemical analysis of membranes. Chapman and Hall Ltd., London.

Maeshima, M. and Beevers, H. (1985). Purification and properties of glyoxysomal lipase from castor bean. Plant Physiol., 79, 489-493.

Mangold, H.K. and Malins, D.C. (1960). Fractionation of fats, oils and waxes on TLC of silicic acid. JAOCS, 37, 383-384.

Marani, A. and Amirav, A. (1970). Effect of delinting and of genetical factors on the germination of cottonseeds at low temperature. Crop Sci., 10, 509-511.

Martin, E.M. and Morton, P.K. (1956). The chemical composition of microsomes and mitochondria from silver beet. Biochem. J., 64, 221-235.

Mattson, F.H. and Volpenheim, R.A. (1963). The specific distribution of unsaturated fatty acids in the triglycerides of plants. J. Lipid Res., 4, 392-396.

Mayer, A.M. and Poljakoff-Mayber, A. (1982). The germination of seeds. 3rd edition. Pergamon Press, New York.

Mazliak, P. (1977). Glyco- and phospholipids of biomembrane in higher plants. In "Lipids and lipid polymers in higher plants." Edited by M. Tevini and H.K. Lichtenthaler. Springer-Verlag, Berlin and New York, 50-51.

Mazliak, P. and Kader, J.C. (1980). Phospholipid-exchange systems. In "The biochemistry of plants". Edited by P.K. Stumpf and E.E. Conn. Academic Press, New York and London, 4, 283-298.

Meara, M.L. (1955). Fats and other lipids. In "Modern methods of plant analysis". Edited by K. Paech and M.V. Tracey. Springer-Verlag, Berlin, 397-402.

Mettler, I.J. and Beevers, H. (1980). Oxidation of NADH in glyoxysomes by a malate-asparate shuttle. Plant Physiol., 66, 555-560.

Mollenhauer, H.H. and Totten, C. (1971a). Studies on seeds. I. Fixation of seeds. J. Cell Biol., 48, 387-394.

Mollenhauer, H.H. and Totten, C. (1971b). Studies on seeds. II. Origin and degradation of lipid vesicles in pea and bean cotyledons. J. Cell Biol., 48, 395-405.

Moore, T.S. (1982). Phospholipid biosynthesis. Ann.Rev. Plant Physiol., 33, 235-259.

Moreau, R.A., Liu, K. and Huang, A. (1980). Spherosomes of castor bean endosperm. Plant Physiol., 65, 1176-1180.

Morré, D.J., Cline, G.B., Coleman, R., Evans, W.H., Glaumann, H., Headon, D.R., Ried, E., Siebert, G. and Windell, C.C. (1979). Markers for membranous cell components. Eur. J. Cell Biol., 20, 195-199.

Mudd, J.B. (1980). Phospholipid biosynthesis. In "The biochemistry of plants." Edited by P.K. Stumpf and E.E. Conn. Academic Press, New York and London, 4, 250-280.

Mukherjee, K.D. (1983). Lipid biosynthesis in developing mustard seed. Plant Physiol., 73, 929-934.

Munn, E.A. (1974). The structure of mitochondria. Academic Press, New York and London, 218-237.

Murray, D.R. (1984). Seed Physiology-Germination and reserve mobilization. Academic Press, London.

Nagahashi, G. and Beevers, L. (1978). Subcellular localization of glycosyl transferases in the cotyledons of Pisum sativum. Plant Physiol., 61, 451-459.

Nagahashi, G., Leonard, R.T. and Thomson, W.W. (1978). Purification of plasma membranes from root of barley. Plant Physiol., 61, 993-999.

Narahara, H.T., Vogrin, V.G., Green, J.D., Kent, R.A. and Gould, M.K. (1979). Isolation of plasma membrane vesicles. Biochim. Biophys. Acta, 552, 247-261.

Nawa, Y. and Asahi, T. (1971). Rapid development of mitochondria in pea cotyledons during the early stage of germination. Plant Physiol., 48, 671-674.

Nichols, B.W. (1963). Separation of the lipids of photosynthetic tissues: Improvement in analysis by TLC. Biochim. Biophys. Acta, 70, 417-422.

Nichols, B.W., Harris, R.V. and James, A.T. (1965). The lipid metabolism of blue-green algae. Biochim. Biophys. Res. Commun., 20, 256-262.

Noggle, G.R. and Fritz, G.J. (1983). Introductory Plant Physiology. Prentice-Hall Inc., New Jersey.

Opute, F.I. (1978). Mesocarp, seed and pollen lipids of raphia palms. J. Sci. Food and Agric., 29, 115-120.

Ory, R.L., Yatsu, L.Y. and Kircher, H.W. (1968). Association of lipase activity with the spherosomes of Ricinus communis. Arch. of Biochem. and Biophys., 264, 255-264.

Ory, R.L. (1969). Acid lipase of castor bean. Lipids, 4, 177-185.

Osagie, A.U. and Kates, M. (1984). Lipid composition of millet (Pennisetum americanum) seed. Lipids, 19, 958-965.

Paech, K. and Tracey, M.V. (1955). Modern methods of plant analysis. Springer-Verlag, Berlin.

Pohl, P., Glasl, H. and Wagner, H. (1970). Zur analytik pflanzlicher Glyko- und Phospholipoide und inhre Fettsauren. J. Chromat., 49, 488-492.

Porter, R.H., Durell, M. and Romm, H.J. (1947). The use of 2,3,5-triphenyl-tetrazolium chloride as a measure of seed germinability. Plant Physiol., 22, 149-159.

Pryde, E.H. (1983). Utilization of commercial oilseed crops. Econ. Bot., 37, 459-477.

Punnett, T. (1959)*. Stability of isolated chloroplast preparations and its effect on Hill fraction measurements. Plant Physiol., 34, 283-289.

Purseglove, J.W. (1968). Tropical crops Dicotyledons. Longmans, Green and Co. Ltd., London.

Quail, P.H. (1979). Plant cell fractionation. Ann. Rev. Plant Physiol., 30, 425-484.

Raie, M.Y., Ahmad, M. Ahmad, I., Khan, S.A. and Athar Jafri, S.A. (1983). Chromatographic studies of cottonseed oils. Fette Seifen Anstrichm, 7, 279-280.

Rest, J.A. and Vaughan, J.G. (1972). The development of protein and oil bodies in the seed of Sinapis alba. Planta, 105, 245-262.

Reynolds, E.S. (1963). The use of lead citrate at high PH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17, 208-212.

Robertson, J.A. (1972). Sunflower: America's neglected crop. JAOCS, 49, 239-244.

Robertson, J.A. (1974). Collaborative study of the determination of total oil in sunflower seed. JAOCS, 51, 223A-226A.

Robertson, J.A. and Morrison, W.H. (1979). Analysis of oil content of sunflower seed by wide-line NMR. JAOCS, 56, 961-964.

Robertson, J.A. and Windham, W.R. (1981). Comparative study of methods of determining oil content of sunflower (Helianthus annuus) seeds. JAOCS, 58, 993-996.

Rossi, C. (1979). Effect of potassium and sodium ions during the germination phase of sunflower seeds (Helianthus annuus). Phyton Rev. Int. Bot. Exp., 37, 49-56.

Roughan, P.G. and Slack, C.R. (1982). Cellular organization of glycerolipid metabolism. Ann. Rev. Plant Physiol., 33, 97-132.

Rouser, G., Kritchevsky, G., Simon, G. and Nelson, G.T. (1967). Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids, 2, 37-40.

Ryhage, R. and Stenhagen, E. (1960). Mass spectrometric studies. IV. Esters of monomethyl-substituted long chain carboxylic acids. Arkiv. Kemi., 15, 291-304.

Salisbury, F.B. and Ross, C.W. (1978). Plant Physiology. 2nd edition. Wadsworth Publishing Co. Inc., California, 70-71.

Scharschmidt, B.F., Keeffe, E.B., Blankenship, N.M. and Ockner, P.K. (1979). Validation of a recording spectrophotometric method for measurement of membrane-associated Mg^{+} and $Na^{+}K^{+}$-ATP-ase acitivity. J. Lab. Clin. Med., 93, 790-799.

Schlenk, H. and Gellerman, J.L. (1960). Esterification of fatty acids by diazomethane on a small scale. Anal. Chem., 32, 1412-1414.

Schlotzhauer, P.F., Ellington, J.J. and Schepartz, A.I. (1977). Thin-layer chromatographic procedure for class separation of plant neutral lipids. Lipids, 12, 239-241.

Schwarzenbach, A.M. (1971). Cited in "Development of oil bodies". In "The biochemistry of lipids." Edited by P.K. Stumpf and E.E. Conn. Academic Press, New York and London, 4, 226-229.

Seiler, G.J. (1985). Evaluation of seeds of sunflower species for several chemical and morphological characteristics. Crop Sci., 25, 183-187.

Shine, W.E., Mancha, M. and Stumpf, P.K. (1976). Differential incorporation of acyl-CoA and acyl-ACP into plant microsomal lipids. Arch. Biochem. Biophys., 173, 472-479.

Silver, J.G., Rochester, C.P., Bishop, D.G. and Harris, H.C. (1984). Unsaturated fatty acid synthesis during the development of isolated sunflower (Helianthus annuus) seeds. J. Exper. Bot., 35, 1507-1514.

Singh, G. and Garg, O.P. (1971). Effect of red, for -red radiations on germination of cottonseed. Plant Cell Physiol., 12, 411-415.

Slack, C.R. Roughan, P.G. and Balasingham, N. (1978). Labelling of glycerolipids in the cotyledons of developing oilseeds by [1-^{14}C]acetate and [2-^{3}H] glycerol. Biochem. J., 170, 421-433.

Slack, C.R., Bertaud, W.S., Shaw, B.D., Holland, R., Browse, J. and Wright H. (1980). Some studies on the composition and surface properties of oil bodies from the seed cotyledons of safflower and linseed. Biochem. J., 190, 551-561.

Slack, C.R. and Roughan, P.G. (1980). A comparison of the polypeptide and phospholipid composition of oil body and microsomal preparations from safflower and linseed cotyledons. Biochem. J., 191, 71-80.

Smidovnik, A., Predovic, I. and Vitez, L. (1981). Characterization of vegetable oils by chromatographic methods. Nova Proizvod, 23, 23-29.

Smith, C.G. (1974). The ultrastructural development of spherosomes and oil bodies in the developing embryo of Crambe abyssinica. Planta, 119, 125-142.

Smith, G. and Stein, J. (1982). Lipid preparation. Techniques in lipid and membrane biochemistry. Elsevier; North-Holland Scientific Publishers Ltd., B406, 1-16.

Sorokin, H.P. (1967). The spherosomes and the reserve fat in plant cells. Amer. J. Bot., 54, 1008-1016.

Sottocasa, G.L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. (1967). An electron transport system associated with the outer membrane of liver mitochondria. J. Cell Biol., 32, 415-438.

Sparace, S.A. and Moore, T.S. (1979). Phospholipid metabolism in plant mitochondria. Plant Physiol., 63, 963-972.

Spencer, G.F., Plattner, R.D. and Miwa, T. (1977). Jojoba oil analysis by high pressure liquid chromatography and gas chromatography/mass spectrometry. JAOCS, 54, 187-189.

Sreenivasan, B. (1968). Component fatty acids and composition of some oils and fats. JAOCS, 45, 259-265.

Stobart, A.K. and Stymne, S. (1985). The regulation of the fatty acid composition of the triacylglycerides in microsomal preparations from avocado mesocarp and the developing cotyledons of safflower. Planta, 163, 119-125.

Stumpf, P.K. (1965). Lipid metabolism. In "Plant biochemistry". Edited by J. Bonner and J.E. Varner. Academic Press, New York and London, 322-345 (see also 3rd ed., 1976, 427-461)

Stymne, S. and Appelqvist, L.A. (1980). The biosynthesis of linoleate and linolenate in homogenates from developing soybean cotyledons. Plant Sci. Lett., 17, 287-294.

Supina, W.R. (1974). The packed column in gas chromatography. Supelco Inc., Bellefonte, Pennsylvania.

Swain, T. (1963). Chemical plant taxonomy. Academic Press, London and New York.

Thomson, J.R. (1979). An introduction to seed technology. Leonard Hill, London.

Travis, R.L. and Booz, M.L. (1979)*. Partial characterization of potassium-stimulated adenosine triphosphatase from the plasma membrane of mature soybean root tissue. Plant Physiol., 63, 573-577.

Trelease, R.N. (1969). Changes and characteristics of lipid bodies during development. Ph.D. Thesis. The University of Texas, Austin.

Trelease, R.N. (1969). Ultrastructural characterization, composition, and utilization of lipid bodies in the maize shoot apex during post-germinative development. J. Cell Biol., 43, 147a-152a.

Trelease, R.N. and Doman, D.C. (1980). Lipid mobilization. In "Seed physiology - Germination and reserve mobilization". Edited by D.R. Murray. Academic Press, London, 2, 201-245.

Tribe, M. and Whillaker, P. (1972). Chloroplast and mitochondria. Edward Arnold, London, 29-31.

Ulbright, C.E., Pickard, B.G. and Varner, J.E. (1982). Effects of short chain fatty acids on radicle emergence and root growth in lettuce. Plant Cell Environ., 5, 303-307.

Van Deenen, L.L.M. (1965). Lipid composition of membrane. Prog. Chem. Fats and Other Lipids, 8, 3-63.

Vaughan, J.G. (1970). The structure and utilization of oil seeds. Chapman and Hall Ltd., London.

Veeger, C. Der Vartanian, D.V. and Zeylemaker, W.P. (1969). Succinate dehydrogenase. Methods in Enzymol., 13, 81-90.

Vicente, M., Noronha, A. and Silberschmidt, K. (1969). Substrate moisture levels for germination testing of some agricultural seeds. Analy. Acad. Braxil. Sci., 41, 633-639.

Vick, B. and Beever, H. (1978). Fatty acid synthesis in endosperm of young castor bean seedlings. Plant Physiol., 62, 173-178.

Vijayalkshmi, B. and Rao, S.V. (1972)*. Unusual fatty acids in higher plants. Chem. Phys. Lipids, 9, 82-86.

Vischer, P. and Reutter, W. (1978). Specific alterations of fucoprotein biosynthesis in the plasma membrane of Morris Hepatoma 7777. Eur. J. Biochem., 84, 363-368.

Vranceanu, A.V. (1974)*. Floarea-Soarelui. Academic Republic Society, Romania.

Waldron, K. (1984). Glucuronyltransferase activity associated with hemicellulose and protein biosynthesis in Pisum sativum. Ph.D. Thesis. University of Glasgow.

Waller, G.R. (1972). Biochemical applications of mass spectrometry. Wiley-interscience; John Wiley and Sons, Inc., New York and London.

Wanjura, D.F. and Buxton, D.R. (1972). Water uptake and radicle emergence of cottonseed as affected by soil moisture and temperature. Agron. J., 64, 427-431.

Wanner, G. and Theimer, R.R. (1978). Membranous appendices of spherosomes (oleosomes). Possible role in fat utilization in germinating oilseeds. Planta, 140, 163-169.

Wanner, G., Formanek, H. and Theimer, R.R. (1981). The ontogeny of lipid bodies in plant cells. Planta, 151, 109-123.

Warthen, J.D. (1975). Separation of cis- and trans-isomers by reserve phase high pressure liquid chromatography. JAOCS, 52, 151-153.

Weber, E.J., De La Roche, I.A. and Alexander, D.E. (1971). Stereospecific analysis of maize triglycerides. Lipids, 6, 525-530.

Weber, E.J. (1973)*. Cited in "Production and uses". In "Symposium proceeding on industrial uses of cereals". Amer. Assoc. Cereal Chem., Minnesota, 161-206.

Weiss, E.A. (1983). Oilseed crops. Longman, London and New York.

Wibo, M., Thines-Sempoux, D., Amar-Costesec, A., Beaufay, H., and Godelaine,D. (1981). Analytical studies of mocrosomes and isolated subcellular membranes from rat liver. VIII. Sub-fraction of preparations enriched with plasma membranes, outer mitochondrial membranes or golgi complex membranes. J. Cell Biol., 89, 456-474.

Wikander, T. (1977). Studies on the dormancy of sugar beet fruit. M.Sc. Thesis. University of Glasgow.

Williams, K.A. (1966). Oils, Fats and Fatty Foods. J. and A. Churchill Ltd., London.

Williams, B.L. and Wilson, K. (1981). A biologist's guide to principles and techniques of practical biochemistry. 2nd edition. Edward Arnold Ltd., London.

Williamson, F.A., Moore, D.J. and Jaffe, M.J. (1975). Association of phytochrome with rough-surfaced endoplasmic reticulum fractions from soybean hypocotyls. Plant Physiol., 56, 738-743.

Wolff, I.A. and Miwa, T.K. (1965). Effect of unusual acids on selected seed oil analysis. JAOCS, 42, 208-215.

Wrenn, J.J. (1960). Chromatography of lipids on silicic acid. J. Chromat., 4, 173-195.

Yamada, M. (1957)*. The fat metabolism of castor bean seeds. Sci. Papers Coll. Gen. Ed. University of Tokyo, 7, 97-105.

Yatsu, L.Y. (1965). The ultrastructure of cotyledonary tissue from Gossypium hirsutum seeds. J. Cell Biol., 25, 193-199.

Yatsu, L.Y., Jacks, T.J. and Hensarling, T.P. (1971). Isolation of spherosomes (oleosomes) from onion, cabbage and cottonseed tissues. Plant Physiol., 48, 675-682.

Yatsu, L.Y. and Jacks, T.J. (1972). Spherosomes membrane; half unit membranes. Plant Physiol., 49, 937-943.

Yoshida, H. (1984). Molecular species and fatty acid distribution of triacylglycerols from germinating soybean cotyledons. Lipids, 19, 936-941.

Zeman, A. and Scharmann, H. (1973)*. Mass spectrometry uses. Fette, Seifen Anstrichmittel, 75, 32-44.

Zimmer, D.E. and Zimmerman, D.C. (1972). Influence of some diseases on achene and oil quality of sunflower. Crop Sci., 12, 859-861.

Zimmerman, D.C. and Klosterman, H.J. (1965). Lipid metabolism in germinating flaxseed. JAOCS, 42, 58-62.

Zimmerman, D.C. and Vick, G.N. (1973). Fatty acid composition of sunflower (Helianthus annuus) oil as influenced by seed position. JAOCS, 50, 273-275.

[References not actually consulted, but cited through another authority, have been denoted with an asterisk.]

APPENDIX

Table 96A Common plant and animal fatty acids

Sources: Christie, 1982, Goodwin and Mercer, 1983

Common name	Systematic name	Symbol
1. Saturated acids		
general formula $CH_3(CH_2)_nCOOH$		
acetic	ethanoic	2:0
propionic	propanoic	3:0
butyric	butanoic	4:0
valeric	pentanoic	5:0
caproic	hexanoic	6:0
enanthic	heptanoic	7:0
caprylic	octanoic	8:0
pelargonic	nonanoic	9:0
capric	decanoic	10:0
-	hendecanoic	11:0
lauric	dodecanoic	12:0
-	tridecanoic	13:0
myristic	tetradecanoic	14:0
-	pentadecanoic	15:0
palmitic	hexadecanoic	16:0
margaric	heptadecanoic	17:0
stearic	octadecanoic	18:0
-	nonadecanoic	19:0
arachidic	eicosanoic	20:0
-	heneicosanoic	21:0
behenic	docosanoic	22:0
lignoceric	tetracosanoic	24:0

Common name	Systematic name	Symbol
2. Monoenoic acids		
general formula $CH_3(CH_2)_mCH=CH(CH_2)_nCOOH$		
lauroleic	cis-9-dodecenoic	12:1(n-3)
myristoleic	cis-9-tetradecanoic	14:1(n-5)
-	trans-3-hexadecanoic	16:1
palmitoleic	cis-9-hexadecanoic	16:1(n-7)
petroselinic	cis-6-octadecanoic	18:1(n-12)
oleic	cis-9-octadecanoic	18:1(n-9)
elaidic	trans-9-octadecanoic	18:1
cis-vaccenic	cis-11-octadecanoic	18:1(n-7)
trans-vaccenic	trans-11-octadecanoic	18:1
gadoleic	cis-9-eicosenoic	20:1(n-11)
gondoic	cis-11-eicosenoic	20:1(n-9)
erucic	cis-13-docosenoic	22:1(n-9)
nervonic	cis-15-tetracosenoic	24:1(n-9)
3. Non-conjugated polyunsaturated acids		
general formula $CH_3(CH_2)_m(CH=CHCH_2)\ (CH_2)_nCOOH$		
linoleic	9,12-octadecadienoic	18:2(n-6)
α-linolenic	6,9,12-octadecatrienoic	18:3(n-6)
homo-γ-linolenic	8,11,14-eicosatrienoic	20:3(n-6)
arachidonic	5,8,11,14-eicosatetraenoic	20:4(n-6)
-	4,7,10,13,16-docosapentaenoic	20:5(n-6)
-linolenic	9,12,15-octadecatrienoic	18:3(n-3)

Common name	Systematic name	Symbol
-	5,8,11,14,17-eicosapentaenoic	20:5(n-3)
-	4,7,10,13,16,19-docosahexaenoic	22:6(n-3)
-	5,8,11-eicosatrienoic	20:3(n-9)

Tables 97A - 103A

Mass spectra of LCFAME and SCFAME

* The molecular ion is underlined

** m/z 74 is a diagnostic ion for methyl esters, which arises by the McLafferty rearrangement

*** m/z 88 is a diagnostic ion for ethyl ester and for α-methyl-methyl ester

Table 97A. Mass spectra of LCFA standards. m/z = mass-to-charge ratio RI = Relative intensity %

C_{14}		C_{15}		C_{16}		$C_{16:1}$		C_{17}		C_{18}		$C_{18:1}$		$C_{18:2}$		C_{19}		C_{20}	
m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI
43	27	43	32	55	52	57	55	59	15	43	19	59	6	55	90	43	27	43	48
57	56	55	21	57	44	59	29	69	75	55	60	69	52	57	40	59	20	55	34
59	26	59	10	59	18	74	100	71	45	57	49	71	32	69	70	71	40	57	28
74	100	74	100	74	100	75	50	74	100	59	18	74	100	74	100	74	100	69	23
87	45	88	8	87	77	87	95	75	72	69	46	75	42	75	50	87	93	74	100
101	28	101	8	88	35	88	21	87	82	71	26	101	23	87	35	88	31	75	30
129	29	129	9	101	41	101	24	88	18	74	100	143	15	88	90	101	28	87	83
186	12	143	24	157	12	129	15	101	20	75	59	180	18	101	95	129	28	88	12
199	32	199	9	199	22	152	30	129	20	87	72	199	6	294	92	143	78	101	12
211	26	213	28	239	40	194	37	143	50	143	31	213	30	-	-	199	13	129	16
242	43	256	53	270	87	199	26	241	42	255	26	296	14	-	-	268	22	143	52
-	-	-	-	-	-	268	13	284	72	298	62	-	-	-	-	312	77	326	26

Table 98A Mass spectra of long-chain fatty acids (LCFA).

Source: Eight peak index of mass spectra (1970). Published by Mass Spectrometry Data Centre, Awre, U.K.

C_{14}		C_{15}		C_{16}		C_{18}		$C_{18:1}$		$C_{18:2}$		C_{19}		C_{20}	
m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI
69	13	41	15	69	15	41	22	41	68	41	91	43	74	43	29
74	100	43	21	71	6	43	27	43	59	55	74	55	22	55	22
75	14	55	16	74	100	55	17	55	71	67	57	57	19	57	19
83	7	74	100	75	16	57	17	69	71	68	57	69	15	69	16
87	61	75	19	83	8	74	100	74	100	69	49	74	100	74	100
143	9	87	73	87	65	75	19	83	59	74	100	75	23	75	28
199	6	143	17	88	6	87	65	97	53	81	47	87	28	87	71
242	7	256	16	143	8	143	16	264	51	87	42	312	23	326	23

Table 99A Mass spectra of LCFAME from dry Helianthus (A) and Gossypium (B) seeds.

(A)												(B)											
C_{16}		C_{18}		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$		C_{20}		C_{14}		C_{16}		$C_{16:1}$		C_{18}		$C_{18:1}$		$C_{18:2}$		$C_{18:3}$	
m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI
55	42	43	16	43	23	55	55	55	43	43	41	43	21	55	48	57	43	43	21	43	42	55	38	55	55
57	30	55	52	55	18	57	35	57	32	55	30	57	43	57	38	59	28	55	55	55	58	57	32	57	22
69	18	57	42	57	16	69	28	74	100	57	28	74	100	59	22	74	100	57	40	59	15	74	100	74	100
74	100	74	100	74	100	74	100	75	54	74	100	87	55	74	100	75	41	74	100	74	100	75	48	75	50
75	22	75	45	75	32	75	45	87	83	75	28	101	20	75	15	87	82	75	42	75	40	87	72	87	82
87	82	87	82	143	18	87	72	292	54	87	81	129	34	87	82	88	16	87	89	83	42	101	61	292	31
88	20	143	28	296	81	88	22			143	42	211	22	88	15	101	14	143	28	143	23	294	88		
143	10	298	65			294	78			326	33	242	53	239	32	194	23	255	26	213	38				
270	86													270	85	199	22	298	58	296	24				
																268	24								

Table 100A Mass spectra of LCFAME from spherosomal preparations of Dry H. annuus (A) and G. barbadense (B).

(A)

C_{16}		C_{18}		$C_{18:1}$		$C_{18:2}$	
m/z	RI	m/z	RI	m/z	RI	m/z	RI
55	43	43	16	69	48	55	65
57	40	55	42	74	100	57	55
74	100	57	38	75	34	74	100
75	20	69	32	101	20	75	30
87	80	74	100	143	18	87	65
88	45	75	51	213	28	88	25
101	26	87	74	296	26	101	35
143	30	143	28			115	25
199	18	298	64			294	45
223	30						
270	25						

(B)

C_{16}		C_{18}		$C_{18:1}$		$C_{18:2}$	
m/z	RI	m/z	RI	m/z	RI	m/z	RI
55	22	43	30	43	22	55	60
57	34	55	45	55	15	74	100
74	100	57	30	69	32	75	25
75	65	74	100	74	100	87	85
87	92	75	25	75	28	88	35
88	40	87	75	83	41	178	28
101	25	143	15	143	15	220	15
129	22	199	18	213	22	263	26
143	35	298	18	296	18	294	30
227	18						
239	28						
270	15						

Table 101A Mass spectra of LCFAME from mitochondria-rich fractions of H. annuus (A) and G. barbardense (B).

(A)

C_{16}		C_{18}		$C_{18:1}$		$C_{18:2}$	
m/z	RI	m/z	RI	m/z	RI	m/z	RI
55	10	43	24	55	61	55	71
57	15	55	54	57	42	57	42
74	100	57	41	74	100	74	100
75	25	74	100	75	22	75	13
87	86	75	43	87	54	87	62
88	42	87	78	143	12	101	28
101	20	143	28	296	25	143	18
115	12	298	43			263	18
143	20					294	29
232	12						
270	15						

(B)

C_{16}		C_{18}		$C_{18:1}$		$C_{18:2}$	
m/z	RI	m/z	RI	m/z	RI	m/z	RI
55	20	43	28	55	45	55	40
57	35	55	48	57	35	57	35
74	100	57	41	74	100	74	100
75	60	59	21	75	15	75	10
87	90	74	100	87	65	87	70
88	55	75	40	88	20	88	40
101	25	87	77	101	15	101	30
129	15			143	10	123	65
143	35	143	28	263	10	178	25
227	15	255	21	296	15	263	20
270	15	298	53			294	25

Table 102A. Mass spectra of short-chain fatty acids (SCFA) m/z = mass-to-charge ratio RI = Relative intensity %

(A). Source: Eight peak index of mass spectra (1970). (B). SCFA standards

C_6		C_8		C_{10}		C_{12}		C_6		C_8		C_9		C_{10}		C_{11}		C_{12}	
m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI
41	21	15	27	41	15	15	20	41	48	41	26	41	40	41	24	41	20	41	20
43	44	27	27	43	23	27	19	55	50	43	28	43	60	43	35	43	20	43	30
55	18	29	29	55	14	29	29	57	16	55	20	55	20	44	51	55	18	44	70
59	26	41	33	59	9	41	36	59	50	57	23	57	25	55	15	57	10	55	20
71	12	43	39	74	100	43	39	69	41	59	21	59	8	57	14	74	100	57	20
74	100	55	24	75	10	55	25	71	49	71	9	74	100	74	100	87	60	74	100
87	33	74	100	87	50	74	100	74	100	74	100	87	50	87	60	129	10	87	60
99	21	87	40	143	13	87	54	75	36	75	13	101	9	101	8	143	25	101	10
								87	60	87	48	129	14	129	12	157	36	129	30
								101	50	101	10	141	15	143	40	169	25	143	45
								130	98	129	11	143	13	155	20	171	10	171	30
								-	-	158	14	172	14	186	15	200	15	214	18

Table 103A Mass spectra of SCFAME from dry Helianthus (A) and Gossypium (B) seeds.

(A)								(B)							
C_8		C_9		C_{10}		C_{12}		C_8		C_9		C_{10}		C_{12}	
m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI	m/z	RI
43	31	43	62	43	22	43	18	43	22	43	50	43	30	43	28
55	22	55	21	55	18	55	22	55	21	55	22	55	12	55	25
74	100	74	100	59	12	74	100	59	18	57	22	57	15	57	18
87	52	87	55	74	100	87	57	74	100	74	100	74	100	74	100
129	22	129	24	75	15	143	22	87	63	87	57	87	62	87	66
143	12	172	14	87	56	214	31	101	19	101	10	101	12	101	12
158	16			143	22			129	20	129	16	129	15	129	22
				186	32			143	14	143	16	143	42	143	38
								158	25	172	26	155	25	183	20
												186	28	214	33