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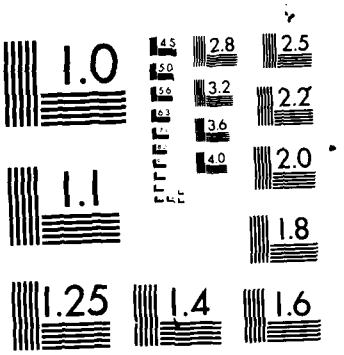
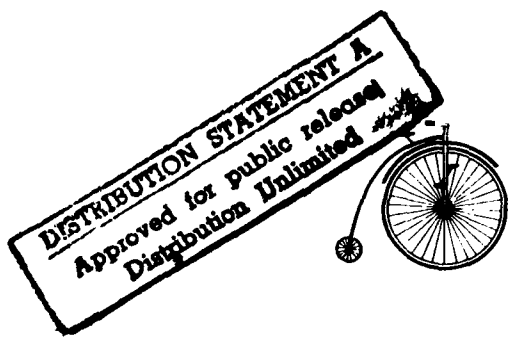


7th INTERNATIONAL SYMPOSIUM
OF THE
STRUCTURE AND FUNCTION
OF PLANT LIPIDS



ABSTRACTS OF PLENARY LECTURES AND POSTERS

Contract N00014-86-G-0051

UNIVERSITY OF CALIFORNIA, DAVIS
JULY 27-AUGUST 1, 1986

Organizing Committee

Chair: P.K. Stumpf

Program Coordinator: J.B. Mudd

Organization Coordinator: W.D. Nes

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We wish to thank the following contributors for their generous financial assistance which made this meeting possible:

National Science Foundation, USA
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and

Arco Plant Cells Research Institute
Calgene
DuPont
Henkel
Monsanto
Proctor and Gamble
Shell Agricultural Chemical Co.

ALL SESSIONS TO BE HELD IN 2 WELLMAN HALL

SUMMARY OF PROGRAM

Time	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
		(1) Biochem. Isoprenoids/ Sterols - Nes, Chair	(3) Structure/Function Lipid - Galliard, Chair	(5) Oxygenases-Desaturases - Axelrod, Chair	(8) Algal Lipids - Benson, Chair	
8:15		Opening Remarks				
8:30		Croteau	Oemel	Vick/Zimmerman	Murata	
9:15		Phinney	Vigh	Hatanaka	Kates	9:00 AM
10:00		Coffee Break	Coffee Break	Coffee Break	Coffee Break	All day
10:10		Schultz	Seigenthaler	Gerhardt	Thompson	wine tour
10:55		Kleinig	Thomson	Stymne	Patterson	
11:40		Posters	Posters	Posters	Posters	
12:30		Lunch	Lunch	Lunch	Lunch	Napa
		(2) Function Isoprenoids/ Sterol - Loomis, Chair	(4) Complex Lipids - Mudd, Chair	(6) Medium-Long Chain F.A. - Yamada, Chair	(9) Molecular Biology - Stumpf, Chair	Valley
1:30		Nes	Huang	Pollard	Downey	
2:15	Registration -	Benveniste	Roughan	Harwood	Jones	-Tickets-
3:00		Break	Break	Break	Break	
3:10	Leach Hall	Parks	Douce	Kolattukudy	Somerville	
3:55		Lichtenthaler	Moore	Cassagne	Ohlrogge	
4:40		Kuc	Mazliak	von Wettstein	Poster Discussion Leader - Still	
5:25		Poster Discussion (1-2) Leader - Goad	Poster Discussion (3-4) Leader - Wintermann	Poster Discussion (5-6) Leader - Perchorowicz	Business Meeting	
6:00-7:15		Dinner (Dorm)	---	Dinner (Dorm)	---	
7:00				---	From 7:00 P.M. Banquet Faculty Club - Tickets -	
7:30	Reception		From 6:30 - Barbeque - Rec Pool Lodge - Tickets -	(7) Interaction - Hess, Chr.		
8:15	Leach Hall	OPEN		Sampson		
9:00				Ong		
10:00				Schmid		

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Session 1 - Biochemistry of Isoprenoids
and Sterols

1

METABOLISM OF MONOTERPENES AND SESQUITERPENES. Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, Washington 99164-6340.

The common monoterpenes and sesquiterpenes are cyclic, contain relatively few simple functional groups, and represent variations on a limited number of skeletal themes. Earlier proposals for the origin of cyclic monoterpenes and sesquiterpenes are reviewed, and the biosynthetic steps involved in the conversion of acetyl CoA to the relevant C_{10} and C_{15} acyclic precursors (geranyl and farnesyl pyrophosphate, respectively) are described. A number of monoterpene cyclizations are discussed, and some of the mechanistic and stereochemical features of these unique enzymatic reactions are noted. A general stereochemical model for the cyclization of geranyl pyrophosphate to monoterpenes is proposed, the key feature of which is the intermediacy of the corresponding tertiary allylic isomer, linalyl pyrophosphate, which is necessitated by the fact that geranyl pyrophosphate cannot cyclize directly because of topological constraints. Subsequent transformations of the parent cyclic products are responsible for the great diversity of monoterpenes found in nature, and a general strategy for the oxidative metabolism of cyclic olefins is presented. The enzymology and mechanisms of these transformations are emphasized. The origin of selected sesquiterpenes is discussed with particular reference to the similarities between monoterpene and sesquiterpene biosynthesis. Evidence for the metabolic turnover of lower terpenes is reviewed, and the catabolism of monocyclic monoterpenes is described in detail. The ultimate fate of the terpenes indicates that these compounds, after having fulfilled a presumptive ecological function, are salvaged as a carbon and energy source at sites distant from the sites of synthesis.

DWARF MUTANTS OF MAIZE - GIBBERELLIN BIOSYNTHESIS AND CLONING FOR ENZYMES OF THE PATHWAY. B.O.Phinney and C.Spray, Department of Biology, University of California, Los Angeles 90024 Cal. U.S.A.

The gibberellins(GAs) are a class of diterpenes that are ubiquitous in higher plants. They act as plant hormones, controlling such diverse responses as the de novo synthesis of α-amylase in cereals and shoot growth in higher plants. The gibberellins originate biosynthetically from copalyl pyrophosphate(CPP) by cyclization to give the tetracyclic kauranoid, ent-kaurene, which is then oxidized and rearranged through a series of steps to give GA_{12}-aldehyde, the common precursor for all of the known GAs (currently 72). At least 5 pathways diverge from GA_{12}-aldehyde to give a series of biologically active GAs. This report will review the basic pathway leading to GA_{12}-aldehyde. We will present recent evidence that only one gibberellin, GA_1, is bioactive per se in the control of shoot elongation in most higher plants. (Other GAs endogenous to the shoot are bioactive only by their metabolism to GA_1). The evidence for this conclusion is based on feeds of doubly labelled intermediates to GA mutants of maize. Preliminary data will also be given on the cloning of genes coding for enzymes of the pathway, using dwarf mutants of maize that originate from the insertion into the genome of Robertson's mutator(a transposable element).

THE SYNTHESIS OF PRENYLQUINONES IN CHLOROPLASTS. G. Schultz, J. Soll*, E. Fiedler and D. Schulze-Siebert, Botanisches Institut, Tierärztliche Hochschule Hannover, and *Botanisches Institut, Universität München.

In higher plants, plastoquinone-9 (PQ-9), α-tocopherol and phylloquinone (vitamin K_1) are the specifically plastidic prenylquinones. Their synthesis occurs in chloroplasts. The prenyl moiety of the quinones is formed by the plastidic isozymes of the mevalonate pathway and the plastidic polyprenyl-PP synthesis predominately producing compounds of the di- and tetraterpenoidic type; the aromatic moiety is formed from E4P and PEP by the plastidic shikimate pathway localized in the soluble phase (stroma) of the chloroplast.

The inner membrane of the chloroplast envelope is the site of prenylquinone synthesis. Both PQ-9 and α-tocopherol are derived from homogentisate which is formed from 4-hydroxyphenylpyruvate by a a dioxygenase. Phytyl-PP and solanesyl-(nonaprenyl-)PP, respectively, is specifically introduced into position C-6 of homogentisate. Thus, the following steps are strongly coordinated. In the synthesis of α-tocopherol, 2-methyl-6-phytyl-1,4-benzoquinol formed from homogentisate either (a) is methylated by S-adenosylmethionine (SAM) to form 2,3-dimethyl-5-phytyl-1,4-benzoquinol; this is then cyclisized to yield y-tocopherol or (b) is cyclisized to form δ-tocopherol; this is then methylated to yield y-tocopherol. Finally, the latter is methylated to α-tocopherol. In the synthesis of PQ-9, 2-methyl-6-solanesyl-1,4-benzoquinol formed from homogentisate is methylated to yield the 2,3-dimethyl quinol PQ-9-H_2. Only the benzoquinols but not the benzoquinones serve as substrates in the enzymic reactions.

Because of minimal rates, investigations of K_1 synthesis are not without problems although the reactions in principal seem identical to those in microorganisms. Leistner et al. (1982 and later) has studied the introductory steps of its synthesis in cell cultures of Galium mollugo which are as follows: Shikimate $\longrightarrow$ Chorismate $\longrightarrow$ Isochorismate $\xrightarrow{\text{Succinylsemialdehyde TPP}}$ 2-Succinylbenzoate $\xrightarrow{\text{ATP, CoA-SH}}$ "Aliphatic" CoA-ester of 2-succinylbenzoate $\longrightarrow$ 1,4-Dihydroxy-2-naphthoate. In preparations of envelope membranes of spinach chloroplasts, it has been shown by us that the naphthoate is prenylated by phytyl-PP to form 2-phytyl-1,4-napthoquinol which is methylated by SAM to yield phylloquinol (K_1-H_2).

Session 2 - Functions of Isoprenoids and Sterols

4

CAROTENOID SYNTHESIS AND CAROTENOGENIC ENZYMES IN PLASTIDS. Hans Kleinig, Institut für Biologie II, Universität Freiburg, Schanzlestr. 1, D-7800 Freiburg, FRG.

Isopentenylpyrophosphate (IPP) is the direct precursor for carotenoid synthesis in plastids which is accepted by the phytoene synthase complex. The formation and its compartmentation in the plant cell of this intermediate will be discussed in the first part of this presentation. The second part will deal with a progress report on the analysis of carotenogenic enzymes. Chromoplasts have been shown to represent the most suited plastid type among the carotenoid forming plastids (chloroplasts, etioplasts, chromoplasts) for the investigation of the enzymes involved in this pathway. In these organelles the phytoene synthase complex (which includes an IPP isomerase, prenyl transferase, prephytoenepyrophosphate synthase and phytoene synthase) exhibits the characteristic features of a peripheral membrane protein which is highly stimulated in its activity in the presence of lipid bilayers or membranes. The following enzymes in the biosynthetic sequence leading from phytoene to β-carotene (dehydrogenase, for steps from phytoene to β-carotene; cis-trans isomerase, on the level of phytofluene; cyclase, two steps from lycopene to β-carotene) are integral membrane proteins. Solubilization from the membrane and fractionation of these proteins can only be achieved in the form of minute micells by using an appropriate detergent. The reconstitution of full enzymatic activities can be recovered by their subsequent incorporation into artificial liposomes.

5

BIOSYNTHESIS AND FUNCTION OF PLANT STEROLS: AN OVERVIEW OF PAST AND CURRENT DEVELOPMENTS. W. David Nes, Plant and Fungal Lipid Group, Plant Development and Productivity Research Unit, Western Regional Research Center, U.S. Department of Agriculture, Albany, CA 94710.

Plants (including the fungi) produce 24-alkylated sterols, a biosynthetic event which animals lack. This overview, in part from a historical perspective, will examine the chemical and biochemical evidence which shows the mechanisms and sequences that are involved in 24-α/β alkyl formation and the functional importance of this side chain substituent. As is now becoming increasingly apparent, evolution and ecology have been impacted by the presence (and absence) and stereochemistry of this grouping. Specific non-alkylated intermediates which do not control evolution may be used as phylogenetic markers of lineage, i.e., cycloartenol and lanosterol. Alternatively, the functional alkylated-end products, whose occurrence crosses evolutionary lines, may have a fundamental role in the evolution of lineages, viz., by regulating (and maintaining) growth and reproduction.

DESIGN OF HIGH ENERGY INTERMEDIATE ANALOGUES INHIBITORS TO STUDY TRITERPENOID BIOSYNTHESIS AND FUNCTION IN HIGHER PLANTS. Pierre Benveniste, U.L.P., Institut de Botanique, Laboratoire de Biochimie Vegetale et de Chimie Enzymatique, 28, rue Goethe - 67083 - Strasbourg Cedex, France.

It has been demonstrated previously that the design of transition state (TS) or HEI analogues could lead to powerful and specific inhibitors of enzymes. We have applied this approach to the following target enzymes : 2,3-oxidosqualene cyclase, S-adenosyl methionine-cycloartenol-C-24-methyltransferase (AdoMet CMT), cycloeucalenol-obtusifoliol isomerase (COI) and $\Delta^{8} \rightarrow {}^{7}$-sterol isomerase. Very potent inhibitors have been obtained in each case. A new example consisted into N-alkyl-8-aza-decalins which were shown to inhibit strongly in vitro the COI and the $\Delta^{8} \rightarrow {}^{7}$-sterol isomerase ($Ki/Km = 10^{-3}$); these molecules were shown to be also very active in vivo : when suspension cultures of bramble (Rubus fruticosus) cells were treated with N-benzyl decalins at low concentrations, dramatic changes of the sterol profile were observed with an almost complete replacement of Δ^{5}-sterols (normally present in control cells) by 9β,19-cyclopropyl sterols and (or) Δ^{8}-sterols. N-[1,5,9-trimethyl-decyl]-4, 10-dimethyl-8-aza-trans-decal-3β-ol was shown to inhibit also the 2,3-oxidosqualene-cycloartenol cyclase in vitro and in vivo. In the same plant material, the 2,3-oxido-squalene-$\beta(\alpha)$-amyrin cyclase was not inhibited. Hence, for the first time, these two cyclases have been discriminated in the same cell-free extract by use of specific inhibitor. The data obtained offer an opportunity to evaluate the physiological and biochemical consequences of the almost complete replacement of Δ^{5}-sterols by cyclopropyl- (or other) sterols in higher plant cells. When locusts were reared with cyclopropyl sterols containing wheat, a dramatic decrease in ecdysteroid content was accompanied with abnormalities in embryonic development. This result suggested that the cyclopropyl sterols contained in wheat treated with fenpropimorph could not be used by the insects in place of Δ^{5}-sterols to make precursors usable for ecdysteroid synthesis.

STUDIES ON THE FUNCTIONS OF STEROLS IN THE YEAST, SACCHAROMYCES CEREVISIAE. Leo W. Parks, Christopher Low, Thomas A. Lewis, and Russell J. Rodriguez, Department of Microbiology, North Carolina State University, Raleigh, NC 27695.

In order to study the physiological functions of sterols in an organism, it must be possible to regulate the qualitative and quantitative pattern of sterols in the test system. The yeast Saccharomyces cerevisiae, because of its cultural and genetic versatility, is ideal for studying sterol functions. We have manipulated the structures and amounts of sterols in yeast, using auxotrophic strains that are incapable of sterol synthesis. With this procedure we have been able to define four different levels of function for sterols in this organism.

Wild-type S. cerevisiae under aerobic conditions is not able to accumulate sterols from the growth medium. Inhibition is dependent on the ability of the organisms to synthesize heme compounds. This situation precludes the uptake of exogenous sterols under most conditions, and assures that only endogenously synthesized sterols are available to the cells. A critical physiological function may be dependent on ergosterol and would be antagonized if non-ergosterol sterols were accumulated. In addition, we have shown that exogenously supplied sterols coordinately regulate specific phospholipid species, fatty acid composition and sterol/phospholipid ratios. Taken together our data support the essential nature of ergosterol in the yeast cell, and its sparing effects under certain cultural conditions.

8

FUNCTIONAL ORGANIZATION OF CAROTENOIDS AND PRENYLQUINONES IN THE PHOTOSYNTHETIC MEMBRANE. Hartmut K. Lichtenthaler, Botanisches Institut (Plant Physiology), University of Karlsruhe, Kaiserstraße 12, D-7500 Karlsruhe, West Germany.

The three types of plant prenyllipids, the chlorophylls, carotenoids and prenylquinones, are integral components of a functional photosynthetic apparatus. The carotenoids of the photosynthetic membranes of higher plants consist of ß-carotene, lutein, violaxanthin and neoxanthin. The prenylquinones of thylakoids comprise plastoquinone-9, α-tocoquinone, its chromanol α-tocopherol and phylloquinone. The functional organization of these prenyllipids within the photosynthetic membrane is far from being well understood. Our present knowledge on the functional association and partition of individual carotenoids and prenylquinones with pigment proteins and/or membrane lipids will be reviewed.

During light-induced biogenesis of photosynthetically active membranes the kinetics of accumulation of carotenoids and prenylquinones differ considerable and provide some insight into the assembly of a functional photochemical apparatus. In chloroplasts carotenoids are bound to various chlorophyll-carotenoid-proteins, the carotenoid composition of which will be presented. Variations in relative pigment-protein levels, due to natural environmental factors, forest die-back processes or herbicide action, are correlated with changes in pigment ratios and in the stacking degree of thylakoids.

Except for plastoquinone 9 the functional role of prenylquinones in the photosynthetic membrane is not clear. The present view of Q-cycles and of special quinones at the donor and acceptor sites of the two photosystems provides possibilities to integrate phylloquinone and α-tocoquinone within the functional membrane.

The importance of galacto- and phospholipids for a functional integration of pigments and prenylquinones in the photosynthetic membrane is underlined by our results with the new grass-herbicide sethoxydim. This herbicide inhibits glycerolipid biosynthesis and blocks the accumulation - but not the biosynthesis - of pigments and prenylquinones.

9

ARACHIDONIC AND EICOSAPENTAENOIC ACIDS, GLUCANS AND CALCIUM AS REGULATORS OF RESISTANCE TO A PLANT DISEASE. Joseph Kuć, Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546.

Incompatible races of Phytophthora infestans elicit a hypersensitive-type disease resistance reaction (HR) in potato tubers. The HR includes rapid death of penetrated potato cells, tissue browing and the accumulation of fungitoxic sesquiterpenoid phytoalexins. Arachidonic and eicocosapentaenoic acids are elicitors of this reaction and the major active components in the mycelium of the fungus. β-Glucans from the fungus, though inactive as elicitors, enhance the activity of AA and EPA but they race-specifically suppress (HR) produced by incompatible races. Saturated 16, 18 and 20-carbon and unsaturated 16 and 18-carbon fatty acids are inactive as elicitors and 20-carbon acids with the 5, 8, 11 double bond configuration are 5-10 times more active than others. Activities of lipids containing esterified AA are consistent with the free acid being the active agent. In the presence of appropriate β-glucans, elicitor activity of AA was detected at the picomole level, and in their presence inactive unsaturated 20-carbon acids, but not 16 or 18-carbon acids, with 1-3 double bonds elicited (HR). AA-elicited HR is inhibited by SHAM, BW755C and ETYA, all inhibitors of lipoxygenase, and structural requirements for elicitor activity suggest the importance of lipoxygenase in HR. Recent evidence from our laboratory indicates Ca^{++} enhances AA-elicited HR and it plays a key role in HR. A comparison between the effect of β-glucans and their receptors and Ca^{++} on the enhancement of 5-lipoxygenase activity and release of leukotriene B_4 from AA in animals and the regulation of HR elicited by P. infestans is intriguing.

10

STEROL BIOSYNTHESIS IN PLANT CELL CULTURES. F.F. Roberto[a], L.M. Shannon[b] and J.B. Mudd[c], [a]Department of Plant Pathology, Univ. of California, Davis, [b]Department of Biochemistry, Univ. of California, Riverside, [c]ARCO Plant Cell Research Institute, Dublin, CA

We recently conducted a study of lipid biosynthesis during a complete culture passage in *Nicotiana glutinosa* cell suspension cultures, employing a variety of radiolabeled precursors, including acetate. While a substantial proportion of the incorporated label was localized, as expected, within fatty acids, over 40% of the label derived from acetate was found within sterols when cultures were examined at the onset of log-phase growth.

Free sterols constituted the major sterol component, but steryl esters and glycosides also represented a substantial proportion at various times within the culture passage.

Radiolabeled mevalonic acid was also used in a parallel experiment where sterols were the only lipid class to accumulate any appreciable label. A similar distribution of label among free sterols, steryl esters and glycosides was seen.

These results suggest a greater demand for sterols in plant cells as they exist in culture, perhaps reflecting some fundamental difference between plant cell membranes *in situ* within the green plant and *in vitro* , which might be revealed upon further investigation. Such an examination might also provide valuable insights concerning the role of sterols in plants.

11

ENDOGENOUS GIBBERELLINS IN WHEAT SEEDLINGS. J. T. Lin and A. E. Stafford, Western Regional Research Center, U. S. Department of Agriculture, ARS, Albany, CA, USA.

Identification of endogenous gibberellins (GAs), a group of plant hormones, in developing wheat (Triticum aestivum L.) grains has been reported. GA_1 and GA_3 have also been identified in the vegetative tissues of mature wheat plants. GAs in wheat seedlings have not been identified previously. The seedlings of Chinese Spring wheat were harvested 3 weeks after sowing. Shoots and roots were separated. The acidic ethyl acetate fractions were extracted from the shoots and roots. The fractions were then purified by sequential polyvinylpolypyrrolidone (PVPP) purification, preparative C_{18} high-performance liquid chromatography (HPLC), preparative silica HPLC and analytical C_{18} HPLC. The fractions from the analytical C_{18} HPLC were bioassayed by the Tanginbozu dwarf rice bioassay. The biologically active fractions were methylated with excess ethereal diazomethane. The trimethylsilyl (TMSi) ethers of the methyl esters of the endogenous GAs were prepared using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA). The derivatized GAs were then identified by capillary gas chromatography-selected ion monitoring (GC-SIM). We have identified GA_{19} in the roots and GA_{19} and GA_{44} in the shoots of wheat seedlings. The conversion of $[^3H]GA_1$ to $[^3H]GA_8$ previously shown in wheat seedlings and the identification of GA_{19} and GA_{44} in the present study suggest the operation of early-13-hydroxylation pathway in wheat seedlings. The concentration of GAs in developing seeds apparently is much higher than the concentration of GAs in vegetative tissues.

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DEVELOPMENTAL REGULATION OF STEROL AND PENTACYCLIC TRITERPENE BIOSYNTHESIS AND COMPOSITION: A CORRELATION WITH SORGHUM FLORAL INITIATION. Rick C. Heupel and W. David Nes, Plant Devel. Prod. Res. Unit, U. S. Department of Agriculture, Albany, CA 94710

Sterols and pentacyclic triterpene (PT) composition and biosynthesis have been examined during the development of the shoot (1986 Lipids 21: 69-75) and panicle of Sorghum bicolor. Free sterol content of the seed (µg/seed) increased somewhat during a 20h germination period. As the plant matured (7 to 48 days), there was a logarithmic increase in leaf sterol content (µg/blade) which plateaued at the onset of floral differentiation (ca. 41 days) and rapidly decreased over the next 18 days. Panicle free sterol content at day 66 was ca. 8x that of the leaf content; the sterol content apparently increases with panicle development. Foliar applications of radiolabeled sterols indicate that the regulatory and bulk sterols of the panicle maybe derived from a leaf sterol pool. With the onset of floral differentiation, leaf PT increased from negligible levels reaching levels that surpassed sterols as flowering progressed; panicle free PT content at day 66 was negligible. Feeding studies with labeled precursors demonstrated that sorghum possesses separate post-cycloartenol pathways to separate steroidal end products which were developmentally influenced. A critical mass of sterol may be required for floral initiation. The decrease in leaf sterol levels and the appearance of PT indicate that PT may replace the sterol in the membrane so that the preformed sterol may be matabolized for other physiologycal functions and/or translocated to other target sites.

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BIOSYNTHESIS OF STEROLS AND STEROL INTERMEDIATES IN DEVELOPING SEEDLINGS OF *CUCURBITA MAXIMA*. INCORPORATION OF [2-^{14}C]MEVALONIC ACID. Vipin K. Garg* and William R. Nes, Department of Biological Sciences, Drexel University, Philadelphia, PA 19104, U.S.A. (*Present address: ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568, U.S.A.)

As part of our investigation on sterols of the family Cucurbitaceae we have examined the incorporation of [2-^{14}C]mevalonic acid into various sterol fractions in seedlings of *C. maxima*. During the first two days of germination, label from MVA appeared primarily in squalene (*ca* 64% of the total), while 4,4-dimethyl-, 4α-methyl and 4-desmethylsterols together contained only about 36% of the total label. It was only by day 4 that an appreciable proportion (54%) of the label was transferred to 4-desmethylsterols through the oxygen-requiring steps. By day 12, desmethylsterols were labelled to an extent of 81%, while squalene was only to an extent of 1.5%. Similar delayed conversion of squalene to sterols has been observed previously during germination of seeds of *Pisum sativum* and *Pinus pinea*. When the 4-desmethyl fraction was further analysed into Δ^5- and Δ^7-sterols, the label was found to be associated only with the Δ^7-components, and failed to become incorporated into Δ^5-sterols. This is consistent with our recent finding that while Δ^5-sterols are present in the seeds of *C. maxima*, they progressively disappear following germination. It is suggested that the Δ^5- sterols have a specific function during germination. The possibility is also raised that the expression of sterol biosynthetic sequences in higher plants is temporally regulated.

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EVIDENCE FOR A 24-ALKYL STEROL REQUIREMENT IN GROWTH OF A CROP PLANT. Beni Tal and W. David Nes, Plant Development-Productivity Research Unit, U. S. Department of Agriculture, Albany, CA 94710

Suspension cultures of Helainthus annuus (sunflower) were shown via GLC, HPLC, MS and PMR to possess a similar Δ^5 -, Δ^7 -, and $\Delta^{7,9(11)}$- sterol profile as previously observed in seeds (Homberg, E.E. and Schiller, H.P.K. (1973) Phytochemistry 12, 1767). When 5.0 ppm of the carbonium ion high energy intermediate enzyme blocker of C-24 transalkylation, ie., 24-epiiminolanosterol, was incubated with dedifferentiated cells of sunflower growth was completely inhibited; a 50 per cent inhibition of growth results from a level of 1.0 ppm inhibitor. The results are interpreted to imply that vascular plants, analogous to ascomycetous fungi, require sparking and/or bulk amounts of 24-alkyl sterol(s) in the vegetative phase of the life cycle.

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PRESENCE OF UNUSUALLY HIGH LEVELS OF CHOLESTEROL IN THE SHOOT-APICES OF FLOWERING PLANTS. Vipin K. Garg, Trevor J. Douglas and Leslie G. Paleg, Department of Plant Physiology, Waite Agricultural Research Institute, University of Adelaide, SA 5064, Australia.

The major sterol components isolated from most plant tissues are sitosterol, stigmasterol and campesterol; cholesterol is a minor component and is sometimes found only in trace amounts. In the course of our work on the sterol profiles of different plant tissues during floral development, we have identified very high levels of cholesterol in the shoot-apices of *Lolium temulentum, Hordeum vulgare* and *Xanthium strumarium*. In each species, 4-desmethylsterols were analysed separately from leaves, stems and shoot-apices (terminal inflorescence carefully dissected-out under a microscope), for several days during flowering. In leaf and stem tissues, as expected, sitosterol was the main sterol (50-80% of the total), followed by campesterol (10-30%) and stigmasterol (5-20%), while cholesterol was mostly a minor component (<5% of the total). In contrast, in the shoot-apices, cholesterol was highly abundant (15-45%) and matched the levels of the other three sterols throughout the sampling period. On a per unit dry weight basis, the cholesterol level of the apical tissue was up to 200 fold higher than that of the leaf and stem tissues. Contrary to the widely held view, these results indicate that cholesterol may not always be of little significance in plants. A specific association of cholesterol with meristematic and/or reproductive tissues is suggested.

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DROUGHT STRESS EFFECTS ON ROOT CELL MEMBRANES
H. Svenningsson, M. Andersson and C. Liljenberg, Department of Plant Physiology, University of Göteborg, Sweden.
Young rape plants (Brassica napus L.) were exposed to repeated water-deficit stress. The water potential of the shoot changed from c. -0.5 to -1.5 MPa during each stress treatment. Three consecutive 24 h stress periods interfoliated with 24 h non-stressed conditions resulted in an increase in the root/shoot dry weight ratio by 69%. A membrane vesicle fraction enriched in plasma membranes of the roots was isolated after 24 h and 3 x 24 h stress periods. The membrane fraction was identified with marker enzymes and electron microscopy and the lipids were analysed. The stress caused a decrease in the level of total phospholipids based on protein by 17% after 24 h stress and by 48% after 3 x 24 h stress compared to the control membranes. The phospholipid composition changed too with repeated stress, leading to a change in the ratio of the major lipids phosphatidylcholine/phosphatidylethanolamine from 1.78 to 1.17 and almost a doubling in the proportion of phosphatidylinositol. Changes in the molecular species of the phospholipids occurred with stress. The molar ratio free sterols/phospholipids was 0.58 in the control membranes and 0.70 after stress.

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PHYTOSTEROL STRUCTURE AND COMPOSITION IN THE CHEMOSYSTEMATICS OF THE CARYOPHYLLALES. J.H. Adler and T.A. Salt, Dept. of Biological Sciences, Michigan Tech. Univ., Houghton, MI and Dept. of Botany, Univ. of Maryland, College Park, MD, U.S.A.

Sterols from 39 species within 7 families of the order Caryophyllales were analyzed. In the family Caryophyllaceae the dominant sterols from 13 species in five tribes were 24-ethyl-Δ^7-sterols. In the tribe Alsineae, 2 species in the subtribe Sabulininae synthesize both 24α- and 24β-ethyl-Δ^7-sterols, whereas 3 species in the subtribe Stellariinae produce 24-alkyl-Δ^5- as well as 24α-ethyl-Δ^7-sterols. In the family Cactaceae the dominant sterol from 8 species in seven genera was sitosterol. The family Chenopodiaceae synthesized a diversity of 24α-ethylsterols within 13 species from eight genera. Two species in the type genus Chenopodium synthesize only Δ^7-sterols, whereas 3 additional species in this genus and 2 in Salicornia produce relatively fixed ratios (1:1) of Δ^5- and Δ^7-sterols. Five species in five additional genera synthesize predominately 24-alkyl-Δ^5-sterols. Five species in four additional families Phytolaccaceae, Aizoaceae, Basellaceae and Portulacaceae sythesize 24-alkyl-Δ^7-sterols. The Caryophyllales, to date, is a predominately Δ^7-sterol producing order. The synthesis of Δ^5-sterols and 24β-ethyl-Δ^7-sterols is sufficiently restricted within families as to be putatively useful in the chemosystematics of the order.

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INHIBITORS OF FUNGAL C-24 STEROL ALKYLATION: 24,25-EPIMINOLANOST-8-EN-3β-OL(I) and 25-AMINOLANOST-8-EN-3β-OL(II). Edward J. Parish and W. David Nes*, Department of Chemistry, Auburn University, Auburn University, Alabama 36849 and *Plant and Fungal Lipid Group, Plant Development and Productivity Research Unit, Western Regional Research Center, USDA, ARS, Albany, CA 94710

We have endeavored to develop rationally designed molecules to selectively inhibit the growth and reproduction of certain pathogenic fungi toward crop plants. In pursuit of this goal, we have designed and chemically synthesized two new carbocationic high energy intermediate blockers which have demonstratable biochemical effects on the sterol pathway. Inhibitor I was prepared by chemical synthesis using commercial "lanosterol" (a mixture of lanosterol and 24,25-dihydrolanosterol) as the starting material. Purification was achieved by chromatography and recrystallization. Treatment of I under conditions of catalytic hydrogenation produced II in high yield. Incubations with Gibberella fujikuroi have indicated that both I and II are highly effective inhibitors of C-24 alkylation and indicate a functional necessity for the C-24methyl substituent.

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THE REGULATION OF TERPENOID BIOSYNTHESIS IN TAPPED LATEX. G. J. Piazza, E. J. Saggese, and M. P. Thompson, USDA, ARS, Eastern Regional Research Center, 600 East Mermaid Lane, Philadelphia, PA 19118.

The biosynthesis of triterpenols (TOH) and their fatty acid esters (TE) can be observed in the tapped latex of Euphorbia lathyris provided with appropriate radiolabeled precursors. Low speed centrifugation of the latex affords a pellet which utilizes mevalonate as a precursor for TE and TOH biosynthesis. Various antagonists of the calcium binding protein, calmodulin, were found to be good inhibitors of latex pellet triterpenoid biosynthesis (e.g., chlorpromazine, I_{50} = 150 μM; trifluoperazine, I_{50} = 55 μM). Several chlorinated phenoxyamine compounds were also tested as inhibitors. Increased chlorine atom substitution increased inhibitor effectiveness. The best inhibitor, 2-(pentachlorophenoxy)ethyl N,N-diethylamine (I_{50} = 35 μM), was also the best antagonist of calmodulin. Therefore, our results suggest the involvement of calmodulin in the regulation of latex triterpenoid biosynthesis.

Recent work has demonstrated that a protein, electrophoretically similar to that of bovine brain calmodulin, occurs in tapped latex. This protein has been isolated from homogenates of E. lathyris and is being characterized.

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SEPARATION AND IDENTIFICATION OF CAROTENOID-ESTERS IN RED PEPPER /CAPSAICUM ANNUM/ DURING RIPENING. P.A. Biacs, J. Bodnár, A. Hoschke, Anna Cs. Pavisa, H. Daood, F. Hajdu and Natalia Kiss-Kutz. Central Food Research Institute, Budapest, Hungary.

Natural pigments of red pepper are mostly bound to fatty-acids as carotenoid esters. Their partial hydrolysis is caused during ripening, processing and storage. Ultraviolet absorbance methods are suggested for stepwise record of the process of ripening and decomposition of carotenoids and their esters. Individual carotenoids and their esters could not be identified by the complex UV-spectra and their effect on stability could not be determined selectively.
Preparative thin-layer chromatography was used for the separation of individual compounds, and the UV, IR and NMR spectra were used for the identification. The quality and quantity of fatty-acids were determined by GLC and HPLC methods.
The changes in the composition of carotenoids, mono- and disaccharides have been measured by HPLC during ripening and storage of red pepper. More than 20 components were measured and their formation and decomposition were followed. The kinetics of autooxidation and oxidation catalysed by lipoxygenase and hydroperoxid-isomerase were followed by UV-spectra of individual pigments.

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TRITERPENOID BIOSYNTHESIS IN EUPHORBIA LATHYRIS. C. Skrukrud, S.E. Taylor, D.R. Hawkins, and M. Calvin, Lawrence Berkeley Laboratory and Department of Chemistry, University of California, Berkeley, CA

Triterpenols and their esters make up 5-8% of the dry weight of E. lathyris. A significant amount of these triterpenoids are synthesized and stored within laticifer cells. We have studied the controls of carbon allocation to these compounds. Preliminary results suggest that the rate of terpenoid biosynthesis is controlled as early as sucrose utilization. The subcellular organization of triterpenoid biosynthesis in latex has been investigated by centrifugation and electron microscopy. Using these techniques, we have found that the conversion of HMG-CoA to MVA is a key step. We have partially purified the enzyme responsible for this step, HMG-CoA reductase. We will also report on the structures and biosynthesis of the latex triterpenoids from squalene.

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FATTY ACID OXYGENASE REGULATION OF MORPHOGENESIS BY THE FUNGUS LAGENIDIUM GIGANTEUM. J.L.Kerwin, C.A.Simmons and R.K.Washino, Department of Entomology, University of California, Davis, CA 95616.

Lagenidium giganteum (Oomycetes:Lagenidiales), a facultative fungal parasite of mosquito larvae, produces a sexual stage, the oospore, in liquid culture media containing exogenous sources of sterols structurally related to cholesterol and esterified unsaturated fatty acids. Oosporogenesis can be inhibited in developmentally synchronized cultures of the fungus by the addition of compounds known to inhibit mammalian lipoxygenases (alpha-naphthol, salicylhydroxamic acid, propyl gallate, nordihydroguiaretic acid) and cyclooxygenases (salicylates, ibuprofen, indomethacin, phenylbutazone). Fatty acid oxygenases occur in both microsomal and cytosolic fractions of the fungus. Preliminary characterization of these oxygenase enzymes and a discussion of their role in fungal morphogenesis is presented.

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METABOLISM OF EICOSAPOLYENOIC ACID LIPIDS IN RACE-SPECIFIC INTERACTIONS BETWEEN PHYTOPHTHORA INFESTANS AND POTATO.

R. M. Bostock. Department of Plant Pathology, University of California, Davis.

Arachidonic (AA) and eicosapentaenoic (EPA) acids are abundant components of the acyl lipids of the fungal plant pathogen, Phytophthora infestans. EPA and AA are efficient elicitors of the hypersensitive response and associated reactions which serve to limit pathogen ingress in potato tuber tissue. It has been proposed that hydrolysis of fungal lipid with the concomitant release of EPA/AA is a definitive early event in incompatible spore-plant tissue interactions. To test this hypothesis, fungal and synthetic lipids containing radiolabelled EPA and AA were applied at concentrations below the threshold of induction of the tuber response to potato disks which were subsequently inoculated with spores of an incompatible (race 0) or compatible (race 1.4) isolate of P. infestans. Lipids were extracted at various times after inoculation and analyzed by TLC and liquid scintillation counting. There was a significant decline in the proportion of label in triglyceride within 24 hr after inoculation and a concomitant increase in polar lipid and free fatty acid pools. The pattern was similar in both interactions and analysis of the fatty acid composition did not indicate a change in the proportion of EPA/AA relative to other fatty acids. No differences were detected between interactions in the polar lipid composition. Acyl hydrolase activity (substrate = p-nitrophenyl palmitate) was similarly affected in both interactions. The data suggest that although hydrolysis of acyl lipid occurs in inoculated tissue, the reactions proceed similarly in both incompatible and compatible interactions.

Session 3 - Structure and Function of Lipids

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STRUCTURAL AND DYNAMICAL ASPECTS OF MEMBRANE LIPIDS. R.A. Demel, Laboratory of Biochemistry, Utrecht, The Netherlands.

Biological membranes appear to be composed of complex mixtures of lipid species, showing many variations in size and charge of the polar head groups, in length and unsaturation of the paraffin chains, and cholesterol content. For a given membrane the lipid composition is characteristic, but between membranes with different functions, large differences can be noticed. This raises the question whether such a complex lipid composition is a functional requirement and provokes the hypothesis that lipids contribute in more specific ways to membrane functions. Lipids, like phosphatidylcholines, phosphatidylserines, sphingomyelin, and diglycerides, spontaneously adopt a bilayer organization whereas unsaturated phosphatidylethanolamines and monoglycosyldiglycerides tend to form a hexagonal H_{II} phase. Transitions between different organizations are possible, and can be induced by temperature, ion concentration, sterols, and lipid-protein interactions.

Catalytic reactions, occurring at the membrane interface, have been found to be strongly dependent on the molecular packing, the composition, and charge density of the membrane lipids. There is evidence that the relative amounts of neutral lipids as cholesteryl esters and triacylglycerols, affect the catalytic hydrolysis of the latter, possibly by affecting the lateral distributions of these lipids.

Lipid transfer proteins can contribute in a specific way to the dynamic properties of membrane by affecting their composition. Some transfer proteins interact with one class of lipids. However, considerable differences have been noted between transfer of fatty acyl chain positional isomers. There is strong evidence that transfer proteins can also be involved in fusion between membranes, enabling a fast mass transfer of phospholipids - but also of sterols.

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STRUCTURE-FUNCTION RELATIONSHIP OF PLANT MEMBRANE LIPIDS: THE ROLE OF FATTY ACID UNSATURATION. László Vigh, Institute of Biochemistry, Biological Research Center of Hungarian Academy of Sciences, H-Szeged, P.O. Box 521.6701, Hungary.

For better understanding the specific role of fatty acid unsaturation in the function and molecular organisation of plant cell membranes series of physiological, biochemical and biophysical studies have been performed recently. In order to modulate the level of unsaturation the improved technique of homogeneous catalytical hydrogenation was applied. In situ hydrogenation of lipids within native biomembranes in the presence of water-soluble catalysts provided us an excellent opportunity to learn more about this subject. Our results indicated:

(1) In vivo catalytic hydrogenation of the cis-double bonds resulted in a shift in the chilling susceptibility of the blue-green alga, Anacystis nidulans. Selective modification of cytoplasmic membrane provided evidence on its determinative role in cold tolerance of Anacystis cells.

(2) Reduction of membrane fluidity in living protoplasts of Nicotiana plumbaginifolia initiated an enhanced rate of 18:1-PC desaturation, simultaneously. This desaturation, competing with hydrogenation, seemed to be a part of self regulatory process for fluidity restoration.

(3) Saturation of polyenoic fatty acids of wheat root microsomes did not influence temperature-dependency of Mg-ATPase activity.

(4) Successive removal of double bonds in pea thylakoids brought about increased orientational ordering of membrane lipids, and appearance of gel-phase. $Pd(QS)_2$ catalyst saturated selectively cis-double bonds of fatty acyl residues and had no effect on double bonds present in chlorophylls, carotenoids and plastoquinone. Progressive saturation primarily inhibited electron transport between the photosystems followed by the inhibition of electron flow around PS-II. PS-I was not affected even by 50% saturation.

(5) Increased lipid saturation reduced the tendency of formation of non-bilayer structures and enhanced thermal stability of pigment-protein complexes in the native thylakoid.

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SPATIAL ORGANIZATION AND FUNCTIONAL ROLES OF ACYL-LIPIDS IN THYLAKOID MEMBRANES. P.A. Siegenthaler, A. Rawyler and C. Giroud, Laboratoire de Physiologie végétale, Université de Neuchâtel, Chemin de Chantemerle 20, CH-2000 Neuchâtel, Switzerland.

The unique acyl-lipid composition of thylakoid membranes is known for many years. However, the organization of these lipids in the membrane and the role which they play in photochemical activities are still not well understood.

The first purpose of this review is to present the different approaches which have been adopted to determine the lateral and transversal distribution of acyl-lipids in thylakoid membranes. Special emphasis will be given to the enzymatic approach, i.e. the use of various specific phospholipases and galactolipases. From all these studies, an asymmetric distribution of acyl-lipids in the thylakoid membrane will emerge.

The second purpose of this review will be to scrutinize the evidences available showing that certain lipids (or part of these lipid molecules) are required for maximal photosynthesis. Several approaches will be presented (enzymatic attack, chloroplast fractionation, reconstitution experiments, etc.) to assess this relationship. However, it will appear that the functional role of lipids in the thylakoid membrane is an ambiguous concept because lipids, in contrast to most proteins, have by themselves no recognized catalytic properties. Rather, they allow the maintenance of an appropriate conformation and orientation of proteins which may express their function under optimal physico-chemical conditions of the membrane (fluidity, packing, etc.) and/or in the presence of certain lipids (or portion of those lipids). Although it is extremely difficult to dissociate the functional from the structural role of lipids, we may propose that each lipid class consists of several topologically distinct pools which may play specific roles in the membrane function.

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ULTRASTRUCTURAL STUDIES ON PLANT MEMBRANES. W. W. Thomson and K. A. Platt-Aloia, Department of Botany and Plant Sciences, University of California, Riverside 92521

Analyses of density patterns produced with transmission electron microscopy and particle distributions observed with freeze-fracture electron microscopy (FEM) of the plasmalemma and tonoplast show that these membranes often display lateral heterogeneity in organization such that in some regions, they may possess a subunit rather than a bilayer organization. Also, lipid enriched microdomains, indicative of lateral phase separations and the formation of gel-phase lipids, develop in the plasmalemma and tonoplast during senescence and chilling. In some instances, sterols also appear to be enriched in the membrane surrounding nuclear pores and often filipin, which binds membrane sterols, appears to induce the formation of microdomains in membranes. These observations support the view that plant membranes are not only asymmetric, but also laterally heterogeneous in organization and presumably in function and composition. Assuming all three properties are integrally related, shifting degrees of organization, such as occurs with senescence, and as influenced by chemicals that interact with membrane compounds would suggest a concommitant shift in membrane function.

Session 4 - Biosynthesis of Complex Lipids

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CHARACTERIZATION AND BIOGENESIS OF LIPID BODIES IN MAIZE SCUTELLA. Anthony Huang, Biology Department, University of South Carolina, Columbia, SC 29208, USA.

We have been using maize scutellum as a model system to study lipid body biogenesis during seed maturation and germination. Our findings include the subcellular location of diacylglycerol acyltransferase, the characteristics of lipid body membrane "structural" proteins (properties, purification, biosynthesis, gene structure and expression), and the properties, purification, and biosynthesis of lipase. These and other biochemical findings, together with electron microscopic observations, will be used to delineate the mechanism of lipid body biogenesis.

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ON THE CONTROL OF FATTY ACYL COMPOSITION AND DISPOSITION WITHIN PLANT GLYCEROLIPIDS. P.G. Roughan, Division of Horticulture and Processing, Mt. Albert Research Centre, DSIR, Private Bag, Auckland, New Zealand.

Until recently, studies on the biosynthesis of complex plant lipids had been confined almost exclusively to the incorporation of polar head groups; there was little interest in the fatty acid compositions of the diacylglycerol or phosphatidate precursors of the glycerolipids synthesised.

And yet, the fatty acid composition of a particular glycerolipid class may be so characteristic as to allow a reasonably confident identification of the lipid in spite of - or possibly because of - the distribution of fatty acids within plant glycerolipids being more complex than that within bacterial or animal lipids.

Plants seem to have their own rules for deciding which fatty acid will go where, and what further processing of those fatty acids is permissible. What are those rules and can we begin to understand how the characteristic fatty acid compositions of different plant glycerolipids is determined?

The complex distribution of fatty acids within the glycerolipids of leaves in particular, is consistent with the occurrence of two major sites of sn-glycerol 3-phosphate acylation in plant cells; each site synthesises a phosphatidate having a different fatty acid at the sn-2 position. Two different types of phosphatidate or diacylglycerol are thus generated within the cell and the way in which these precursors may be subsequently utilised in the net synthesis of complex lipids will be discussed. Particular emphasis will be placed upon recent research into the control of the fatty acid composition of phosphatidylglycerol in chilling-resistant and chilling-sensitive plants.

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LIPID DISTRIBUTION AND SYNTHESIS WITHIN THE PLANT CELL. Roland Douce and Jacques Joyard, DRF-Biologie Végétale-ERA 847, Centre d'Etudes Nucleaires, 85X F 38041 Grenoble Cedex, France.

The development of fast and reliable methods to purify plant cell organelles in good physiological state together with the preparation of their limiting membranes in a reasonably pure state has allowed us to study thoroughly lipid distribution and synthesis within the plant cell.

We have analyzed the lipid composition (polar lipids, prenylquinones, pigments) of membranes isolated from several types of plastids (chloroplasts, proplastids...), mitochondria and peroxisomes. Indeed, and in contrast with what was commonly thought few years ago, one can conclude that each plant membrane has a unique lipid composition which is probably genetically determined. One of the most striking feature which also emerge from these studies is the specific enrichment in phosphatidylcholine of the membranes (such as the outer membranes of chloroplasts and mitochondria) in close contact with the cytosol. The situation is particularily obvious in chloroplasts, which are mostly devoid of this phospholipid except in the outer leaflet of the outer envelope membrane.

Owing to their limiting membranes, most of the plant cell organelles contain all the enzymes involved in the acylation of glycerol 3-phosphate, which provide phosphatidic acid for further synthesis of polar lipids. Finally, using plant cells in suspension culture, we demonstrated that β-oleoyl-glycerolipids present in all cell membrane systems are the most likely candidates for the conversion of oleoyl to a linolenyl product, and that the desaturases involved exhibited a low affinity for molecular oxygen.

The figure which emerge from all these studies is the occurence of multiple sites of lipid synthesis and desaturation within the plant cell.

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REGULATION OF PHOSPHOLIPID HEADGROUP COMPOSITION IN CASTOR BEAN ENDOSPERM

Thomas S. Moore, Jr., Department of Botany, Louisiana State University, Baton Rouge, LA 70803

It is well established that phospholipid headgroup composition can have a profound effect on the structure and physical properties of biological membranes. Indeed, the gross phospholipid composition of those membranes is highly conserved, with repetitive isolations of the membranes, often from different ages, having the same phospholipid compositions. Such facts suggest a requirement for regulation of phospholipid synthesis. Despite this, the mechanism of regulation of phospholipid synthesis in plants has been little examined, and such studies still are in a primitive state.

Several potential means of regulation of phospholipid synthesis exist. Some which have been examined in plants include changes in the levels of the respective enzymes, enzyme and precursor compartmentalization, general enzyme requirements, precursor and product effects, and others. These will be discussed in general, but more specifically for the castor bean endosperm system. In the castor bean, the enzymes for phospholipid synthesis which have been examined increase in activity from very low levels in the dry seed to high activity within 3 to 4 days. Such increases appear to reflect enzyme synthesis and the enzyme activities generally are in proportion to the phospholipid concentration of the cell. Various cations affect the activities, as with other plants, and substrate requirements generally are in the umolar range.

We recently have been examining in more detail the regulation of synthesis of the major phospholipid of plants, phosphatidylcholine. Our efforts have centered on CDPcholine: diacylglycerol cholinephosphotransferase and CTP: cholinephosphate cytidyltransferase. The cholinephosphotransferase occurs primarily in the endoplasmic reticulum, where it is tightly bound. It can be released by the addition of octyl-B-D-glucopyranoside (OGP) and certain other detergents; even so it remains partially aggregated and loses up to 80 to 90 percent of its activity. The activity of OGP-treated ER can be reconstituted, with the best and most consistent results having been obtained by the addition of its lipid substrate, diacylglycerol. The characteristics of the detergent-treated, reconstituted enzyme will be described. The cytidyltransferase also is ER-bound and may be rate limiting, thereby controlling the production of the second substrate for cholinephosphotransferase, CDPcholine. The *in vitro* characteristics of this enzyme also will be described.

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MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINE IN PLANTS : BIOSYNTHESIS AND ROLE IN OLEATE DESATURATION OR FREEZING RESISTANCE.

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Molecular species of phosphatidylcholine (PC) from several plant tissues have been separated by means of HPLC. The biosynthesis of these molecular species has been studied in potato tuber and pea leaf microsomes, using CDP- ^{14}C choline as a precursor. No discrimination between the endogenous species of diacylglycerols was displayed by CDP-choline phosphotransferase.

Furnishing ^{14}C oleoyl-CoA to microsomes from aged potato slices, it was possible to follow the acylation of ^{14}C oleoyl residues into six molecular species of PC. When NADH was added, in presence of O_2, the main desaturation products were the predominant molecular species of PC in potato tuber microsomes : 1-palmitoyl-2-linoleoyl-PC and 1-linoleoyl-2-linoleoyl-PC.

A positive relationship has been established between freezing resistance in apple embryos and the accumulation of some molecular species of PC.

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PHASE TRANSITION BEHAVIOUR OF MONOGALACTOSYLDIACYLGLYCEROL.

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The kinetics and mechanisms of phase transitions in aqueous dispersions of saturated monogalactosyldiacylglycerol from spinach leaves have been investigated by time-resolved X-ray diffraction using synchrotron radiation. The kinetics of transition from the lamellar-gel phase and two lamellar crystalline phases to the liquid-crystalline phase were found to be in the order of 15 to 20 s. These transitions are about one order of magnitude slower than the rate observed for phase transitions in phospholipid-water systems. The liquid-crystalline phase was characterized by a single low-angle diffraction at about 30 nm and a wide-angle reflection characteristic of disordered hydrocarbon chains. Freeze-fracture electron microscopy showed this to be an amorphous phase.

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CORRELATION OF METABOLIC RATE CHANGES AND MEMBRANE TRANSITIONS DETERMINED BY MICROCALORIMETRIC METHODS.

R.W. Breidenbach and R.S.Criddle, Plant Growth Laboratory and Department of Biochemistry, University of California, Davis, CA.

The hypothesis that low temperature induced injury to chilling sensitive plant species is due to the physical properties of their membranes has been frequently criticized. These criticisms fall into three general categories. Firstly, "breaks" in Arrhenius plots of various biochemical or physiological processes have often been supported by insufficient data to satisfy statistical criteria. Secondly, measurements of the physical properties of membranes or membrane lipids, showing transitions at temperatures correlated with the "critical temperature" for injuring are usually indirect, relying on reporter molecules, such as fluorophores or spin labels. Thirdly, few of the physical measurement have been made on "native" membranes.

A differential scanning calorimetric technique was developed to measure the rate of metabolic heat production by cells as temperatures are varried. This allowed us to acquire large numbers of data points for examining rates of metabolism of cultured tomato cells over the range of 0 to 20C. A sharp break in the slope of the Arrhenius plot of these data occured at 12 C. Differential scanning experiments were also used to measure thermal transitions in isolated tomato lipid preparations, microsomal membrane vessicles, and intact tomato cells immediately following their treatment with cyanide. These preparations all exhibited distinct exothermic transitions centered near 12C. These results clearly demonstrate a relation between altered metabolism and membrane properties coincident with the temperature where injury is induced.

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PROPERTIES OF ACYL-(ACYL-CARRIER PROTEIN):GLYCEROL-3-PHOSPHATE ACYL-TRANSFERASE FROM GREENING SQUASH COTYLEDONS I. Nishida[1], M. Frentzen[2] and N. Murata[1], [1]National Institute for Basic Biology, Okazaki, Japan. [2]Institute für Allgemeine Botanik, Universität Hamburg, FRG.

Acyl-(acyl-carrier protein):glycerol-3-phosphate acyltransferase of plastids is proposed to be a key enzyme which controls the biosynthesis of saturated molecular species of phosphatidylglycerol in chilling-sensitive plants.

In order to evaluate the validity of this hypothesis, we investigated the properties of this enzyme from squash, a chilling-sensitive plant. In greening cotyledons of this plant the plastidial acyltransferase occurs in different isomeric forms. These isomers were purified, and their substrate selectivities were compared. The relationship between the substrate selectivity of the isomers and the fatty acid composition of squash phosphatidylglycerol will be discussed.

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A STUDY ON LIPIDS FROM RICE TISSUES IN RELATION TO CHILLING SENSITIVITY. S. Toriyama[1], K. Hinata[1], I. Nishida[2] and N. Murata[2], [1]Laboratory of Plant Breeding, Faculty of Agriculture, Tohoku University, Sendai 980, and [2]National Institute for Basic Biology, Okazaki 444, Japan.

Rice is one of the most chilling-sensitive plants. Since the contents of saturated lipid molecules in the cellular membranes are assumed to be responsible for the chilling injury, we analyzed the glycerolipids from rice tissues. The fatty acid compositions of the anther lipids were unique in high contents of stearic acid which amounted to about 8% of the total fatty acids in the total lipids and most of individual lipid classes, whereas in the leaf lipids this fatty acid comprised only 2%. Surprisingly high contents of stearic acid amounting to 25% was found in lipids from anther walls. These high contents of stearic acid in the anther walls may be related to the finding that the pollen formation within the anther is the stage which is the most sensitive to low temperature among all the growth and reproductive stages of rice plants.

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PLASMA MEMBRANE LIPID ALTERATIONS ASSOCIATED WITH LOW TEMPERATURE ACCLIMATION OF WINTER RYE. D.V. Lynch, X. K. Young and P.L. Steponkus, Agronomy Dept., Cornell University, Ithaca, NY 14853

The plasma membrane plays an important role in determining the cryobehavior of plant cells and is the primary site of freezing injury. Cold acclimation alters the cryobehavior and increases the cryostability of the plasma membrane. The lipid profiles of highly enriched plasma membrane preparations isolated from leaves of nonacclimated (NA) and cold acclimated (ACC) rye seedlings were characterized. Free sterols accounted for approximately 34 and 45 mole % of total lipid in NA and ACC samples, respectively. The relative proportion of β-sitosterol increased from 64 to 74% during acclimation, whereas the levels of campesterol and stigmasterol decreased from 27 to 23% and 7 to 3%, respectively. The glycolipid content of the plasma membrane decreased with acclimation, from 37 to 14 mole % of total lipid; however, the relative proportions of the major constituents, sterol glycoside, acylated sterol glycoside and an unknown, did not change significantly. The phospholipid content increased during acclimation, from 30 to 42 mole %. The relative proportions of PC (45%), PE (35%), PG (5.5%), PS (3.3%) and PI (< 2%), remained relatively constant after cold acclimation. Although there were only small differences in the proportions of the component acyl chains of the total phospholipids, there were pronounced differences in the molecular species. Increases in di-unsaturated molecular species (18:2/18:2, 18:2/18:3, 18:3/18:3) of PC and PE were associated with cold acclimation. The major species of PG, 16:0/18:2 and 16:0/18:3, increased at the expense of 32 carbon species (primarily 16:0/16:0).

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LONG-CHAIN TRIACYLGLYCEROL ACYL HYDROLASE ACTIVITY IN WHEAT GRAIN.
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Although triacylglycerol acyl hydrolase (TG lipase) activity of wheat germ develops during germination, the germ component of ungerminated wheat does not contain lipolytic activity towards long chain triacylglycerols (LCTG). The classical wheat germ 'lipase' does not hydrolyse LCTG. Nevertheless, the bran of sound, ungerminated wheat does contain TG lipase activity and this is responsible for the poor storage properties of food materials containing bran. Differing levels of enzyme activity in samples of grain account for variability in shelf-life of such products. Unlike most enzymes, the TG lipase of bran is active at very low water activities because the substrate (oil) can diffuse through dry material. Moreover, at low moisture levels the enzyme is resistant to heat inactivation. The poster will illustrate some properties of wheat bran TG lipase and will show that some widely-used assays for 'lipase' are not appropriate for materials containing this type of enzyme.

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MOLECULAR SPECIES COMPOSITION OF PHOSPHATIDYLGLYCEROL IN LEAVES OF CAMELLIA SPECIES AND CHILLING SENSITIVITY. J. Sekiya*, H. Koiso, A. Morita and A. Hatanaka, Department of Agricultural Chemistry, Yamaguchi University, Japan

The object is to investigate relationship between molecular species of membrane lipids and chilling sensitivity in evergreen camellia species. The MGDG isolated from these plant was 18:3 type and 18:3 accounted for 80-90% of fatty acid constituents. PG contained 30% of 18:1 in addition to (3E)-16:1. (3E)-16:1 exclusively bound to C-2 position of PG and 18:1 to C-1 position. To examine major molecular species of PG, PG was converted to 3,5-dinitrobenzoyl(DNB) derivatives by the aid of phospholipase C and 3,5-dinitrobenzoylchloride, and subjected to HPLC analysis. Major molecular species was 1-18:1-2-(3E)-16:1-PG. This species accounted for 70% in summer tea leaves and 40% in winter leaves. Thus, molecular species composition may correlate with chilling sensitivity in evergreen camellia leaves, but in the manner different from that in annual plants. * Present address: Faculty of Agriculture, Okayama University, Tsushima, Okayama 700, Japan.

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'ILLING SENSITIVITY OF CUCUMBER PROTOPLASTS. M.K. Pomeroy and J.B. Mudd, ARCO Plant Cell Research Institute, Dublin, CA

Studies using the chilling sensitive cucumber (Cucumis sativus L.) have shown that protoplasts isolated from cotyledons of one week-old seedlings provide a suitable system for examining the mechanism of chilling tolerance and susceptibility. Freshly isolated protoplasts are extremely sensitive to damage when chilled at 4° C in light, but suffer significantly less injury when chilled in dark at the same temperature. Furthermore, damage is greater when protoplasts which have been chilled either in light or dark are transferred directly to 27° C in light, than when they are initially placed in dark for at least 24 hours. If freshly isolated protoplasts are pre-chill conditioned at 27° C in either light or dark for several hours prior to exposure to various chilling stresses, subsequent chilling damage is markedly reduced. Experiments utilizing the cell wall synthesis inhibitor, dichlorobenzonitrile, showed that initiation of cell wall synthesis during the pre-chill conditioning period at 27° C was not responsible for the enhanced chilling tolerance observed in these protoplasts. The role of lipid metabolism in chilling sensitivity and in the pre-chill conditioning phenomenon observed in cucumber protoplasts has been examined. Changes in lipid and fatty acid composition, and in the pattern of uptake and distribution of $NaH^{14}CO_3$ under various treatment conditions will be discussed.

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ON THE FUNCTION OF METHYL-BRANCHED CHAIN FATTY ACIDS IN PHOSPHOLIPIDS OF CELL MEMBRANES OF HIGHER PLANTS. A. Radunz, Universität Bielefeld, Lehrstuhl Zellphysiologie, D-4800 Bielefeld 1, FRG

The phospholipids: PC (Phosphatidyl choline), PE (Phosphatidyl ethanolamine) and PI (Phosphatidyl inositol) of leaves and petals from Antirrhinum majus-and Nicotiana tabacum-mutants contain 16 to 24% mono-methyl branched chain fatty acids of the iso- and anteiso-types with chain lengths of 12 to 22 carbon atoms. After an enzymatic digestion with phospholipase A_2 it was found that the iso- and anteiso-acids are esterified in phospholipids in position 2 of the glycerol, just as unsaturated fatty acids. A higher content of methyl-branched fatty acids in lipids increases the fluidity of bi-membranes, just as a higher content of unsaturated fatty acids, because methyl-branching of the hydrocarbon chain of the fatty acids influences the lipid molecule packing in the lipid double layer. A higher fluidity of the membranes guarantees better ion transport between cell compartments and modifies the activity of phospholipid dependent enzyme systems. This is due to the fact that enzymic reactions depend on the chemical composition of the lipid matrix.
The branched fatty acids were investigated by means of comparative GLC with authentic f.a., by I.R. spectroscopy, NMR spectroscopy as by means of the measured optical activity.

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CALCIUM CHLORIDE EFFECT ON LIPID METABOLISM IN OLIVE TREE LEAVES.
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In the last years, many works about the effect of mineral ions on the plant membrane physiology have been made. Some of these studies have examined the calcium action on the plant lipid membranes. In the present work, we have investigated the effect of calcium chloride on the polar glycerolipids and their fatty acid biosynthesis in olive tree leaves using a labelled precursor: the (1-^{14}C) acetate. For this, young olive tree plants were irrigated with nutrient solution at various $CaCl_2$ concentrations: 0, 25, 50, 75 and 100 mM. Leaves were harvested their glycerolipids were extracted and separated by TLC; the lipid groups' fatty acid methyl esters were analysed by GLC.

Fatty acid analysis showed that high $CaCl_2$ concentrations (>50 mM) provoked a decrease in the amounts of total fatty acids and particularly the linolenic one. In addition, the in vivo labelling experiments with (1-^{14}C) acetate have confirmed the above results and showed a decrease of (^{14}C) acetate incorporation in total lipids, in galactolipids and main phospholipids when calcium chloride concentrations increased in the culture medium. Whereas, label in phosphatidic acid seemed to increase in treated leaves. Concerning the synthesis of fatty acids under saline condition, there was a decrease in labelled linolenic acid percentage to the advantage of labellel oleic acid one. The level of labelled linoleic acid increased slightly.

These results on the effect of $CaCl_2$ in olive tree leaves are in agreement with previous studies on other higher plants and showed that the calcium excess caused an inhibition of lipid metabolism enzymes and mainly those responsible of $C_{18:3}$ synthesis.

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LIPID CATABOLISM IN MEMBRANE SENESCENCE. Y.Y. Leshem, Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

With progress of normal senescence of pea foliage membranes free fatty acid:sterol ratios increase, this reflecting increment of phospholipolytic activity. The effect is simulated by exogenous application of phospholipase A_2 in the presence of Ca^{2+} and calmodulin and inhibited by the calmodulin inhibitor fluphenazine. This trend is accompanied by an increase in membrane microviscosity and transition temperatures and, as evidenced by wide angle X-ray diffraction data, indicates a change from the liquid crystalline phase to a solid gel lipid bilayer configuration.

Ethylene production, associated with plant senescence, closely follows the above biophysical changes. Membranes also manifest lipoxygenase activity, increasing with age and upon application of Ca^2, calmodulin and phospholipase A_2 the increment being inhibited by the more specific calmodulin inhibitor calmidazolium. Moreover the polyunsaturated linolenic acid enhanced phospholipase A_2-induced ethylene production whereas the saturated stearic acid was ineffective.

Results are interpreted as a Ca^{2+}-triggered and calmodulin mediated phospholipase A_2 activation whereby membrane integrity is impaired by the release of the 2-situated polyunsaturated fatty acids which in the freed form induce lipoxygenase and specific Ca^{2+} ionophoretic activity during the process of which an ethylene forming mechanism is activated.

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FREEZING RESISTANCE AND LIPID CHANGES IN CHOLINE-TREATED WHEAT SEEDLINGS. W.P. Williams, I. Horvath, L. Vigh, P.G. Thomas and P.J. Quinn, Department of Biochemistry, Biological Research Center of Hungarian Academy of Science, H-Szeged, P.O. Box 521.6701, Hungary.

Wheat seedlings grown in hydroponic cultures using media containing choline chloride exhibit an increased resistance to freezing damage. This increase is associated with an increase in phospholipid/protein ratios, and a decrease in the sterol/phospholipid ratio, of plasma membrane preparations of leaf cells. At the same time overall leaf growth is reduced and substantial changes occur in the water-balance of the cells. Comparison of results obtained with a wheat cultivar exhibiting a high capacity for cold-hardening (Miranovskoja 808) with one showing a more limited capacity for hardening (Brigand) suggest that the capacity for choline-induced changes matches their ability to cold-harden. These observations are explained in terms of a hierarchical model involving control of cold-hardening, and lipid metabolism, at both the genetic and metabolic levels.

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COCOA BUTTER BIOSYNTHESIS - COCOA SEED DIACYLGLYCEROL ACYLTRANSFERASE: STUDIES ON THE MICROSOMAL BOUND ENZYME. Paul J. Fritz and Lauren McHenry, Dept. of Pharmacology, M.S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

Diacylglycerol acyltransferase (DAGAT), the final of three acyltransferases in the four enzyme triacylglycerol biosynthetic pathway, was measured in cocoa seed microsomal preparations at developmental stages ranging from liquid endosperm, about 100 days after pollination (DAP), to full maturity, about 180 DAP. Relative to other proteins in microsomal fractions, DAGAT activity increased as much as five fold in a 30 day developmental period peaking around 130 DAP. This peak activity corresponds to times when the rates of seed triacylglycerol accumulation are at a maximum. Thereafter the activity fell rapidly as the seeds approached maturity.

Microsomes prepared from seeds approximately 120 DAP could support triacylglycerol synthesis from glycerol-3-phosphate, in contrast to mature seeds. Substrate concentration experiments revealed that microsomal preparations contained endogenous diacylglycerol in almost half-saturating amounts for DAGAT. Apparent Km values for palmitoyl, stearoyl, and oleoyl CoA were all in the range of 100 micromolar. When offered separately palmitoyl CoA was the preferred fatty acid donor followed by stearoyl and then oleoyl CoA, however when allowed to select from an equimolar mixture of either palmitoyl and oleoyl or stearoyl and oleoyl CoA, the unsaturated fatty acid was favored. This effect was accentuated at low assay temperatures (15-25°C) but the trend was reversed at higher temperatures (30-40°C). These results may be related to observed lower melting point cocoa butter obtained from seeds grown in cooler climates.

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ALTERATIONS IN THE PLASMA MEMBRANE COMPOSITION OF WHEAT CELLS DURING COLD ACCLIMATION. I.Horváth, Z.Oláh* L.Vigh, Institute of Biochemistry and Biophysics*, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

To gain more detailed information on the molecular mechanism of cold acclimation and freezing injury of plant cells,the chemical composition of plasma membrane fraction isolated from tissue cultured wheat cells (Triticum monococcum L.,cell line TM 1066) was analysed. The degree of fatty acid unsaturation increased markedly during cold acclimation meanwhile the content of free sterols and proteins decreased. As a result of cold treatment the ratio of PC to PE almost doubled. Significant change in the distribution patterns of polypeptides was also demonstrated. Since all of the compositional alterations observed in the plasma membrane are considered to reduce the membrane microviscosity (increase in the degree of unsaturation, phospholipid to sterol and phospholipid to protein ratios, respectively), it tempts us to speculate that fluidity properties of plasma membrane might be involved in cold acclimation. Further studies are needed to clarify which of these modifications are contributed to the development of enhanced freezing tolerance.

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GLYCOPROTEIN NATURE OF LIPOLYTIC ACYL HYDROLASES IN POTATO TUBERS AND LEAVES. Robert A. Moreau, Gerald Nagahashi, and Thomas S. Seibles, Eastern Regional Research Center, 600 E. Mermaid Lane, Phila., PA. 19118

In a recent report (Can. J. Bot 62:1640, 1984) lipolytic acyl hydrolase activity (hydrolysis of p-Nitrophenyl laurate) was shown to copurify with patatin, a glycoprotein which comprises about 20% of the soluble protein in potato tubers. We compared the rates of hydrolysis of PNP-Laurate by patatin with those of 3 other lipid substrates (4 methyl umbelliferyl laurate, C_6-NBD-phosphatidyl-choline, and dipalmitoyl phosphatidylcholine). Greater than 90% of the PNP-laurate hydrolase activity was associated with the patatin fraction, as previously reported. However, with the other 3 assays lower proportions of the enzyme activities were associated with patatin. Patatin was also found to contain other enzyme activities (acid phosphatase and β-N-acetylglucosaminidase). The distribution of enzyme activities in the patatin and non-patatin fractions was found to vary when different cultivars of potato tubers were tested. Since potato leaves also contain high levels of lipolytic enzymes similar experiments were conducted with leaves. In potato leaves the lipolytic enzyme activities were associated with nonglycosylated proteins.

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SUBSTRATE SPECIFICITY OF LIPASES FROM CORN AND OTHER SEEDS. Y.H. Lin, Julie Olsen, R. Qu, Anthony Huang. Biology Department, University of South Carolina, Columbia, SC 29208, USA.

Lipases from several seed species were shown to be relatively specific on triacylglycerols containing the major fatty acid components of the storage triacylglycerols in the same species. In a direct comparison using individual triacylglycerol as well as mixed triacylglycerol preparations, highest activities were observed for corn lipase with trilinolein and triolein, castor bean lipase with triricinolein, rapeseed lipase with trierucin, and elm seed lipase with tricaprin. This pattern of fatty acyl specificity was also observed in diacylglycerols, monoacylglycerols, and fatty acyl 4-methylumbelliferone, although the pattern became less distinct. The seed lipases were inactive with lecithins. Corn lipase was more active with tri- than di- or monolinolein, and released linoleic acids from both primary and secondary positions. As judged from the kinetics of hydrolysis of rac-glyceryl-2,3-stearate-1-oleate and rac-glyceryl-1,3-stearate-2-oleate, and of trilinolein and dilinolein, corn lipase exerted some degree of preference in releasing fatty acid from the primary rather than the secondary position of a triacylglycerol. At the primary position, corn lipase was more active with oleyl ester than stearyl ester.

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REGULATION OF PHOSPHOLIPASE ACTIVITY IN POTATO LEAVES BY PROTEIN PHOSPHORYLATION-DEPHOSPHORYLATION. Robert A. Moreau, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, Pennsylvania 19118

We have recently reported that calcium and calmodulin stimulate the rate of autolysis of phospholipids in potato leaf homogenates (Plant Science 40:95, 1985). The results of the current study indicate that calmodulin also stimulates phospholipase activity (measured with a fluorescent phospholipid analogue, C_6-NBD-phosphatidylcholine) in potato leaves. Because many calmodulin-mediated cellular events are actually controlled indirectly by calmodulin-dependent protein kinases, experiments were also designed to investigate this possible mechanism. Potato leaf phospholipase activity was stimulated by the addition of protein kinase (the catalytic subunit of cyclic-AMP dependent protein kinase) and Mg^{2+}-ATP. Comparable levels of stimulation of phospholipase activity (30-50%) were observed with the addition of either calmodulin or protein kinase. The durations of both types of stimulations were short (20-30 minutes) but could be prolonged by adding sodium fluoride (a phosphatase inhibitor).

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FRAGRANCE COMPOUNDS IN AMBRETTE (*Hibiscus abelmoschus*) SEEDS. M.R. Pollard, S. Jamil-Panah, and T.Y. Nee. Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.

Ambrette seeds contain fragrance compounds which are monoesters. The principal compounds are decyl, dodecyl and tetradec-5-enyl acetates; 14-tetradec-5-enolide, 16-hexadec-7-enolide and 18-octadecen-9-olide (macrocyclic lactone musks); and farnesyl acetate. Preparation of cells from whole seeds suggested that the monoesters are located in the outer integument of the seed coat. However, the cells were not viable, preventing their use in metabolic experiments. The monoesters are formed during the period of embryo maturation. In order to examine their biosynthesis intact, developing seeds were incubated with various [^{14}C] precursors and the labelled lipids analysed. Acetate gave the best incorporation into monoesters, but most of the label was present as farnesyl acetate (both sesquiterpene and acetyl moieties), with very little in the macrocyclic musks. The seed coat outer integument had a high capacity to synthesis acyl lipids, as shown by the incorporation of acetate into palmitate, oleate and linoleate in typical acyl lipids (tri- and di-acylglycerols and phosphatidyl choline). Current evidence suggests that the most likely route of biosynthesis of the macrocyclic musks involves the synthesis of oleoyl lipids, and subsequent utilisation of this oleate pool for the chain shortening, omega-hydroxylation and cyclisation reactions that then produce the musks.

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HYDROLYSIS OF PALM OIL BY PANCREATIC LIPASE AND BY A YEAST LIPASE. H. T. Khor, N. H. Tan and C. L. Chua, Department of Biochemistry, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia.

Palm oil is increasingly being used as a source of dietary fat in many countries. Pancreatic lipase is the key enzyme involved in the digestion of dietary fat. In this study, the hydrolysis of palm oil by pancreatic lipase under optimal conditions was investigated. Our results show that palm oil can be effectively hydrolysed by bovine pancreatic lipase in vitro.

Fatty acids are important raw materials for the oleochemical industry. To expand the end uses of palm oil, the hydrolysis of palm oil by a yeast lipase was investigated. Our results show that the yeast (Candida rugosa) lipase is a suitable enzyme for the hydrolysis of palm oil into free fatty acids.

The kinetics of hydrolysis of palm oil by the two lipases is shown and the potential of using immobilised lipase for the splitting of palm oil is also discussed.

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TRIACYLGLYCEROL BIOSYNTHESIS IN DEVELOPING COTYLEDONS OF SAFFLOWER (Carthamus tinctorius). G. Griffiths[1], S. Stymne[2] and A.K. Stobart[1], [1]Department of Botany, The University of Bristol, Bristol, England, [2]Department of Food Hygiene, Swedish Univeristy of Agricultural Sciences, Uppsala, Sweden.

Triacylglycerol deposition in developing seeds of safflower occurs over a narrow but well defined period during maturation. The utilisation of (^{14}C)glycerol at different stages of development (16-28 days after flowering) has been used to assess the effect of cotyledon age on lipid metabolism. Cotyledons which differed by only a few days in development have different patterns of (^{14}C)glycerol incorporation into complex lipids. The results demonstrate the need for using synchronised and well defined experimental material in studies of triacylglycerol biosynthesis in oil seeds.

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COMPLETE AMINO ACID SEQUENCE OF NON-SPECIFIC LIPID TRANSFER PROTEIN FROM CASTOR BEAN SEEDS. M. Yamada and S. Watanabe, Department of Biology, The University of Tokyo, Tokyo 153 and K. Takishima and G. Mamiya, Department of Biochemistry, National Defense Medical College, Saitama 359, JAPAN

A non-specific lipid transfer protein which carries both phospholipids and galactolipids between subcellular membranes was purified to a single protein. The complete amino acid sequence of this protein was determined by automatic sequencing of Staphylococcus aureus proteinase-digested peptides and tryptic peptides. The protein has 92 amino acid residues, a molecular weight of 9313 and an isoelectric point of higher than 10.5. Sequence microheterogeneity was found at residues 42 and 50, suggesting the occurrence of two genes for this protein or the allelic variation of the same gene. Of 14 acidic and basic amino acids, 12 were located on the latter half of the sequence, suggesting that the former and latter halves of the peptide chain interact with non-polar and polar moieties of the lipid, respectively. The first 20 residues of this protein have a homology (45%) with the residues 2-21 of the spinach non-specific lipid transfer protein. In the amino acid sequence of phosphatidylcholine transfer protein from bovine liver the repetition of β-sheet structure was shown, whereas there is no repetition of such a structure in the castor bean protein. In addition, difficulty of the binding of this protein with the lipid suggests different mechanism of lipid transfer between specific and non-specific lipid transfer proteins.

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PROPERTIES AND *IN VITRO SYNTHESIS OF PHOSPHOLIPID* TRANSFER PROTEINS F.Tchang,F.Guerbette,D.Douady, M.Grosbois,C.Vergnolle,A.Jolliot, J.P.Dubacq and J.C.Kader, Laboratoire de Physiologie Cellulaire (UA 1180) Université Pierre et Marie Curie,4 place Jussieu, Paris,France.

Spinach leaves and maize seeds contain phospholipid transfer proteins able to facilitate an intermembrane transfer of lipids. These proteins have been purified to homogeneity by low-pressure chromatography and, recently, by high performance liquid chromatography. These proteins possess similar properties : low molecular mass (8 - 9 kDa), high pI (around 9) and non-specificity towards phospholipids. In order to study the in vitro synthesis of these proteins, RNA (poly A+) have been extracted from spinach leaves or maize seedlings. After translation with reticulocyte lysate and labelled methionine, newly synthesized protein was detected by high performance liquid chromatography. These experiments open new perspectives in the study of the biogenesis of these proteins.

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GALACTOLIPID SYNTHESIS AND TRANSPORT IN ISOLATED CHLOROPLASTS. J-P Dubacq[1] R Mackender[2] and P Mazliak[1]. Laboratoire de Physiologie Cellulaire, Université P. et M. Curie, 75230 Paris Cedex (France)[1] and Department of Botany, Queens University of Belfast, N. Ireland, U.K.[2]

UDPgal transferase activity has been reported to be localized in the outer chloroplast envelope membrane (OM) in pea (18:3) and in the inner envelope membrane (IM) in spinach (16:3). To examine this discrepancy we have carried out the following experiments: (1) Intact pea chloroplasts were incubated for upto 20 min with UDP-^{14}C- gal and then fractionated to give OM, IM and thylakoid membranes (TM). MGDG and DGDG became labelled in all membrane fractions within 30 secs. The sp. act. of the MGDG in OM > IM > TM and increased throughout the incubation whereas it reached a maximum in about 15 min in the IM. Although the sp. act. of the galactolipids in the TM is low, > 50% of the activity incorporated is present in them within 30 sec. (2) OM, IM and TM fractions have been isolated from unlabelled chloroplasts and analysed for UDPgal transferase and long chain fatty acid acyl CoA synthetase (LFACoA synth) activities and diglyceride content and molecular species. UDPgal transferase activity was present in OM and IM fractions but had a different Km in each. On the basis of LFACoA synth activity < 10% the transferase activity in the IM fraction can be ascribed to OM contamination. TM are devoid of LFACoA synth. activity and almost all transferase activity in the absence of added diglyceride. The localization of galactolipid synthesis and its transport within plastids will be discussed.

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FREE RADICAL OXIDATION AND ALLOMERIZATION OF THE CHLOROPHYLL Merzlyak M.N., Kovrighnyh V.A., Reshetnicova I.V., Gusev M.V. Moscow State University, Faculty of Biology, Moscow, USSR.

The appearance of allomerized products during the chlorophyll interactions with free radicals generated in model systems was observed. Similar products were found after illumination of the isolated chloroplasts and liposomes containing pigments, but were absent in the case of chlorophyll photobleaching in solution. It was shown that allomerization may be initiated by the chlorophyll interaction with O_2^-. Chlorophyll allomerization involved with enzymatic activities of lipoxygenase and peroxydase was demonstrated. It was found that chlorophyll allomerization occured during detached leaves dark aging or after the treatment of plants with SO_2 and diquat. The significance of the free radical reactions in chlorophyll degradation is discussed.

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EVIDENCE FOR DIFFERENT ACYL LIPID DOMAINS IN SPINACH AND OAT THYLAKOID MEMBRANES SUPPORTING VARIOUS PHOTOSYNTHETIC FUNCTIONS.

P.A. Siegenthaler, C. Giroud and J. Smutny, Laboratoire de Physiologie végétale, Université de Neuchâtel, Chantemerle 20, CH-2000 Neuchâtel, Switzerland.

One approach to studying the role of acyl lipids in thylakoid membranes has been to incubate intact thylakoids with lipolytic enzymes and then to determine the relationships between the changes occuring in their lipid content and composition and, simultaneously, in their photochemical activities. Whilst phospholipases hydrolyze specifically the two phospholipids encountered in the thylakoid membrane (PG, phosphatidylglycerol and PC, phosphatidylcholine), lipolytic acyl hydrolases deacylate not only glycolipids (MGDG, DGDG, SQDG; monogalactosyl-, digalactosyl-, sulphoquinovosyl-diacylglycerol) but also phospholipids. The non-specificity of the enzymes used does not allow an unequivocal assessment of function to each of these lipids. Moreover, in most studies, the hydrolysis of acyl lipids have been carried out under conditions allowing the attack of all acyl lipids, i.e. lipids which are localized indistinctly in both monolayers of the membrane. However, it is now well established that all acyl lipids of the thylakoid membrane present a transversal asymmetric distribution, suggesting that different acyl lipid pools may sustain different functions. Thus, it would be of great interest to establish relationships between the hydrolysis of specific acyl lipids in the outer membrane monolayer only and the resulting changes occurring in certain photochemical activities.

To this aim, we have designed conditions under which (1) only PG + PC or MGDG are hydrolyzed in the outer leaflet of the thylakoid membrane; (2) no transbilayer movement of acyl lipids occurs and (3) the deleterious hydrolysis products (e.g. free fatty acids and lysophospholipids) are withdrawn from the membrane by bovine serum albumin. Simultaneously, we have measured several photochemical parameters (various electron transport activities and fluorescence characteristics, photooxidation and dark reduction of cytochrome f, etc.).

Our results show that the selective hydrolysis of certain acyl lipids in the outer monolayer of the thylakoid membrane causes preferential effects on the photochemical activities. These observations give further support on the photochemical activities. These observations give further support to our proposal that acyl lipids are likely to be organized into distinct and descrete pools, each of them having a structural or a functional role in the thylakoid membrane.

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A MEMBRANE LOCATED, CALCIUM-CALMODULIN ACTIVATED PHOSPHOLIPASE STIMULATED BY AUXIN. D. James Morré and Barbara Drobes, Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907 USA

Lipase-catalyzed hydrolyses of membrane phospholipids yield diglycerides, calcium ions and inositol phosphates, all implicated as second messengers capable of amplifying and translating a received hormonal stimulus into a response cascade potentially important to growth control. Our work demonstrates that membrane vesicles freshly isolated from etiolated hypocotyls of soybean contain an auxin-responsive phospholipase activated both by calcium and by calmodulin, all at micromolar concentrations. The response is seen with endogenous phospholipids as measured by loss of radioactivity from membranes prelabeled with [^{3}H]inositol or [^{14}C]choline or with an exogenous phospholipase C substrate, *p*-nitrophenylphosphorylcholine. The optimum response to auxin is about 0.5 to 1 μM with greater or lesser amounts of auxin resulting in a diminished response. The response of the phospholipase to auxin concentration parallels the concentration dependency of the growth response to auxin with excised tissue segments floated on solutions to suggest physiological significance. The response is rapid and stimulated several-fold by auxin-calcium-calmodulin with about 25% of the labeled phosphatidylinositol or phsophatidylcholine of the membranes being cleaved in 10-15 min. Pretreatment of tissue sections with auxin for several hours enhanced rather than reduced the auxin responsiveness of the isolated membranes. Yet, turnover (incorporation) of choline or inositol in situ as a result of auxin treatment of tissue sections was accelerated only 25-30% over 4 h of incubation with 1 μM auxin. While suggested by the cell-free results, endogenous operation of a closed cycle of rapid phosphatidylcholine or -inositol turnover is not supported by the in situ findings as a general response of plant membranes to auxin. Work supported in part by the National Science Foundation PCM 8260222.

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THE ROLE OF LIPIDS IN THE ASSEMBLY AND FUNCTION OF PHOTOSYNTHETIC MEMBRANES Denis J. Murphy, Department of Botany, University of Durham, U.K.

Essentially all non-acyl lipids of photosynthetic membranes, e.g. chlorophylls, carotenoids, xanthophylls, are tightly bound to membrane protein. Circular dichroism studies indicate that the long axis of the tetrapyrolle ring of chlorophyll is orientated at 30° to the bilayer normal, as is the major axis of the trans-bilayer α-helical domains of the chlorophyll-proteins e.g. PSI, PSII. It is proposed that chlorophyll molecules align themselves one to each side of the bilayer, parallel to the α-helical protein domains. This is supported by findings of an approximate 2:1 stoichiometry between chlorophyll and trans-bilayer α-helical protein regions in purified pigment-proteins.

The molecular packing properties of the acyl lipid and protein components of thylakoid membranes have been examined. In the granal regions, protein accounts for 86% of the membrane by wt and the molar ratio of acyl lipid: protein is only 74:1, hence about 70% of the acyl lipid will be adjacent to a protein surface at any given time. ESR studies have confirmed that the vast majority of the PC and PG pools of granal regions are motion-restricted. PC and PG are significantly better reconstituting agents for energy transfer and electron transfer than are the glycolipids. Thus, while most of thylakoid acyl lipid may be obligatorily associated with membrane protein surfaces, it may be that some acyl lipids, e.g. PC and PG, form stronger functional interactions with specific proteins.

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LIPID BIOSYNTHESIS IN DEVELOPING SEEDS OF OILSEED RAPE.
Denis J. Murphy, Department of Botany, University of Durham, U.K.

Microsomal extracts of oilseed rape, Brassica napus L. incorporated oleate from oleoyl CoA into triacylglycerol via a Kennedy-type pathway (G3P→ LPA→ PA→ DG → TG) and/or a phosphatidylcholine (PC) pathway. Only in the latter case was desaturation observed. In the absence of exogenous acyl acceptors, both pathways operated. In the presence of exogenous lyso-PC (10 μM), almost all of the added oleate was incorporated into PC. The relative efficiency of different lyso-PC molecular species as acyl acceptors was 14:0 > 16:0 > 18:1 > 18:0. The oleoyl CoA: lyso-PC acyl transferase exhibited Michaelis-Menten kinetics with respect to oleoyl CoA and was found to have a K_m of 15 μM and a V_{max} of 29.4 nmoles, mg^{-1} protein min^{-1}. In the presence of exogenous lyso-PA (100 μM), virtually all added oleate was incorporated into PA. With equimolar mixtures of exogenous lyso PA + lyso PC most of the oleate was still incorporated into PA. It is proposed that the relative activity of these two acyl transferases may channel fatty acids to TG via either PC or PA and hence determine the acyl quality of the storage oil of rapeseed.

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Ca^{2+} AND INTER-MOLECULAR BRIDGING OF MEMBRANAL PHOSPHOLIPIDS AND PROTEINS.
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Membranal stability, microviscosity ($\bar{\eta}$) and bulk lipid phase state may be controlled by Ca^{2+}. All negatively charged phospholipids (PL's) such as phosphatidylinositol or phosphatidylserine, by virtue of electrostatic crosslinking with the $^{-}O-\overset{|}{P}-OH$ section of the headgroup induce physiologically meaningful structural rearrangements in membranal architecture. Three types of molecular bridging may occur:
1) A multiple PL-PL bonding between PL's themselves [$^{-}O-\overset{|}{P}-O-Ca-O-\overset{|}{P}-O^{-}$] inducing partial separation of charged PL's into discrete domains. At high Ca^{2+} concentrations this causes membrane rigidification.
2) PL-protein binding between the membranal PL's and the carboxyl tails of membrane proteins [$^{-}O-\overset{|}{P}-O-Ca-OOC-protein$] which restricts protein motility.
3) A direct PL-cytoskeleton bridging of identical chemical nature as in '2', or an indirect effect promoting PL-cytoskeleton binding via the γ-glutamyl-ε-lysine bridges which is Ca^{2+}-activated transglutaminase mediated. Such 'anchoring' besides limiting the protein's motility may also cause its deeper insertion within the bilayer and thus less exposure for interaction with effectors on the membrane's exterior.

The acidic nature of several hormones (IAA, GA and ABA) may induce displacement of bridging Ca^{2+}. Low hormone concentrations would decrease membrane rigidity and $\bar{\eta}$ and promote liquid crystalline configurations. At higher ones the Ca^{2+} stabilizing would be lost, anchored protein hormone receptors could be ejected from the bilayers and released Ca^{2+} triggers the "phospholipase-lipoxygenase cascade" resulting in ethylene production and senescence.

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ISOLATION AND CHARACTERISATION OF ENVELOPE MEMBRANES FROM PURIFIED PEA ETIOPLASTS. Jürgen Soll, Botanisches Institut der Universität München, 8000 München, West Germany.

The envelope membrane is a feature common to all members of the plastid family. It is conservatively retained as a two membrane barrier, consisting of inner and outer envelope, at all levels of plastid development, e.g. proplastids, etioplasts, chromoplasts and chloroplasts. Studies on chloroplast envelopes from pea and spinach have revealed its essential role in the proper function of the organelle. No data are available so far on the function of the plastid envelope in chloroplast development, though some initial reports have been published from etioplasts of cereals. These results are difficult to compare with data obtained from dicotyledons. We have therefore developed a method to purify etioplasts from dark grown pea plants and to isolate envelope membranes of these plastids, thus enabling us to compare directly data from different developmental stages.

The results demonstrate that the envelope membrane fraction contains already the enzymes responsible for galactolipid and prenylquinone biosynthesis, as marker enzymes for the inner envelope membrane, as well as a protein kinase and a galactolipid-galactolipid-galactosyltransferase, as marker enzymes for the outer envelope membrane. Analysis of the fatty acid composition of the polar lipids showed a high content of C-14 fatty acids, which were not detectable in envelope membranes from chloroplasts. We have also found significant differences in the carotenoid composition, where envelope membranes from etioplasts contained high amount of antheraxantin. The prenylquinones α-tocopherol and plastoquinone-9 were present in higher concentrations in etioplast envelope than in mature chloroplast envelope. Further data on the enzyme characterisation which is active in lipid and prenyl-quinone biosynthesis is available and will be presented. More detailed analytical results on the lipid pigment composition will also be given.

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GALACTOSYLTRANSFERASE ACTIVITIES IN INTACT SPINACH CHLOROPLASTS AND ENVELOPE MEMBRANE

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Since it is known that GGGT produces not only digalactolipid, but also the artificial higher homologues, tri- and tetragalactosyldiacylglycerol, and because, on this ground, its physiological significance in vivo has raised questions, we have studied the synthesis of galactolipids in isolated chloroplasts, and compared the results with those obtained with envelope membranes. It was found that in intact chloroplasts, the operation of UDGT and GGGT was largely comparable to that seen in isolated envelopes, but that the galactolipid synthesis was limited by diacylglycerol. GGGT can control the rate of galactolipid formation by supplying diacylglycerol. Moreover, the ratio of digalactolipid/(tri- + tetragalactolipid) appeared dependent on the ionic composition of the incubation medium. From these results the physiological significance of GGGT dependent digalactolipid synthesis in vivo is discussed. We believe that the physiological role of this enzyme is not limited to catabolism and autolysis.

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CHARACTERIZATION OF GALACTOSYLTRANSFERASES IN SPINACH CHLOROPLAST ENVELOPE MEMBRANES

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Two galactosyltransferases have been demonstrated unequivocally in chloroplast envelope membranes. UDPGal:diacylglycerol galactosyltransferase (UDGT) and galactolipid:galactolipid galactosyltransferase (GGGT). Both are involved in the galactolipid metabolism of the chloroplast. Specific assays were developed for both UDGT and GGGT, so that the enzymes could be measured separately from each other. The monogalactolipid synthetizing UDGT, located in the inner envelope membrane, was stimulated by magnesium and manganese ions; and could be inhibited rather specifically by UDP and N-ethyl maleimide. GGGT could be localized definitively in the outer envelope membrane. GGGT was strongly stimulated by various divalent and monovalent cations, including calcium, barium, magnesium, iron (II) and manganese ions. Stimulation by divalent cations was abolished by chelating anions. Low concentrations of zinc ions inhibited specifically. The influence of different molecular species of the lipidic substrates for UDGT and GGGT was tested. Mass-conversions of galactolipids in isolated envelope membranes could be followed for both enzymes, using a newly developed method for galactolipid separation and quantification by HPLC. Finally, monogalactolipid synthesis in the absence of UDPGal will be discussed.

Session 5 - Oxygen Requiring Systems -
Oxygenases and Desaturases

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THE LIPOXYGENASE PATHWAY. Brady A. Vick and Don C. Zimmerman, U.S. Department of Agriculture, Agricultural Research Service, Metabolism and Radiation Research Laboratory, Fargo, North Dakota 58105.

Both plants and animals have enzyme systems for the production of oxygenated fatty acids from polyunsaturated fatty acids. In animals the major substrate for oxygenation is arachidonic acid. Two major pathways, known as the arachidonic acid cascade, operate in mammalian systems: (1) the cyclooxygenase pathway that leads to the synthesis of leukotrienes and lipoxins. Most of the mammalian oxygenated fatty acid metabolites have high biological activity. Plants have a similar version of the arachidonic acid cascade, but the substrates are linoleic of linolenic acid, and only the lipoxygenase pathway is known to operate. Lipoxygenase catalyzes the incorporation of molecular oxygen into polyunsaturated fatty acids to form fatty acid hydroperoxides. The fatty acid hydroperoxide products have been proposed to participate in the production of stress ethylene, the regulation of enzyme activity, and the degradation of membranes during senescence. They also serve as a central intermediate in the polyunsaturated fatty acid cascade reactions of plants. At least three enzyme systems diverge from the hydroperoxide intermediate. Hydroperoxide lyase results in the formation of aldehydes and oxoacids. Although the physiological significance of the reaction is not clear, the aldehydes are known to have fungicidal and bactericidal properties. The oxoacids may have a role in the promotion of callous tissue as part of a wound response. Hydroperoxide isomerase produces α- and γ-ketols, whose physiological roles are not understood. Hydroperoxide cyclase leads to the formation of 12-oxo-phytodienoic acid, a prostaglandin-like fatty acid that is further metabolized to jasmonic acid, which has potent growth-regulating properties in plants. Although the physiological role of these pathways is not clear, the powerful biological activity of oxygenated fatty acids in mammalian systems suggests that fundamental knowledge of similar pathways in plants could provide important information about plant regulatory mechanisms.

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ENZYMIC OXYGENATIVE-CLEAVAGE REACTION OF LINOLENIC ACID IN LEAVES. A. Hatanaka, T. Kajiwara and J. Sekiya, Department of Agricultural Chemistry, University of Yamaguchi 753, Japan.

Leaf alcohol, (3Z)-hexenol and leaf aldehyde (2E)-hexenal are widely distributed in fresh leaves, vegetables and fruits, and they are responsible for the green odor characteristics of leaves. These compounds are derived from (3Z)-hexenal which is formed from linolenic acid via 13-L-hydroperoxylinolenic acid by the sequential reactions in tea chloroplasts with lipoxygenase (LPO) and hydroperoxide lyase (HPOL). The (3Z)-hexenal-producing enzyme system (LPO-HPOL) is bound to lamellae of chloroplasts in tea leaves. The membrane bound HPOL is selectively solubilized from the lamellae with Tween 20. Properties including substrate specificities of LPO and HPOL, and distribution in some plants and the seasonal variation of activities for LPO and HPOL are represented. A mechanism for the oxygenative-cleavage reaction is discussed.

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FATTY ACID B-OXIDATION IN HIGHER PLANTS. B. Gerhardt, Botanisches Institut, Universitaet Muenster, Germany.

The classic mitochondrial B-oxidation system has yet to be demonstrated unequivocally for higher plants. Higher plant cells possess an extramitochondrial B-oxidation system located in the peroxisomes. The enzymes involved in fatty acid activation and B-oxidation have been demonstrated in the peroxisomes from a wide range of plant tissues and some of these enzymes have been characterized. Specific features of the peroxisomal B-oxidation system are, among others, an acyl-CoA oxidase which catalyzes the first oxidative reaction and produces H_2O_2, and a bifunctional protein with enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities. The acyl-CoA synthetase is active on long-chain fatty acids, the acyl-CoA oxidase on long- and short-chain acyl-CoAs. The ability of the peroxisomes to produce ^{14}C-acetyl-CoA from ^{14}C-fatty acids has been proven. The peroxisomal B-oxidation system is carnitine-independent.

Substrates for fatty acid B-oxidation result from the mobilization of storage triacylglycerols in the fatty, gluconeogenetic tissues of germinating seeds and may result from membrane lipid and protein turnover in other plant tissues. Some enzymes additionally required for complete degradation of these substrates, i.e. unsaturated, oxygenated and branched-chain fatty acids, have been demonstrated in plant peroxisomes.

The fate of the acetyl-CoA generated by the peroxisomal B-oxidation is only known for the glyoxysomes in which the acetyl-CoA enters the glyoxylate cycle. With respect to the reoxidation of the NADH produced in the peroxisomes during B-oxidation, there exist different models. There are yet other unsolved problems concerning the operation of the peroxisomal B-oxidation system in the cell.

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THE DESATURATION OF FATTY ACIDS ON PHOSPHOLIPID SUBSTRATES. S. Stymne and A.K. Stobart, Department of Food Hygiene, Swedish University of Agricultural Sciences, Uppsala,Sweden and Department of Botany, The University of Bristol, Bristol, England

There is now strong evidence that, in plants, linoleate can be synthesised from oleate whilst esterified to phosphatidylcholine. This desaturation step is localised in the endoplasmatic reticulum of the cell. Linoleate may also be formed in the plastid from oleoyl-monogalactosyldiacylglycerol. Information about the further desaturation of linoleate to alpha-linolenate is scarce. Available data, however, suggests that linoleoyl-phosphatidylcholine in the endoplasmatic reticulum and linoleoyl-monogalactosyldiacylglycerol in the plastid can serve as substrate for the linoleate desaturases. The relative contribution of the galactolipid and phospholipid desaturases may differ considerably between both tissue types and plant species. It is generally believed, however, that, in both photosynthetic and non-photosynthetic tissues, phosphatidylcholine is a donor of polyunsaturated fatty acids to complex lipids.

The concept of fatty acid desaturation with a phospholipid substrate evokes many questions concerned with the regulation of the desaturation and the metabolism of the phospholipid molecule. For instance, why are certain fatty acids desaturated on complex lipids whilst others on an acyl-CoA or an acyl-ACP substrate? How is the level of desaturation controlled when the desaturase enzyme moves within a bilayer of the substrate? Which are the mechanisms that channel acyl groups to phosphatidylcholine for desaturation and how are the desaturated acyl groups made available for the synthesis of other lipids in the cell? Some of these questions will be discussed in light of recent experimental data.

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FATTY ACID SYNTHESIS IN MATURING OILSEEDS. Michael R. Pollard and Sheo S. Singh, Arco Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568.

The biochemistry of lipid deposition in developing oilseeds will be reviewed. Although the entire process from sucrose unloading to triacylglycerol formation will be covered, particular emphasis will be placed on the acyl carrier protein dependent reactions of fatty acid synthesis. Areas where our current understanding is lacking will also be highlighted. These include induction and coordinate regulation of the biosynthetic enzymes during embryo development; key regulatory enzymes and metabolite channelling; purification of membrane-bound enzymes; mechanisms of transfer of acyl groups and lipids between organelles; and molecular genetics. Recent work in our laboratory on the enzymology of fatty acid synthesis will be described. This includes a study of malonyl-CoA and malonyl-ACP decarboxylation reactions. Malonyl decarboxylation activity is high in extracts from developing oilseeds. Experiments are underway to probe whether this activity is associated with the plastid fraction and thus whether it can be considered as an integral part of fatty acid synthesis or not. Work on medium-chain fatty acid biosynthesis will also be described.

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MEDIUM AND LONG CHAIN FATTY ACID SYNTHESIS

J.L.Harwood, Department of Biochemistry, University College, P.O.Box 78, Cardiff CF1 1XL, U.K.

De novo synthesis of fatty acids in algae and plants is catalysed by the combined operation of the acetyl CoA carboxylase and fatty acid synthetase complexes.

Recent successes in attempts to purify the carboxylase and the proteins catalysing the partial reactions of fatty acid synthetase have led to an increase in our knowledge of fatty acid synthesis. The final product of de novo synthesis in different plant tissues varies. This can be due to a premature termination of the chain-lengthening mechanism as well as to the coordinated operation of palmitate elongation and fatty acyl desaturation.

A comparison will be made between the results obtained with preparations of acetyl CoA carboxylase, acyl carrier protein and the various proteins of fatty acid synthetase from different plants. This will include a discussion of enzymic properties, control of the proteins' synthesis and possible regulatory mechanisms. The detailed subcellular localisation of these proteins and the possible implications of this for lipid formation will be discussed.

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Cuticular Lipids in Plant-Microbe Interaction. P. E. Kolattukudy, William F. Ettinger and Joseph Sebastian; Institute of Biological Chemistry; Washington State University; Pullman, Washington 99164-6340.

Cuticle is the first barrier to be penetrated by fungi and the biopolyester, cutin, is the major structural component of the cuticle. It has been shown that infecting fungi use an extracellular enzyme, cutinase, to break the polymeric barrier and that inhibition of cutinase can prevent penetration and thus infection. The mechanism of action of fungal cutinase has been established. The complete primary structure of the enzyme was deduced from the nucleotide sequence of cloned cDNA for cutinase from Fusarium solani pisi and Colletotrichum capcisi and conserved regions of the enzyme are becoming apparent. The mechanism by which the fungal spore senses its contact with the plant surface to induce cutinase synthesis was established. The small amount of cutinase carried by the spore generates monomers from cutin upon contact with the plant surface and the most unique cutin monomers activate transcription of the cutinase gene. Cutinase might also play a role in the interaction of phyllospheric bacteria with plants. A fluorescent Pseudomonas was co-isolated with a nitrogen-fixing Corynebacterium from the phyllosphere. The former can be induced to generate cutinase and thus provide a carbon source from cutin while the latter provides fixed nitrogen for the growth of both. This bacterial cutinase was purified to homogeneity and characterized. Its molecular size and other properties are quite different from those of the fungal enzyme although the catalytic mechanism of the bacterial enzyme is similar to that of the fungal enzyme. The bacterial cutinase gene was cloned in λgt11.

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LONG CHAIN (C20 C30) FATTY ACID BIOSYNTHESIS IN HIGHER PLANTS.
Claude Cassagne, Institute Biochem. et Neurochimie, Universite Bordeaux II, 1 rue Camille - Saint Saens, F-33000 Bordeaux, France.

1. Saturated long chain fatty acids (C20 to C30, LFA), important plant wax components and common constituents of the plasma membrane in higher plants are synthesized in vivo by elongation of a variety of precursors. In vitro LFA synthesis occurs exclusively in membrane fractions ("microsomes"), chiefly in ER. The LFA synthesis requires reduced pyridine nucleotides and malonyl-CoA. Endogeneous substrates may be elongated provided ATP is added. Alternatively, exogeneous saturated acyl CoAs (and not free fatty acids and acyl ACP) or unsaturated acyl CoA are elongated. ACP and acetyl ACP are inhibitors.

The LFAs are released from the elongases as acyl CoAs, and only then are they transferred to polar lipids, chiefly PC.

2. LC and acyl CoA elongases are solubilized by non-ionic detergents. After gel filtration or ultracentrifugation, two acyl CoA elongases may be resolved, the first one elongating exclusively C18 CoA and the second one chiefly C20 CoA. The apparent molecular weights of the detergent elongase mixed micelles are compatible with the occurrence of two non-dissociated multienzymes.

3. If LFA are synthesized by ER in vivo, and not by the plasma membrane (PM), they have to be transferred to their intracellular site of accumulation (PM) before reaching the wax layer. Pulse chase experiments, followed by membrane fractionation and purification, demonstrate the LFA synthesis in, and the intermembrane transfer along, the endomembrane system. The nature of the transfer is discussed.

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How many elongases participate in synthesis of the fatty acyl chains composing epicuticular waxes?

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On plant surfaces are found a wide range of relatively non-polar lipids which can serve as a raincoat. Most epicuticular wax classes are composed of a series of fatty acyl chains having 20 or more carbons among which those having either even or odd numbers dominate. Enzyme systems known as elongases synthesize the very long carbon chains by consecutively adding C_2-units to an intermediate or product of a fatty acid synthetase (fas) or another elongase. Before arriving on the cuticle surface, the resulting chains are usually modified by other enzymes thereby giving rise to various lipid classes. Both elongases and associated enzyme systems are located in the plasma lemma or walls of epidermal cells. Modifications of the biosynthesis of the wax carbon chains by inhibitors, genetic mutation and photoperiodic treatments are best interpreted by the existence of several elongases within a cell. To illustrate: the most clearly differentiated are the barley acyl and 8-ketoacyl elongases. The former uses C_{16} and/or C_{18} fatty acyl chains as substrates for the spectrum of carbon chains composing the alkanes, whereas the latter utilizes primarily a C_{18} 3-oxoacyl intermediate in constructing carbon skeletons of the 8-diketones. Condensing activity of the 8-ketoacyl elongase is part of the multifunctional polypeptide determined by the gene cer-cqu. Analyses of waxes from cer-n mutants not only reveals that the two elongases have factors in common, perhaps analogous to fas and C_{16} elongase, but also that a third elongation system functions in synthesis of the C_{23}-C_{41} alkenes.

Poster Abstracts Pertaining to Sessions 5 - 6

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ACETYL-CoA CARBOXYLASE AND BIOTIN-CONTAINING PROTEINS IN CARROT SOMATIC EMBRYOGENESIS. Basil J. Nikolau, J. Croxdale*, T. H. Ulrich, and E. S. Wurtele. NPI, 417 Wakara Way, Salt Lake City, Utah 84108 and *Botany Dept., University of Wisconsin , Madison, WI 53706.

Carrot cells in suspension culture were induced to form embryos by removal of the auxin from the media. Fractions enriched in globular, heart, and torpedo embryos, and non-embryogenic cells were obtained. Cuticular wax deposits were found on the embryos at all stages of development, but not on the non-embryogenic cells. Associated with the increase in cuticular wax deposition in embryos is an increase in the specific activity of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid biosynthesis. Since acetyl-CcA carboxylase is the only biotin-containing protein known in plants we also examined the abundance of these proteins in the fractionated embryos by a Western blotting procedure using ^{125}I-streptavidin as a probe. The relative abundance of the biotin-containing proteins correlated with the specific activity of acetyl-CoA carboxylase. Most significantly, biotinylated proteins of molecular weights 50,000, 35,000, and 33,000 are present in globular, heart, and torpedo embryos, but are completely absent in non-embryonic cells which contain a 70kDa biotin-containing protein. We have optimised a purification procedure for the 70kDa biotinylated protein using avidin-affinity chromatography and are currently investigating the relationship these four biotinylated proteins have with acety-CoA carboxylase.

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THE EFFECT OF TEMPERATURE ON DESATURATION OF GALACTOLIPID FATTY ACIDS IN BRASSICA NAPUS. J.P. Williams, K. Mitchell and M. Khan. Department of Botany, University of Toronto, Toronto, Canada, M5S 1A1

Brassica napus (a 16:3-plant) has two distinctly different pathways of monogalactosyl diacylglycerol (MGDG) biosynthesis; one involving a cytosolic component, the other contained within the chloroplast. The first pathway results in predominantly 18/18C molecular species, the second in mainly 16/18C molecular species. $^{14}CO_2$-tracer labelling experiments of intact leaf tissue show that radioactivity is rapidly incorporated into 16/18C molecular species and more slowly into 18/18C molecular species. Desaturation of the radioactive fatty acids can be traced by incubation over time. It is, therefore, possible to determine rates of desaturation of fatty acids in the 16/18C group of molecular species if analyses are performed within very short periods of post-feeding incubation.

Results of experiments involving plants grown and incubated at different temperatures have been used to determine the effect of growth temperatures and incubation temperatures on rates of desaturation of fatty acids in the 16/18C group of molecular species.
In plants grown at one temperature, but fed $^{14}CO_2$ and incubated at several temperatures, the rate of desaturation was always more rapid at the higher temperature of incubation. However, plants grown at low temperatures have significantly higher rates of desaturation than plants grown at higher temperatures if fed $^{14}CO_2$ and incubated at the same temperature.

Our results show that the rate of desaturation in MGDG fatty acids is largely dependent on the temperature of growth of the plants.

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IS MONOGALACTOSYL DIACYLGLYCERIDE INVOLVED IN THE PACKAGING OF LIGHT HARVESTING CHLOROPHYLL PROTEINS IN THE THYLAKOID MEMBRANE?
P. Dominy and W.P. Williams, Department of Biochemistry, King's College, London, Campden Hill, Kensington, London W8 7AH, United Kingdom.

The presence of up to 50% of the non-bilayer forming lipid MGDG in the thylakoid membrane has led to the proposal of two hypotheses regarding its biochemical role. One suggests the physical shape of the MGDG molecule aids membrane curvature and it is therefore located primarily at the edges of the granal stacks. The other suggests that MGDG is involved in the efficient packaging of membrane bound proteins into the lipophillic environment.

It is now well established that chlorophyll is associated with the thylakoid-bound light harvesting chlorophyll proteins which constitute a large fraction of the total thylakoid proteins. In this study attempts were made to determine the class of lipid most closely associated with these proteins by inducing chlorophyll photobleaching in thylakoids isolated from Phaseolus vulgaris. Photobleaching results from the production of an activated oxygen species which leads to photooxidation of lipids in the immediate vicinity. Determination of the losses of all lipid classes from thylakoid membranes with increased levels of photobleaching using TLC was not possible due to autooxidation of the samples and interference of the breakdown products. However, it appears that MGDG is more rapidly lost than DGDG suggesting MGDG may be associated with the packaging of chlorophyll proteins. A more detailed analysis of the possible roles of the different lipid classes using HPLC techniques is currently in progress.

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REGULATION OF ACETYL COENZYME A SYNTHESIS IN CHLOROPLASTS. K.-P. Heise and H.J. Treede, Institut für Biochemie der Pflanzen, University of Göttingen, Federal Republic of Germany.

Photosynthetically active chloroplasts of spinach, peas and maize (mesophyll) are capable of synthesizing acetyl-CoA either from acetate via acetyl-CoA synthetase (ACS) or from pyruvate via the pyruvate dehydrogenase complex (PDC). As a possible control mechanism, determining the relative involvement of both pathways within the different types of chloroplasts, the following events are discussed:

1. Variations in the stromal pools of their substrates which in turn appear to depend on their cellular concentrations and on the mechanism of their uptake.
2. Feedback control of the PDC by their end products.
3. Differences in the stromal cofactor levels which, like MgATP, influence both pathways either in a stimulatory or an inhibitory manner.

Nonaqueous fractionation studies support the findings that acetate originates in the mitochondrial and pyruvate in the cytosolic compartment. Moreover the previously controversial general occurrence of the PDC in chloroplasts has been resolved by partial purification of this multienzyme complex from all of the chloroplast types investigated above. Desintegration of the highly labile PDC from spinach chloroplasts has been compensated by reconstitution of its dihydrolipoyl dehydrogenase subcomplex, dissociated during purification procedures.

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RAPID PURIFICATION OF A HIGH MOLECULAR WEIGHT SUBUNIT POLYPEPTIDE FORM OF SPINACH LEAF ACETYL COA CARBOXYLASE. P. Heathcote[1], N.J. Darrell[1] and A.R. Slabas[2]. School of Biological Sciences, Queen Mary College, (University of London), Mile End Road, London E1 4NS[1]. Protein Chemistry Group, Biosciences Division, Unilever Research, Colnworth House, Sharnbrook, Bedford MK44 1LQ[2].

Spinach leaf acetyl coA carboxylase has previously been reported to have a subunit polypeptide ≈ 61,000 daltons (Mohan, S.B. & Kekwick, R.G.O. (1980) Biochem. J., 187, 667-676). However, by using a more rapid purification procedure of ammonium sulphate fractionation and affinity chromatography on monovalent avidin sepharose and blue sepharose a high molecular weight subunit polypeptide has been isolated from rape seed of 220,000 daltons (Slabas, A.R. & Hellyer A. (1985) Plant Science 39, 177-182). This technique has been applied to spinach leaf extract and a 220 000 daltons polypeptide has been isolated. The inclusion of glycerol is necessary to improve stability and the inclusion of standard protease inhibitors is not sufficient to eliminate proteolysis. Analysis by SDS polyacrylamide gel electrophoresis of the final preparation shows virtual homogeneity. Kinetic properties of the enzyme will be described.

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LIPID BIOSYNTHESIS IN EPIDERMAL, GUARD, AND MESOPHYLL CELL PROTOPLASTS FROM LEAVES OF *VICIA FABA* L.

N. Sato, Department of Botany, University of Tokyo, Japan

Protoplasts of epidermal cells, guard cells and mesophyll cells (ECPs, GCPs and MCPs) were prepared enzymatically from leaves of light-grown broad bean plants, and then purified by Percoll step gradient centrifugation. Surprisingly, the ECPs contained small chlorophyllous plastids, which were much smaller than the chloroplasts in GCPs and MCPs.

Comparable rates of the incorporation of [2-^{14}C]acetate into the lipid fraction per cell was obtained for ECP, GCP and MCP. Major labelled lipid classes were PC and FFA in MCP, whereas, in GCP and ECP, TG and PC were efficiently labelled. Although all types of cells could synthesize 16:0 and 18:1, 20:0 and 22:0 were synthesized only in GCP and ECP but not in MCP.

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LIPID PRECURSORS IN PLANT CELLS - BIOSYNTHESIS AND COMPARTMENTATION

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At present, the intracellular origin and compartmentation of the precursor molecules leading to the lipophilic domains of lipid molecules in plants - namely acetyl CoA and isopentenylpyrophosphate - is not definitively understood, and, for this reason, controversial ideas have been developed on this topic (1).

Several hypotheses have been advanced in order to explain the supply with acetyl CoA of the plastidal fatty acid synthesis. One rather favoured conception implies that the plastidal acetyl CoA synthetase activates the acetate anion which derives from the mitochondrion. As an alternative pathway the intraplastidal formation of acetyl CoA via a pyruvate dehydrogenase complex (PDHC) will be presented and the controversial existence of the glycolytic enzymes linking the primary photosynthate 3-phosphoglycerate with the PDHC's substrate pyruvate will be discussed.

Usually, the presence of a glycolytic path in combination with a plastidal PDHC has been considered to be restricted to so-called proplastids. Here, it will be shown that a young plant, the growing and developing mustard seedling, is bearing all the enzymatic activities in question within its cotyledon's chloroplasts.

(1) Liedvogel, B. (1986), J. Plant Physiol. (in the press)

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BIOSYNTHESIS OF SULFOQUINOVOSYLDIACYLGLYCEROL BY ISOLATED CHLOROPLASTS. K.F. KLEPPINGER-SPARACE and J.B. Mudd, ARCO Plant Cell Research Institute, Dublin, CA

Chloroplasts isolated from spinach leaves (Spinacia oleracea L. var hybrid 424) are capable of incorporating $^{35}SO_4^{2-}$ into sulfoquinovosyldiacylglycerol (SQDG), provided that the chloroplasts are intact and illuminated. The synthesis of SQDG by chloroplasts maintained in darkness can be supported by exogenous ATP or by an ATP generating system dihydroxyacetone phosphate (DAP), oxalacetate (OAA), and (Pi). These systems produce less other polar sulfolipids (OSL) as well as SQDG. The production of OSL is greatly diminished in the ATP generating system by the inclusion of Pi. The intact chloroplasts use ^{35}S-APS and ^{35}S-PAPS for the synthesis of SQDG. These reactions require neither illumination nor ATP. They are stimulated by DAP, but the other components of the ATP generating system (OAA and Pi) are not necessary for this stimulation. The function of DAP in this system is not yet clarified. Broken chloroplast systems incorporate $^{35}SO_4^{2-}$, ^{35}S-APS, and ^{35}S-PAPS only poorly into SQDG.

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THE CONTROL OF CTP: CHOLINEPHOSPHATE CYTIDYLYLTRANSFERASE IN PEA STEMS. M.J.Price-Jones and J.L.Harwood, Department of Biochemistry, University College, P.O.Box 78, Cardiff, CF1 1XL, U.K.

Studies on the effects of the plant growth-promoting compound, indol-3-yl acetic acid (IAA) on pea (Pisum sativum L.) stems have shown that within one hour of treatment a decrease of incorporation of [^{14}C]choline into phosphatidylcholine occurred. Measurements of in vitro activities of enzymes in the phosphatidylcholine synthetic pathway and in vivo pool sizes of phosphatidylcholine precursors showed that this decrease in incorporation was due to inhibition of CTP: cholinephosphate cytidylyl-transferase (EC 2.7.7.15) activity which suggested that the cytidylyl-transferase was rate-limiting in the pea stem tissue. Several control mechanisms for the cytidylytransferase have been suggested in animal tissues including aggregation of the cytosolic enzyme (mediated by oleate or acidic phospholipids) and translocation to the endoplasmic reticulum, where it was activated, and a phosphorylation-dephosphorylation cycle. We have investigated these mechanisms in the pea stem tissue together with the possibilities of regulation by alterations in the levels of enzyme protein or allosteric regulation.

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LIPID METABOLISM IN POTATO LEAF DISKS: EFFECT OF CALMODULIN ANTAGONISTS.
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Potato leaf disks were cut and floated on various aqueous test solutions for 0 to 24 hours. At desired time intervals the disks were removed, the lipids were extracted and the levels of phosphatidylcholine (PC) and digalactosyldiglyceride (DGDG) were measured. In the H_2O control very little breakdown (< 4%) of either lipid species was observed during 18 hours in the dark. When 4 common calmodulin antagonists were tested (dibucaine, chlorpromazine, trifluoperazine, and w_7) the rates of breakdown of PC and DGDG were significantly stimulated (17-85% degraded in 18 hours). Among the various inhibitors which were tested, dibucaine was the most potent. Although trifluoperazine stimulated lipid breakdown, trifluoperazine sulfoxide had no effect. This observation is suggestive of an authentic calmodulin interaction rather than a nonspecific effect of the drugs. Curiously, these results are the exact opposite of those we recently observed with potato leaf homogenates (Plant Science 40:95, 1985), where calmodulin antagonists inhibited the rate of membrane lipid breakdown (and calmodulin stimulated it).

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Epicuticular wax formation on needles of *Picea abies and Pinus cembra*.

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Youngest needles of 2 healthy trees at the alpine timberline (*Picea abies, Pinus cembra*) were plucked monthly (July - March). The development of their epicuticular wax was investigated quantitatively and qualitatively. Observations made by SEM suggested, that wax layers of different structure follow each other. Monthly analyses of the wax composition showed the development of the different fractions and that certain components appear only one, two or three months after flushing. At the end of the vegetation period, the following fractions were found in the wax of *Picea abies*: ω-hydroxy fatty acids (HFA), fatty acids (FA), nonacosane-5,10-diol (NDI), nonacosan-10-ol (NON), estolides (EST), short chain esters (SE), 3 ketones (?) (3U), hentriacontan-4-ol (HEN), 2 sec. alcohols (2SA), longchain, unknown hydrocarbons (LHC), p-hydroxy acetophenone (p-HAP), in the wax of *Pinus cembra*: HFA, FA, NDI, NON, EST, SE, 3U. During winter months composition did not change.

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LOCATION AND PROPERTIES OF CHOLINEPHOSPHATE: CYTIDYLYLTRANSFERASE IN CASTOR BEAN ENDOSPERM. A.J. Kinney and T.S. Moore Jr., Department of Botany, Louisiana State University, Baton Rouge.
The reaction catalyzed by CTP:cholinephosphate cytidylyltransferase (E.C. 2.7.7.15) has been postulated to be a control reaction in the synthesis of phosphatidylcholine (PC) in many plant and animal cells. A number of studies utilizing animal tissues have indicated that the enzyme activity is regulated by a translocation of a soluble enzyme to microsomal membranes, where it becomes activated. We have investigated the location and properties of this enzyme in castor bean endosperm. Following sucrose density gradient fractionation, 60-70% of the enzyme activity was associated with the endoplasmic reticulum (ER) fraction. Activity was also observed in another membrane fraction ("band A"), which was possibly a subfraction of the ER. No activity was observed in the soluble fractions. The apparent Km (2.5mM) of the ER enzyme for cholinephosphate was ten-fold higher than the endogenous levels of this substrate (0.19mM). The Km for CTP was 0.22mM, close to the estimated levels of CTP in this tissue. Based on these results we suggest that in plant tissues cytidylytransferase activity is regulated by intracellular CTP levels rather than by cytoplasm-membrane translocation. In castor bean endosperm it is unlikely that CTP is rate-limiting and we suggest that this enzyme does not regulate the rate of PC biosynthesis in this tissue.

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CHOLINE KINASE ACTIVITY IN CASTOR BEAN ENDOSPERM.
A.J. Kinney and T.S. Moore Jr., Department of Botany, Louisiana State University, Baton Rouge.
The reaction catalyzed by choline kinase (E.C. 2.7.1.32 ATP:cholinephosphotransferase) is the first commited reaction of the nucleotide pathway for phosphatidylcholine (PC) biosynthesis. As part of our studies on the regulation of PC biosynthesis in castor bean endosperm we have investigated the properties of this enzyme. The enzyme activity was restricted to the 100,000xg-60min supernatant, which we concentrated by ultrafiltration. The activity was reversible, having a Vmax in the forward direction of 150nmol/h/mg protein and in the reverse direction of 400nmol/h/mg protein. The Km for cholinephosphate (4.0mM) was four times higher than the Km for choline (1.0mM). The Km's for ATP and ADP were 0.29mM and 1.0mM respectively. The endogenous levels of these substrates were measured after freezing the endosperm in liquid nitrogen. From these data we conclude that the forward reaction will be favored in this tissue. The enzyme was inhibited by hemicholinium-3, which also inhibited *in vivo* incorporation of ^{14}C-choline, but not ^{14}C-choline phosphate, into PC. Since the Km for ATP was close to the endogenous levels of this substrate (0.23mM) it is possible that choline kinase activity, and hence PC biosynthesis, may be regulated by the energy charge in some plant cells. However, in developing castor bean endosperm, energy charge, and hence choline kinase activity, was not rate-limiting for PC biosynthesis.

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MOLECULAR SPECIES COMPOSITION OF GRANAL AND STROMAL MEMBRANE LIPIDS. H. A. Norman,[1] J. B. St. John,[1] F. E. Callahan,[2] A. K. Mattoo,[2] and W. P. Wergin,[3] Weed Science Laboratory,[1] Plant Hormone Laboratory,[2] Plant Stress Laboratory,[3] USDA, ARS, Beltsville, MD 20705

We have observed heterogeneity in the molecular species composition of the major lipid constituents of chloroplast membranes. Whole thylakoids of Spirodela oligorrhiza were fractionated into granal and stromal lamellae by a detergent solubilization and differential centrifugation method (Leto et al. 1985, EMBO J. 4:1645-1653). Both fractions were more than 98% homogeneous based on electron microscopic examination. SDS-PAGE revealed that PSII and PSI associated polypeptides were enriched in the granal and stromal membranes, respectively. The relative proportion of mono- and digalactosyldiacylglycerol (MGDG and DGDG) in both granal and stromal membranes was 3:1. MGDG from granal membranes was enriched in molecular species with 18C/16C fatty acids paired in the sn-1 and sn-2 positions, respectively. The ratio of these "procaryotic" molecular species to the 18C/18C "eucaryotic" molecular species was 2.53. The stromal membranes were composed of the same molecular species but in reverse proportions; the ratio of 18C/16C to 18C/18C molecular species was 0.53. Thus, granal lamellae contain predominantly "procaryotic" lipids while stromal lamellae contain predominantly "eucaryotic" lipids.

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PLASMA MEMBRANE AND TONOPLAST FRACTIONS ISOLATED FROM SPINACH LEAVES BY PREPARATIVE FREE-FLOW ELECTROPHORESIS: EFFECT OF PHOTOINDUCTION. Guy Auderset, Anna Stina Sandelius, Claude Penel, Andrew Brightman, Hubert Greppin and D. James Morré, Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907, Laboratory of Plant Physiology, University of Geneva, Geneva, Switzerland and Department of Plant Physiology, University of Göteborg, Göteborg, Sweden.

Membrane fractions enriched in plasma membrane and tonoplast vesicles were isolated from green leaves of Spinacia olearaca L. by preparative free-flow electrophoresis. The identification of the fractions was based on membrane morphology, and on the presence or absence of biochemical markers. The plasma membrane fraction consisted of thick (9-11 nm) membranes that bound N-1-napththylphthalamic acid (NPA), and reacted with phosphotungstic acid at low pH on thin sections by electron microscopy. The tonoplast fraction was enriched in vesicles with 7-9 nm thick membranes that neither bound NPA nor reacted with the phosphotungstic acid at low pH. Both the plasma membrane and the tonoplast fractions were about 90% pure, with a cross contamination of not more than 2%. In leaves of photoinduced plants (24 h light period), the plasma membranes were thicker than in control leaves (8 h light, 16 h dark). The plasma membrane fraction obtained from photo-induced leaves by free-flow electrophoresis retained this increase in thickness. The results show not only that photoinduction alters plasma membrane structure but that the change in stable to isolation. The biochemical basis for the structural modification is under investigation. Supported in part by a grant from the National Science Foundation PCM 8260222.

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MULTIPLE PATHWAYS OF LINOLENIC ACID SYNTHESIS OPERATE AND INTERACT IN LEAF TISSUE. H. A. Norman and J. B. St. John, Weed Science Laboratory, USDA, ARS, Beltsville, MD 20705

A combined genetic and chemical approach established the operation of multiple pathways of linolenic acid (18:3) synthesis in leaf tissue. Synthesis of polyunsaturated galactolipids in Arabidopsis thaliana involved a chloroplastic pathway for production of 18:3 at the sn-1 position of monogalactosyldiacylglycerol (MGDG) utilizing 18:2/16:2 MGDG as substrate. A eucaryotic pathway involving desaturation of 18:2 on phosphatidylcholine served as the source of 18:3 for the sn-2 position of MGDG. The chloroplastic pathway was deficient and the eucaryotic pathway predominated in an Arabidopsis mutant containing reduced levels of unsaturated fatty acids in leaf lipids. The desaturation reactions involved in the chloroplastic pathway were preferentially affected by a substituted pyridazinone known to inhibit fatty acid desaturation. Exogenously-incorporated ^{14}C-18:1 entered the pathway leading to synthesis of procaryotic 18:2/16:2 and 18:3/16:3 MGDG only in the presence of the pyridazinone suggesting that the two pathways closely interact to provide chloroplast membranes with requisite molecular species for maintenance of structure and function.

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INVOLVEMENT OF PHOSPHOLIPID METABOLISM IN SIGNALTRANSDUCTION AND DEVELOPMENT OF PLANTS. E. Hartmann and H. Pfaffmann, Institut für Allgemeine Botanik der Universität Mainz, Saarstrasse 21, 6500 Mainz, Federal Republic of Germany.

Plants show, as all organisms, a broad range of different sensory reactions, and the involvement of different effectors in the regulation of cellular responses corresponding with the transduction of the signal. In contrast to animals, it was in no case possible to solve the complex situation of signal transduction in plants. One reason may be the great ignorance about organization and molecular function of plant membranes. The progress in understanding is increasing in the last years but still slow.

The involvement of membranes in primary reactions of signal transduction in different plant cells and tissues is becoming more and more obviously. A general biological function seems to play the metabolization of special lipids in interaction with membrane localized receptors. Phospholipid turnover is a key for the understanding of signal transduction in animal cells.

In higher plants (bean hypocotyls), and in tissues of lower plants (mosses) an involvement of phospholipid turnover could be detected. An important compound of this metabolism seems to be phosphatidylinositol. The degradation products of this phospholipid play an important role in regulation of animal cell metabolism stimulating protein kinase C activity, and generating calcium release from calcium stores. We found results, which support a comparable function in plant cells. The biochemical characterization, and physiological modulation of a phosphatidylinositol specific phospholipase C (E.C.3.1.4.10) gave additional criteria.

Mosses which contain in contrast to higher plants high amounts of polyunsaturated 20C-fatty acids, like arachidonic and eicosapentaenoic acid, show significant changes in the levels of these fatty acids depending on different stress factors. The question has to be solved if these changes have to do with the uncommon capability of mosses to stand extreme environmental conditions. A good support for this assumption is the quite different composition of the membrane lipids of moss tissue. The presented data underline the role of lipids in signal transduction, and developmental processes.

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LIPIDS OF SOYBEAN INOCULATED WITH MICROSYMBIONTS. R. Pacovsky and G. Fuller, USDA-ARS, Western Reg. Res. Ctr., Albany, CA 94710, U.S.A.

The composition of extractable fatty acids in soybeans that were dependent on microbial symbionts for N and P was compared to plants given inorganic fertilizer. Plants were grown in sterilized soil and were either left noninoculated or were inoculated with Rhizobium, a vesicular-arbuscular mycorrhizal (VAM) fungus, or with both symbionts. Noninoculated plants were given N and/or P fertilizer at rates required to produce plants comparable to inoculated plants. Fatty acids were extracted using hexane:isopropanol (3:2, v:v), converted to methyl esters with BF_3:methanol, and analyzed using gas chromatography.

At week 9, total lipid content of VAM roots was 2.1% (w/w) but only 0.6% in non-VAM roots. Over 40% of this increase in VAM roots was due to the presence of an unusual isomer of palmitoleate, 16:1(7). VAM roots also contained traces of polyunsaturated fatty acids (20:3, 20:4 and 20:5) that were absent in non-inoculated plants. In all treatments α-linolenic acid (18:3) accounted for over 50% of all leaf fatty acid. At week 9, leaves of VAM plants had significantly less 18:3 and proportionately more 18:1 and 18:2, but this difference disappeared by week 15. Pod and seed total lipid and α-linolenic acid (16-18% of total) was highest in the Rhizobium treatments. This indicates that the presence of these root-inhabiting microbes can alter the composition and the total amount of C being allocated into lipid in a manner not duplicated by fertilizer alone.

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PYRUVATE REVERSAL OF S-ETHYL DIPROPYLCARBAMOTHIOATE (EPTC) INHIBITION OF PYRUVATE DEHYDROGENASE COMPLEX.
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Previously, fatty acid synthesis was reported to be inhibited by EPTC in 'aged' redbeet (Beta vulgaris L.) root discs. The inhibition was reversed by 2,2-dichloro-N,N-di-2-propyleneacetamide (dichlormid).

Fatty acid synthesis in wheat (Triticum aestivum L. cv Stacy) chloroplasts was inhibited by EPTC. This inhibition was competitively reversed by [2-^{14}C] acetate and [2-^{14}C] pyruvate. Similarly, dichlormid inhibition of wheat chloroplast fatty acid synthesis was competitively reversed by [2-^{14}C] acetate concentration. Pyruvate utilization by wheat mitochondria was inhibited by EPTC at 10^{-13} (10% inhibition) and 10^{-6}M (90% inhibition). Similarly, 2-oxoglutarate dehydrogenase complex was inhibited 65% by 0.033 μM EPTC.

Combinations of decreased isoprenoid and fatty acid syntheses, light intensity influence on carbamothioate response in wheat and corn, and C_3 v C_4 enzymology explains the differential selectivity and activity of the carbamothioates and antidotes.

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RESPONSE OF SYSTEMIC INSECTICIDES IN LIPID COMPOSITION OF BRASSICA JUNCEA L. SEEDS. P.S. Sukhija, S.K. Munshi and D.R.C. Bakhetia, Dept. of Biochemistry, Punjab Agricultural University, Ludhiana 141004 India

The systemic insecticides - phorate, metasystox and rogor decreased oil content in the developing Raya (Brassica juncea L.) seeds and in the mature seeds, these showed an increase. The relative proportion of triacylglycerols and glycolipids decreased significantly ($P < 0.05$) while that of phospholipids and free fatty acids increased in the developing seeds. In the mature seeds, the proportion of triacylglycerols did not change appreciably from control. The erucic acid synthesis which was less at 10 and 20 DAF (Days after fertilization), increased at 30 DAF with metasystox and rogor; phorate was ineffective. The proportion of erucic acid increased at the cost of linoleic and linolenic acid in the mature seeds. All the insecticides significantly reduced the rate of acetate ($1-^{14}C$) incorporation into lipids both in the in vivo and in vitro studies. In the in vivo experiment (insecticides applied in the field), the synthesis of polar lipids was enhanced at 10 and 20 DAF while that of triacylglycerols decreased. In the in vitro experiment (insecticides added in the incubation medium containing $1-^{14}C$-acetate, 4 μCi), the results were similar as obtained in the in vivo experiment.

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ACYL-CoA ELONGATION SYSTEMS IN Allium porrum MICROSOMES. R. Lessire, J.J. Bessoule and C. Cassagne, Institut de Biochimie Cellulaire et Neurochimie du CNRS, 1 rue Camille Saint-Saëns, 33077 Bordeaux cedex, France.

It has been demonstrated that C_{18}-CoA and C_{20}-CoA are the best substrates for exogenous acyl-CoA elongation, using Allium porrum epidermal cell microsomes as the enzyme source. The effects of detergents (n-octyl-beta-D glucopyranoside, Triton X-100 and deoxycholate) suggested the presence of two different elongation systems. In order to test this hypothesis, the elongation activities were solubilized and the presence of two different elongation systems was demonstrated. The C_{18}-CoA elongases were separated by sucrose density centrifugation as well as by gel filtration on a Sephacryl S-300 column. The purification of the solubilized acyl-CoA elongases was undertaken by using ion-exchange and gel filtration chromatographies.

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ONTOGENETIC VARIATIONS IN THE CHEMICAL COMPOSITION OF MAIZE SURFACE LIPIDS. P. Avato, G. Bianchi and *F. Salamini, Dipartimento di Chimica Organica, Università di Pavia, I-27100 Pavia (Italy) and *Max Planck Institut für Züchtungsforschung, D-5000 Köln 30 (FRD).

Morphological variations of wax surface structures during the ontogeny of many plants have been often observed. Seedlings of maize are typically covered by a wax layer up to the fifth-sixth leaf stage of growth. From there on the leaf surface of wildtype seedlings assumes a glossy appearance, which is maintained throughout the plant life.

Ultrastructural modifications of waxes induced by age are frequently associated with changes in their chemical composition. Thus, comparison of waxes from young seedlings and mature plants of maize revealed well defined differences in the chemistry of their waxes.

Results obtained from the compositional analyses of the epicuticular waxes from maize at the two different stages of growth are the subject of the present contribution. Experimental data will be also correlated with earlier observations on the biochemical pathways involved in the synthesis of maize surface lipids.

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MECHANISM OF BIOSYNTHESIS OF ß-DIKETONES IN WAXES. Giorgio Bianchi, Department of Organic Chemistry, University of Pavia, Italy

Long chain ß-diketones are common constituents of plant surface lipids. They were first isolated in the leaf waxes of Eucalyptus, Acacia and Dianthus caryophyllus species as early as 1962 and have since been identified as classes of compounds of the waxes of cereals and other graminaceous species. In waxes from the Gramineae they are sometimes accompanied by hydroxy ß-diketones, oxo ß-diketones and, in Agropyron species, by hydroxyoxo ß-diketones. Hentriacontan-14,16-dione occurs as the major ß-diketones of cereals and grasses whereas tritriacontane-16,18-dione is the dominant constituent of the ß-diketones from Eucalyptus and Acacia species. The structural pattern of these molecules has provoked the very reasonable suggestion that the biosynthesis of ß-diketones involves condensation of the appropriate ß-ketoesters and esters, via the "biological Claisen reaction". An alternative route has been suggested according to which ß-diketones are generated by an elongation-decarboxylation reaction on a protected ß-ketoacid precursor. Possible mechanisms of formation previously advanced and a new hypothesis are presented and discussed in relationship to feeding experiment data, chemical-genetic evidence and abiological biomimetic synthesis of ß-diketones.

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EFFECT OF LIGHT AND INHIBITORS OF PHOTOSYNTHESIS ON LINOLEATE DESATURATION IN PEA LEAVES. A.N. Grechkin, T.E. Gafarova and I.A.Tarchevsky, Kazan Institute of Biology, USSR Acad. Sci.

There were studied effects of light and of some inhibitors of photosynthesis on the formation of polyenoic fatty acids and molecular species of polar lipids in pea leaves. After incorporation of ^{14}C-18:1 and 18:2 the main labelled molecular species of phosphatidylcholine (PC) were 18:2/18:2; 18:1/18:2 + 16:0/18:2 and 18:3/18:3. Darkness didn't alter the labelling of PC species. In the light the predominant labelled molecular form of monogalactosyldiacylglycerol (MGD) was 18:3/18:3. In the dark formation of 18:3/18:3 and 18:2/18:3 species of MGD was largerly depressed and accumulation of 18:2/18:2 was observed. Specific radioactivity of PC species was higher, than that of correspondent MGD species, even of 18:3/18:3. Diuron and metronidazole didn't affect desaturation of 18:1, but inhibit 18:3 formation by 50 and 80%, respectively. Together with light dependence of 18:3 formation, these results suggest that at photosynthetic conditions desaturation is supported by electron transport from H_2O through the photosystem II and a donor part of photosystem I. These result is consistent with prevously established localization of linoleate desaturase in chloroplast thylakoids.

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LIPID BIOSYNTHESIS IN OIL PALM PROTOPLASTS. Ravigadevi Sambanthamurthi*, K.C. Oo+ and A.S.H. Ong*, *Chemistry & Technology Division, Palm Oil Research Institute of Malaysia. +Department of Biochemistry, University of Malaya.

Protoplasts were isolated from oil palm (*Elaeis guineensis* and *Elaeis oleifera*) mesocarp 16 - 20 weeks after anthesis. Embryoids (*Elaeis guineensis*) at different stages of development were also used as source material for protoplast isolation. Incubation of the protoplasts with ^{14}C-acetate resulted in its incorporation into lipids and water-soluble compounds and the release of $^{14}CO_2$. Incorporation into lipids by mesocarp protoplasts increased with the age of the fruit and was maximum at 20 weeks. The embryoid protoplasts were more active in lipid biosynthesis compared to mesocarp protoplasts and incorporated higher levels of acetate into lipids. Analysis of the lipids showed the presence of all the lipid classes involved. The fatty acid composition of the total lipids showed that lipid biosynthesis had been altered in the protoplasts. A novel fatty acid, palmitoleic acid (C16:1) was identified which accounted for 8-39% of the total fatty acids. The ratio of stearic acid (C18:0) to oleic acid (C18:1) in mesocarp protoplasts varied with the age of the fruit. The results suggest that both C16:1 and C18:1 are formed by a common desaturase whose specificity is altered during protoplast formation.

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ELECTON TRANSPORT AND FATTY ACID DESATURATION, N.W. Lem, Department of Biology, University of Waterloo, Canada.

In higher plant cells, desaturation of fatty acids to produce 18:3 has been characterized as either "prokaryotic" or "eukaryotic" in nature, depending upon the degree of participation of the chloroplast and the cytoplasm. These two compartments have different electron transport systems. Can fatty acid desaturation in a prokaryote, such as Anabaena variabilis, be separated into compartments?

Cells of A. variabilis, grown at 29°C, were pulse-chased with $NaH^{14}CO_3$ and incubated in the light, in the dark or in the presence of chemicals which inhibit either photosynthetic or respiratory electron transport. Preliminary evidence indicated that respiratory electron transport was required for (16:0 and) 18:0 desaturation whereas desaturation of 18:1 to form 18:2 required photosynthetic electron transport.

Supported by NSERC.

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A POSSIBLE DIFFERENTIAL ROLE FOR PLANT ACYL CARRIER PROTEIN ISOFORMS IN HIGHER PLANTS. D. J. Guerra, J. B. Ohlrogge and M. Frentzen.* Northern Regional Research Center, Agricultural Research Service. U.S. Department of Agriculture, Peoria, IL 61604; and *Institut fur Allgemeine Botanik und Botanischer, Garten Universitat (Hamburg, West Germany).

The occurrence of at least two acyl carrier proteins (ACP) in higher plant plastids has raised questions regarding the role of isoform expression in the regulation of fatty acid synthesis (FAS) and metabolism. We have investigated the cosubstrate and coenzyme reactivity of spinach ACP I, ACP II and E. coli ACP in de novo FAS, malonyl-CoA:ACP transacylase (MCT), oleoyl-ACP thioesterase and glycerol-3-phosphate acyl transferase. Both plant ACP isoforms were active in de novo FAS and MCT with only minor differences in reactivity as compared to E. coli ACP. However, the oleoyl-ACP thioesterase possessed a 10-fold lower Km for spinach ACP I as compared to ACP II. In contrast, the acyl transferase possessed a 5-fold lower Km for oleoyl-ACP II as compared to oleoyl-ACP I. Data will be presented which suggest that the expression of plant ACP isoforms may partially regulate the flow of long-chain fatty acid between the CoA reactions and ACP reactions of plant lipid metabolism.

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ACTION OF BORON ON ETHYLENE PRODUCTION AND LIPOXYGENASE ACTIVITY IN MICROSOMES FROM SUNFLOWER COTYLEDONS. A. Belver, M.P. Rodriguez, M. Roldán and J. P. Donaire, Department of Biochemistry, Estación Experimental del Zaidín, C. S. I. C., Granada (Spain).

Ethylene production by microsomes isolated from sunflower cotyledons developed in a medium with 50 ppm of boron, was strongly increased relative to membranes from tissues grown under 10 ppm or without boron. The presence of SHAM, BHT and SOD in the reaction medium provoked a high inhibition of ethylene, mainly in microsomes from cotyledons developed in 50 ppm of boron. A strong increase in ethylene takes place upon incubation linolenic acid with crude microsomes or with a protein fraction -which includes the lipoxygenase activity- obtained by fractionation on Sephadex G50 of solubilised crude microsomes; however, no increase in ethylene was detected upon incubation of this fatty acid with the fraction corresponding to ethylene-forming enzyme, in presence of ACC. The lower capacity of ethylene formation observed in intact cotyledons and in crude microsomes prepared from tissues grown in a medium with 10 ppm of boron, would be explained by the development of a protective system against peroxidative reactions. These finding suggest that boron has an important role in the growth of sunflower cotyledons by regulating the ethylene production derived from lipid membrane degradation.

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MANGANESE INDUCED PEROXIDATION OF THYLAKOID LIPIDS AND CHANGES IN CHLOROPHYLL-a FLUORESCENCE DURING AGING OF CELL FREE CHLOROPLASTS IN LIGHT. **S. Panda, A.K. Mishra and U.C.Biswal, Laboratory of Biochemistry and Biophysics, Sambalpur University, Jyoti Vihar, Burla-768 019, INDIA**

Cell free chloroplasts exhibit a significant level of peroxidation of thylakoid lipids duringaging in light. Manganese at its different oxidative states (Mn^{3+} and Mn^{4+}) modulates peroxidation of thylakoid lipids differently. The ion does not show any significant effect on chlorophyll-a fluorescence in chloroplasts immediately isolated. However, on aging of chloroplasts the ion significantly reduces chlorophyll-a fluorescence. A correlation is established between manganese induced enhancement in thylakoid lipid peroxidation and ion induced quenching in chlorophyll-a fluorescence during aging.

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ACYL LIPID METABOLISM IN RHODOTORULA GRACILIS (CBS 3043) AND THE EFFECTS OF METHYL STERCULATE ON FATTY ACID DESATURATION.

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[2]Cadbury Schweppes PLC., The University, Reading, U.K.

The oleaginous yeast, Rhodotorula gracilis accumulates large quantities of triacylglycerols during N-limited growth. Under such growth conditions, acyl lipids contain high proportions of C18 polyenoic fatty acids, indicating the presence of Δ9, Δ12, and Δ15 desaturase enzymes. ^{14}C-Acetate pulse chase studies suggested that oleoyl-phosphatidylcholine is the probable substrate for Δ12 desaturation in whole cells. However, the simultaneous accumulation of {^{14}C}linoleic acid in phosphatidylcholine and phosphatidylethanolamine during labelling studies with {1-^{14}C}stearoyl CoA in cell free extracts suggested that phosphatidylethanolamine could act as a substrate for Δ12 desaturation in this system.

Preliminary studies on acyl lipid metabolism in the presence of methyl sterculate demonstrated a decrease in the oleic acid content of both triacylglycerols and phospholipids. However, the total linolenic acid content of these acyl lipids increased. Further studies on the inhibition of Δ9 desaturation by sterculate and its effects on linoleate and linolenate formation will be discussed.

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MODULATION OF FATTY ACID SYNTHESIS IN PLANTS BY THIOLACTOMYCIN.

M. Yamada, M. Kato, I. Nishida, K. Kawano and A. Kawaguchi, Department of Biology, The University of Tokyo, Tokyo 153, JAPAN

The antibiotic thiolactomycin (TLM) inhibited both ACP-acetyltransferase and 3-oxoacyl-ACP synthase. In spinach chloroplasts and greening Avena leaves, 18:1 synthesis was more strongly inhibited by TLM than 16:0 synthesis. The addition of TLM to the medium of Pseudomonas aeruginosa M-507, in which anaerobic unsaturation path is operative, resulted in more inhibition for the synthesis of 18:0 and 18:1(11) than that of 16:0 and 16:1(9). These results suggest that TLM is more inhibitory for the elongation of 16:0 to 18:0 than de novo synthesis of 16:0. Pea seeds synthesize fatty acids from 16:0 to 24:0 at early stage of germination. TLM was also more inhibitory for the elongation of 16:0 to 18:0 than for de novo synthesis of 16:0. However, further elongation of 18:0 to 24:0 was less inhibited by TLM with the chain length of fatty acids produced.

When greening Avena and Brassica leaves were incubated with TLM, developed chloroplasts were modulated in respect to lipid composition. The amount of MGDG and PG in both leaves markedly changed: 18:2 and 18:3 in MGDG and 16:1(3t) in PG decreased. However, the amount of PC in both leaves was unchanged. The fatty acid composition of PC in Avena was affected by TLM but not in Brassica.

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A MODEL TO STUDY THE INTRACELLULAR TRANSFER OF LIPIDS TO THE PLASMALEMMA. P. Moreau, H. Juguelin, R. Lessire and C. Cassagne. I.B.C.N. (CRNS), 1 rue Camille St Saëns, 33077 BORDEAUX CEDEX - FRANCE.

In vitro experiments have suggested that very long chain fatty acids (VLCFA) could be transferred from their intracellular site of synthesis (ER) to the plasmalemma. Using seven day-old etiolated leek seedlings, we have determined experimental conditions which allow the study of intermembrane transfer events *in vivo*.

The intermembrane transfer of lipids and particularly of VLCFA from a light membrane fraction (1.08-1.09 g/cm^3) to heavier membranes (1.16-1.18 g/cm^3) has been demonstrated (1).

By means of biochemical markers, it has been determined that plasmalemma is confined to the 1.16-1.18 g/cm^3 fraction which is however relatively heterogeneous. The plasmalemma has been further purified in an aqueous two polymer phase system and a transfer of lipids (PC, PE, neutral lipids) and fatty acids (C16 to C24) to the plasmalemma has been evidenced *in vivo*.

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SOME PROPERTIES OF TOMATO LIPOXYGENASE. P.A. Biacs and H. Daood, Central Food Research Institute, Budapest, Hungary.

Lipoxygenase /E.C. 1.13.11.12/ was extracted from tomato fruits /*Lycopersicon esculentum* var. *ventura*/ by modified procedure. The enzyme activity increased proportionally with the progress of ripening at the last stages. The crude and partially purified enzyme was found to be stable under refrigeration at pH 7 for a week without any remarkable loss in activity. The enzyme resisted heat treatment of 60°C for 5 min., but lost all of its activity at 80°C for 2 min. Kinetic properties, effects of EDTA and reaction products of the enzyme support the suggestion that a true lipoxygenase exists in tomato extract, not other lipid oxidizing agents. The enzyme had an optimum pH at 4.0-4.5 and $K_m = 0.027 \times 10^{-4}$ with linoleic acid substrate. The enzyme activity in the supernatant of 0.6 saturation with /NH_4/SO_4 was 3 times more than in the crude extract indicating the removal of some inhibitor during fractionation process. The fraction of 0.3 - 0.6 saturation was found to have an inhibitory effect on partially purified enzyme.

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RAPID ENZYMIC PEROXIDATION OF POLYUNSATURATED FATTY ACIDS ON HYDRATION OF WHEAT MILLING PRODUCTS. T. Galliard, S.P.C. Tait, R.H.M. Research Ltd., Lord Rank Research Centre, High Wycombe, Bucks. U.K.

The accompanying poster describes the lipolytic activity during storage of wheat milling products that causes accumulation of free fatty acids, approximately 65% of which are polyunsaturated (PUFA). When such materials are hydrated, e.g. in the preparation of food products, the PUFA are oxidised very rapidly, due to the action of lipoxygenase which is concentrated in the germ component of wheat. With wholemeal flour, the major products of this reaction are fatty acid hydroperoxides and the extent of the reaction is usually limited by the availability of dissolved oxygen. This process has several practical implications; e.g. the hydrated material rapidly becomes anaerobic, nutritionally-desirable PUFA are depleted and replaced by hydroperoxides, the nutritional and physiological significance of which is not clear. Also, analysis of lipid peroxidation products in the dry materials (as commonly practised in rancidity testing of food materials) does not identify the incipient oxidative rancidity which is expressed only on hydration.

Session 7 - Interaction of Federal, Industrial and Academic Research

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ACADEMIC AND INDUSTRIAL COLLABORATION: A KEY TO FUTURE SUCCESS. R.L. Sampson, Queensland Innovation Centre Limited, Brisbane, Queensland, Australia.

There is a worldwide explosion of technology and information occuring. About 90 percent of man's entire base of scientific knowledge was developed in only the last 30 years. Importantly, the entire pool of man's science is expected to double from today's level in only the next 10 years. With over 100,000 technical journals published worldwide each year, there are immense problems for scientists and engineers to stay at the forefront of their respective fields of technology.

Like other fields of high technology, biotechnology is changing and advancing at an enormous and ever-increasing rate. This high rate of change is creating new vistas of opportunities for discoveries of immense potential value from both academic and commercial viewpoints. Of particular note, research relating to plant lipids appears to be on the threshold of major developments, which might be of much commercial importance in oleochemical and other markets. Not only do plant lipids offer prospects to provide competitive alternatives to certain petroleum-based chemicals, they also offer promise for providing new materials of value which are not readily attainable from any other source. In net, never before have the prospects for fundamental research in plant lipids offered as much potential for industrial application as they do now.

In most areas of technology and innovation, collaboration between both the public and private sectors, and particularly between academia and industry, is increasing dramatically. This is in part a response to the competitive challenges facing all sectors, organisations and disciplines. To remain competitive, in either research or commercial arenas, immense efforts and investments are required. In an age wherein resources of any organisation are limited, collaboration provides a means to achieve progress which otherwise might not be attainable.

Collaboration between academia and industry poses many complex and difficult issues. Superficially, the objectives of each type of organisation almost seem diametrically opposed to those of the other. Fundamentally different positions on issues such as secrecy versus publication, and knowledge versus profit, must be reconciled. However, increasingly these issues have been found to be not as contradictory as they appear, and ample room has been found for collaboration to occur without compromise of either organisation's objectives. Indeed, combinations of the resources of both academic and industrial organisations are being found to yield complementary, even synergistic, benefits and facilitate achievement of significant intellectual and commercial results.

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THE PALM OIL RESEARCH INSTITUTE OF MALAYSIA — A UNIQUE RESEARCH UNIT.
Professor Augustine Ong, Deputy Director-General, Chemistry and Technology, Palm Oil Research Institute of Malaysia, P.O. Box 10620, 50720 Kuala Lumpur, Malaysia.

In 1979, by an Act of Parliament, the Palm Oil Research Institute of Malaysia (PORIM) was founded for the purpose of conducting and promoting research into the production, extraction, processing, storage, transportation, marketing, consumption and uses of palm oil and oil palm products. In February 1984, permanent headquarters were built on 25 acres of land south of Kuala Lumpur, housing administration, chemistry and technology and biology divisions. A new biotechnology building is now under construction. Present research and future goals will be discussed. Examples will be given to indicate the scope of the research effort and impact of this unique Institute on the oil palm industry.

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BIOTECHNOLOGY OF LIPIDS IN INDUSTRY, R.D. Schmid, Department of Biotechnology, Henkel KGaA, Düsseldorf, W.-Germany

Within the past 10 years, several potentially important contributions of biotechnology to the fats and oils industry have emerged. 1) Tissue culture techniques and molecular genetics might speed up and extend traditional breeding thus providing improved oil crops as well as novel types of oils, 2) Biotransformation reactions using immobilized enzymes or microorganisms might ameliorate unit-operations in oleochemistry (e.g. lipase -catalyzed fat-splitting), give improved access to oleochemicals (e.g. esters), and allow the manufacture of novel specialty chemicals (e.g. biosurfactants). The scope, the economic limitations and aspects of international competition in this field will be discussed.

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UNIQUE CHARACTERISTICS OF CYANOBACTERIAL GLYCEROLIPIDS. N. Murata, Department of Regulation Biology, National Institute for Basic Biology, Okazaki 444, Japan.

The cyanobacterial glycerolipids are characterized as follows: (1) The cyanobacteria contain monogalactosyl, digalactosyl and sulfoquinovosyl diacylglycerols and phosphatidylglycerol as major lipid components, and in addition, monoglucosyl diacylglycerol as a minor one. The monoglucolipid is produced as a precursor of galactolipids in cyanobacteria, and is converted to the monogalactolipid by epimerization at the C-4 of the sugar unit. The thylakoid membrane and cytoplasmic membrane are both active in synthesizing the monoglucolipid from diacylglycerol and UDP-glucose. (2) Some cyanobacteria contain saturated and monounsaturated fatty acids, whereas others additionally contain di- and trienoic acids. Cyanobacteria do not contain (trans-3)-hexadecenoic acid, which is esterified to the sn-2 position of phosphatidylglycerol in chloroplasts. It is likely that cyanobacteria produce only saturated fatty acids in the form of acyl-acyl carrier protein, and that, after these fatty acids are esterified to the lipids, they are desaturated. (3) A decrease in temperature stimulates desaturation of fatty acids which have been esterified to the lipids, and suppresses the de novo synthesis of lipids. An increase in temperature suppresses the desaturation, and stimulates the de novo synthesis of lipids.

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LIPIDS OF DIATOMS AND OF HALOPHILIC DUNALIELLA SPECIES. M. Kates, Department of Biochemistry, University of Ottawa, Ottawa, Canada.

Both marine and freshwater diatom species have been found to contain three novel sulfolipids identified as: 1) Sterol sulfate, 2) deoxyceramide sulfonic acid and 3) phosphatidyl sulfocholine (PSC). The latter is a sulfonium analog of phosphatidyl choline (PC) in which the nitrogen atom is replaced by a sulfur atom; it is present in proportions of 1-25% (or greater) of the PC component. In one of the species examined so far, Nitzschia alba, PSC completely replaces the PC. PSC has also been detected in a Euglena species and may be present in other algae in low proportions. Biosynthesis of PSC in diatoms probably occurs by a route analogous to that for synthesis of PC, in which choline is replaced by sulfocholine.

Polar lipids of both halotolerant and halophic Dunaliella species were characterized by high levels of glycolipids, chiefly mono- and di-galactosyldiacylglycerols and sulfoquinovosyldiacylglycerol and low levels of phospholipids, chiefly phosphatidylglycerol. All Dunaliella species examined also contained major amounts of a zwitterionic non-phospholipid, diacylglycerol-O-trimethyl homoserine, and free fatty acids; neutral lipids were mainly triacylglycerol. Halophilic species of Dunaliella had lower levels of phospholipids and higher levels of glycolipids (including several minor unidentified ones) than the halotolerant species. Both species contained high proportions of 16:0, 16:4, 18:2n6 and 18:3n3 acids and lacked C_{20} or C_{22} polyunsaturated acids.

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THE REGULATION OF MEMBRANE LIPID BIOSYNTHESIS IN DUNALIELLA SALINA. Guy A. Thompson, Jr., Helen A. Norman, and Sung Ho Cho, Department of Botany, University of Texas, Austin, TX 78712.

The unicellular green alga Dunaliella salina contains the same organelles found in higher plant cells. It is, we believe, a useful model system for studying the role of lipid metabolism in regulating the structure and function of the diverse membranes coexisting in plants. Therefore, we have begun a systematic characterization of Dunaliella membrane lipid composition and metabolism, paying special attention to those metabolic reactions which may be most important in the acclimation of plants to environmental stress.

Lipid metabolism in Dunaliella, like other plants, is locallized mainly in the chloroplasts and endoplasmic reticulum. An ongoing dissemination of lipids and lipid precursors between these two major compartments is essential for the normal programmed assembly of membranes. In cells that are stressed by exposure to low temperature, enzymes of the endoplasmic reticulum seem more capable of retaining their activity and altering their specificity in ways that lead to new lipid products, while chloroplast enzymes are more strongly inhibited.

One of the first noticeable changes in lipid metabolism after the cells are chilled is an alteration of phospholipid molecular species composition in the endoplasmic reticulum. Evidence suggests that some of these lipids may be imported by the chloroplast, thus slowly altering its composition also.

The endoplasmic reticulum contains a very active lipid acyl hydrolase that is relatively specific for phosphatidylglycerol and phosphatidylethanolamine. This enzyme requires Ca^{2+} and is regulated by Ca^{2+}-calmodulin. It becomes activated when cells are chilled to low temperature and may account for the observed active metabolism under these conditions.

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STEROL BIOSYNTHESIS AND DISTRIBUTION AND ALGAL PHYLOGENY. Glenn W. Patterson, Department of Botany, University of Maryland, College Park, Maryland 20742.

The wide range of structural types of sterols in algae has been long recognized. Along with the possibility of several biosynthetic pathways, especially in the side chain biosynthesis, these different structures in the various algal taxa provide strong evidence for particular phylogenetic affiliations. As with other photosynthetic plants, all algae examined synthesize sterols through the cycloartenol pathway. During side chain alkylation, however, occurrence of both the 24(28) pathway and the 25(27) pathway have been demonstrated. Unlike in higher plants, 24β-alkyl sterols are dominant in algae, although 24α-alkyl sterols are known in some diatoms. Most of the algal divisions can be easily characterized on the basis of their sterol composition. For instance, the red algae are unique in containing primarily cholesterol or related C-27 sterols. Blue-green algae either contain no sterols or contain only small quantities of sterols. Brown algae contain almost exclusively fucosterol. Green algae are perhaps the most diverse with a very wide range of structural types that suggest phylogenetic relationships. Pyrrophyta contain sterols with extra side chain methyl groups not found in other algae. Chrysophyta appear to be a diverse group with many internal consistencies in sterol composition. As data become more accurate and plentiful, it is clear that sterols can play an important role in determining the relationships between algae and in their evolutionary relationships with other organisms.

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R. Keith Downey. Agriculture Center Research Station, 107 Science Crescent, Saskatoon, Saskatchewan, Canada S7N 0X2.

Combining conventional breeding with fast accurate analytical techniques such as GLC, HPLC, wide line NMR and NIR have made possible rapid increases in oil content and led to the production of entirely new and natural oil compositions in the Brassica oilseeds, sunflowers, safflower, flax and other oilseeds. The market impact of these new oil compositions will be discussed. The application of biotechnologies hold promise for accelerating and extending the range of fatty acid composition within a species.

Conventional plant breeding has also lead to the development of hybrid sunflowers with seed yield advantages over conventional varieties of about 25%. A similar yield advance should also occur in rapeseed/canola as hybrid systems, now under development, are commercialized about 1990. In rapeseed/canola conventional breeding has also eliminated the antinutritional factors in the oilseed meal and introduced cytoplasmically inherited herbicide tolerance. Such examples illustrate the remarkable degree to which oilseeds may be modified to meet market demands.

The application of biotechnologies should permit even greater changes and at an even more rapid pace. However, the promise of biotechnology in crop improvement has yet to be fulfilled and regardless of how successful these new technologies may be, conventional plant breeding systems will continue to serve as the base from which they must build and the outlet for the finished product.

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BIOTECHNOLOGY IN THE IMPROVEMENT OF THE OIL PALM. L. H. JONES
Unilever Research, Colworth Laboratory, Colworth House,
Sharnbrook, Bedford MK43 7HD, U.K.

It is now 10 years since the first clonal oil palms produced from tissue culture were planted in Malaysia. The first clones from unselected seedling stock demonstrated the uniformity of performance and the distinct characters of different clones. We have now developed improved clones from selected palms and are evaluating them for performance against good seedling progenies. Increased yields can be obtained from clonal palms and there are considerable differences between clones in their fatty acid composition. Clonal performance in different environments shows systematic differences in oil composition between Malaysia and Cameroon. Clones are being selected for disease resistance, ease of harvesting, drought tolerance, ability to grow on low fertiliser regimes or give a strong response to fertiliser. Although the primary objective is to maximise grower's returns in terms of maximum oil for minimum cost, the possibility of cultivating clones with improved quality oils is being considered. We are now able to clone palms from hybrids of Elaeis guineesis and E.oleifera with high yield and more liquid, unsaturated oils than normal palm oil. In the future it may become possible to introduce new characters via cell genetics and the use of recombinant DNA technology. At present this is not practicable for the oil palm, but it is becoming a reality in many annual crop species and with improved techniques will become so for oil palm. The major need is to be clear about the objectives of gene manipulation and to identify the genes to be transferred.

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GENETIC ANALYSIS OF THE BIOSYNTHESIS AND FUNCTION OF LEAF LIPIDS IN ARABIDOPSIS THALIANA (L.). C.R. Somerville, P. McCourt, L. Kunst, B. Moffatt and J. Browse*, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824, *DSIR, Plant Physiology Division, Palmerstron North, New Zealand.

The development of facile techniques for the genetic manipulation of plant lipid metabolism would afford many novel opportunities in both basic and applied areas of research. As a step in this direction we have isolated a series of mutants of the small crucifer A. thaliana with specific alterations in leaf fatty acyl composition by quantitative analysis of lipid extracts from several thousand mutagenized plants. On the basis of genetic and biochemical criteria, the mutants have been grouped into six distinct classes. Two classes of mutants appear to have lesions which prevent conversion of phosphatidic acid to diacylglycerol. As a result, these mutants appear to lack the "prokaryotic" pathway of lipid biosynthesis which leads to the synthesis of lipids containing trienoic sixteen carbon fatty acids. The four other classes of mutants appear to be deficient in various fatty acyl desaturase activities. By determining which lipid species are affected by the mutations it has been possible to obtain new insights into the specificity of some of the leaf desaturases, and to obtain estimates of the exchange of acyl groups between the chloroplast and extra-chloroplast compartments. Several of the mutants are extremely cold sensitive whereas others exhibit little or no obvious effect of the changes in membrane lipid composition. Thus, the mutants promise to be useful for examining the functional significance of acyl group composition. The mutants may also be useful in facilitating the cloning of the genes which have been genetically marked by the mutations.

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ACYL CARRIER PROTEIN AS A PROBE OF THE MOLECULAR BIOLOGY OF PLANT FATTY ACID SYNTHESIS. John B. Ohlrogge, Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604.

Because of their relatively simple structure and ease of purification acyl carrier proteins (ACP) are the best understood proteins in plant lipid metabolism. From the studies of ACP we have learned that the organization of plant fatty acid synthesis is more closely related to bacteria than to fungi or animals; that de novo fatty acid synthesis in leaves is localized exclusively in chloroplasts; and that isoforms of fatty acid synthetase proteins are expressed in a tissue specific fashion. Because ACP has now been cloned we can anticipate that our first glimpse into the structure and regulation of plant fatty acid synthetase genes will be available soon. As an adjunct to efforts to obtain cDNA and genomic clones for plant ACP we are also constructing a totally synthetic spinach ACP gene. It is hoped that expression of this gene in E. coli will provide an abundant supply of plant ACP for future enzymatic and structural studies.

Poster Abstracts Pertaining
to Sessions 8 - 9

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LIPIDS OF *CHATTONELLA ANTIQUA* (RAPHIDOPHYCEAE). N. Sato, Y. Nemoto and M. Furuya, Department of Botany, University of Tokyo, Japan

Chattonella antiqua is a unicellular marine species of raphidophyceae, a member of Chromophyta (characterized by chlorophylls a and c), which also includes brown algae, diatoms, etc. As an attempt to use *C. antiqua* as a model organism in studying chromophytan lipid metabolism, we analyzed the composition of lipids and fatty acids of *C. antiqua*.

Lipid classes found were MGDG, DGDG, SQDG, PC, PG, PI, PE, PI, DPG, PS, DGTS (diacylglyceryltrimethylhomoserine) and an unidentified acyl lipid (Lipid D) (major ones are underlined). Fatty acids detected were 14:0, 16:0, 16:1(9), 16:1(3t), 16:2(9,12), 18:0, 18:1(9), 18:2(9,12), 18:3(9,12,15), 18:4, 20:0, 20:4(5,8,11,14), 20:5, 22:0, 22:4, 24:0.

C. antiqua is thus similar to other species of Chromophyta in its high content of 20:5, but distinct from them in that it contains DGTS.

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BIOSYNTHESIS OF LIPIDS IN ACETABULARIA MEDITERRANEA. W. Eichenberger and A. Gerber, Department of Biochemistry, University of Bern, Switzerland.

According to a generally accepted scheme, diacylglycerols (DAG) incorporated into the glycerolipids of green plants are synthesized either by the chloroplast (prokaryotic pathway) or by the cytoplasm (eukaryotic pathway). The cytoplasmic synthesis of DAG involves PC as an intermediate.

Acetabularia mediterranea, like a few other algae, shows a non-typical lipid pattern characterized by the presence of DGTS (homoserine lipid) as an additional component, but of only trace amounts of PC. In order to find out how lipids are synthetisized in this type of plant, the lipid composition was analyzed and labelling experiments were carried out.

The fatty acid pattern consists of C_{16}(33%), C_{18}(59%), C_{20}(5%) and C_{22}(3%) acids which are unequally distributed among different lipids: MGDG mainly contains α18:3 and 18:4 and only minor amounts of C_{20} and C_{22} acids. Based on the positional distribution of C_{16} fatty acids in the glycerol moiety it is concluded that MGDG is mainly synthesized in the cytoplasm, while both DGDG and SQDG are predominantly of chloroplastic origin. DGTS, however, is almost exclusively produced in the cytoplasm and contains considerable amounts of C_{20} and C_{22} fatty acids (30%). On incubation of cells and homogenates with [^{14}C]-glycerol-3-phosphate the labelling kinetics of DGTS resemble very much those of the other glycerolipids suggesting that the glycerol pool is the same for all of the glycerolipids present. 2-[^{14}C]-acetate is incorporated most intensively into DGTS and PG suggesting a special role of these lipids in fatty acid desaturation and/or redistribution.

THE EFFECT OF ENVIRONMENTAL CONDITIONS ON FATTY ACID COMPOSITION OF PORPHYRIDIUM CRUENTUM. Z. Cohen, A. Vonshak and A. Richmond, Algal Biotechnology Laboratory, Jacob Glaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede-Boger Campus 84990, Israel.

The unicellular red microalga Porphyridum cruentum belongs to a small group of algae containing the rare and important fatty acids - arachidonic (AA) and eicosapentaenoic (EPA). The fatty acid composition of P. cruentum appears to be very erratic and cultures that are cultivated under seemingly identical conditions resulted in very different fatty acid compositions.

We have investigated the effects of temperature, light intensity, growth phase and nitrogen starvation on fatty acid composition by cultivation under controlled conditions.

At increasing temperatures, the EPA percent of total fatty acids decreased while that of AA increased. The total fatty acid content also increased with temperature resulting in a steep increase in the AA content (percent of dry weight). At 15°C the AA/EPA ratio was as low as 0.3 while at 35°C a ratio of 2.7 was obtained.

Cultivation under low light intensity resulted in a predominancy of AA (AA/EPA=2.3) while under high light conditions, EPA was the main polyunsaturated fatty acid (AA/EPA=0.75).

Cultures reaching the stationary phase had a high AA content and a high AA/EPA ratio (up to 3.6). Under nitrogen starvation conditions a similar effect was observed. Finally, means for controlling the fatty acid composition in P. cruentum will be discussed and a possible explanation for the apparently erratic behaviour of Porphyridium's fatty acids will be suggested.

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LIPID ACCUMULATION IN SILICON-DEFICIENT DIATOMS. P.G. Roessler, Biotechnology Branch, Solar Energy Research Institute, Golden, Colorado.

Silicon deficiency led to an accumulation of total cellular lipids in the diatoms Cyclotella cryptica and Cylindrotheca fusiformis, but not in Thalassiosira pseudonana. The neutral lipid content of all three species increased by at least two-fold under Si-deficient conditions, however.

The percentage of newly photoassimilated ^{14}C partitioned into lipids doubled after only 4 h of Si-deficiency in C. cryptica cells, while the percentage partitioned into storage carbohydrate (chrysolaminarin) decreased by 50%. A slow conversion of non-lipid materials (mainly chrysolaminarin and other unidentified substances, including low molecular weight compounds) into lipids was also observed. Partitioning of newly assimilated carbon into triacylglycerols also increased in response to Si-deficiency. In addition, pulse-chase experiments with $H^{14}CO_3^-$ indicated that polar lipids were slowly converted to triacylglycerols in Si-deficent cells. These changes were not observed in Si-replete C. cryptica cells.

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LIGHT AND TEMPERATURE EFFECTS ON THE LIPIDS OF TWO MARINE RED ALGAE.

T.R.Pettitt and J.L.Harwood, Department of Biochemistry, University College P.O.Box 78, Cardiff CF1 1XL, U.K.

The lipids of two red macroscopic marine algae, Chondrus crispus and Polysiphonia lanosa, have been quantified and their fatty acid patterns determined. The major lipids in both algae were diglycosyldiacylglycerol, monoglycosyldiacylglycerol, sulphoquinovosyldiacylglycerol and phosphatidylcholine ([^{35}S]- labelling indicated that the sulphur analogue, phosphatidylsulphocholine was probably also present). Incubations with [^{14}C]acetate showed that illumination increased the incorporation particularly into diglycosyldiacylglycerol and phosphatidylglycerol. Illumination increased the relative rate of synthesis of 18:1 (n-9) and 18:3 (n-6). The lipid labelling pattern at optimum growth temperatures (about 15°C as determined by photosynthetic measurements) was compared to that at less favourable temperatures.

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COMPOSITIONS AND POSITIONAL DISTRIBUTIONS OF FATTY ACIDS IN LIPIDS FROM THE DIATOM Phaeodactylum tricornutum. A. Kawaguchi, T. Arao, and M. Yamada, Department of Biology, The University of Tokyo, Tokyo 153, JAPAN

The major fatty acid constituents in the total lipids of the marine diatom Phaeodactylum tricornutum were eicosapentaenoic (20:5), palmitoleic, and palmitic acids. The major lipid components were monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulphoquinovosyldiacylglycerol (SQDG), phosphatidylcholine (PC) and phosphatidylglycerol (PG). Trace amounts of triacylglycerol and phosphatidylinositol were also present. The distribution of fatty acids in the C-1 and C-2 positions of sn-glycerol molecule of the lipids was studied by enzymic hydrolysis with Rhizopus lipase. 20:5 was almost exclusively located at the C-1 position of all lipid components, whereas C16 acids were found at both C-1 and C-2 positions. The C-2 position of MGDG, DGDG, SQDG and PG were essentially occupied with C16 acids. Hexadecatrienoic acid was predominantly located at the C-2 position of MGDG. trans-3-Hexadecenoic acid was exclusively localized at the C-2 position of PG. The C18 acids were abundant at the C-2 position of PC. The positional distribution of fatty acids in PC was different from those of other lipids.

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NILE RED: A FLUOROPHORE USEFUL IN ASSESSING THE RELATIVE LIPID CONTENT OF SINGLE CELLS. K.E. Cooksey, D. Berglund, B. Cooksey and L.R. Priscu. Department of Microbiology, Montana State University, Bozeman, MT, 59717.

A program designed to isolate and characterize oleaginous microalgae has been organized for the Solar Energy Research Institute. The program necessitated the screening of algal cultures for lipid content. We have used the fluorescence of Nile Red - stained cells, to assess the relative ability of various algal species to form neutral lipid under nitrogen limitation. Cells are stained with Nile Red (1-10ug/ml) and analyzed in a Becton Dickinson fluorescently activated cell sorter. The average fluorescence per cell is proportional to the cellular lipid content (r=0.95). The fluorescence fades in 20 min. unless the cells are fixed using formaldehyde (4%). The method shows considerable promise as a non-destructive method for lipid determination on small populations of single cells. Nile Red-stained cells are viable.

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IDENTIFICATION OF A POLYPEPTIDE ASSOCIATED WITH CHANGES IN LINOLENATE CONTENT OF SOYBEAN COTYLEDONS. X. M. Wang, D. F. Hildebrand and G. B. Collins, Dept. of Agronomy, University of Kentucky 40546. U.S.A

The effects of a mutation resulting in low linolenate content and a substituted pyridazinone, San 9785, on the protein synthesis of developing soybean cotyledons have been examined in an attempt to identify the gene products involved in the control of linolenate levels. The nuclear mutant, C1640 (Wilcox and Cavins, TAG '85) had more than a 50% reduction in linolenate content. San 9785 was also found to specifically decrease the accumulation of this fatty acid in soybean cotyledons developed in vitro. The newly synthesized proteins from immature cotyledons of the mutant and control with and without San 9785 treatment were labeled with ^{35}S met and resolved using two dimensional gel electrophoresis. A polypeptide has been found to show distinct differences on 2-D gels in both the low linolenate mutant versus the control and in the cotyledons cultured in the presence and absence of San 9785. This protein was reduced substantially in the linolenate mutant and its synthesis was strongly inhibited by San 9785. This polypeptide appears to have a molecular weight of 100,000 kd and isoelectric point of 5.5. Further study has revealed that this altered protein cosegregates with low linolenate individuals in F2 progeny, suggesting that this polypeptide is associated with the alteration of linolenate level in soybeans. Results of studies on the subcellular localization and the purification and biochemical characterization of this peptide will be presented.

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A PRELIMINARY CHARACTERIZATION OF PLANT HOLO-ACYL CARRIER PROTEIN SYNTHASE. Salah A. Elhussein,* Jan A. Miernyk and John B. Ohlrogge, Seed Biosynthesis Research Unit, NRRC, ARS, USDA, 1815 N. University St., Peoria, IL 61604; *Agricultural Research Corporation, P.O. Box 126, Wad Medani, Sudan.

Holo-ACP contains a 4'-phosphopantetheine prosthetic group donated by coenzyme-A to apo-ACP. The attachment of the prosthetic group is catalyzed by the enzyme holo-ACP synthase. A 2-stage discontinuous assay was developed to measure holo-ACP synthase activity. Apo-ACP was prepared by chemically removing the prosthetic group from E. coli ACP. After incubation of an enzyme preparation from spinach leaves or developing castor oil seed endosperm with apo-ACP, Mg^{2+}, and CoA, the reaction was stopped by heating at 70°. Holo-ACP formed in the first stage of the assay was acylated with ^{3}H-palmitate using E. coli acyl-ACP synthase. The final product was spotted onto discs of DE-81 paper, washed to remove free fatty acids, and the radioactivity counted. The identity of the product was verified by ion-exchange HPLC. Spinach leaf holo-ACP synthase required 0.5 mM CoA and 10 mM Mg^{2+} for maximal activity, and had a pH optimum of 8.0-8.5. We previously observed that spinach leaf ACP was synthesized in vitro as a larger precursor. This observation is consistent with a primary transcript containing a transit peptide necessary for uptake into the chloroplast from the site of synthesis in the cytosol. Preliminary data from cell fractionation studies showed holo-ACP synthase located in the cytosol. We suggest that preACP is synthesized and the prosthetic group added in the cytosol prior to uptake and proteolytic processing in the plastid.

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ACYL CARRIER PROTEIN AS A PROBE OF THE MOLECULAR BIOLOGY OF PLANT FATTY ACID SYNTHESIS. J. B. Ohlrogge, P. D. Beremand, D. J. Hannapel, and D. H. Kuhn.* NRRC, ARS, USDA, Peoria, IL 61604; and *Purdue University, West Lafayette, IN 47907.

Because of their relatively simple structure and ease of purification acyl carrier proteins (ACP) are the best understood proteins in plant lipid metabolism. ACP has now been cloned and we can anticipate that our first glimpse into the structure and regulation of plant fatty acid synthetase genes will be available soon. As an adjunct to efforts to obtain cDNA and genomic clones for plant ACP we are also constructing a totally synthetic spinach ACP gene. The synthetic gene is being constructed from thirteen oligonucleotide fragments of 30-45 base length. A plant codon usage table was constructed from 18 sequenced plant genes and used to assign codons for the amino acid sequence. Direct or inverted repeat sequences containing 7 of 8 matches were identified by computer analysis and eliminated by adjustment of the codon usage. One half of the synthetic gene has been successfully cloned into M13 vectors and sequenced. It is hoped that expression of the entire synthetic gene in E. coli will provide an abundant supply of plant ACP for future enzymatic and structural studies.

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A POSSIBLE DIFFERENTIAL ROLE FOR PLANT ACYL CARRIER PROTEIN ISOFORMS IN HIGHER PLANTS. D. J. Guerra, J. B. Ohlrogge and M. Frentzen.* Northern Regional Research Center, Agricultural Research Service. U.S. Department of Agriculture, Peoria, IL 61604; and *Institut fur Allgemeine Botanik und Botanischer, Garten Universitat (Hamburg, West Germany).

The occurrence of at least two acyl carrier proteins (ACP) in higher plant plastids has raised questions regarding the role of isoform expression in the regulation of fatty acid synthesis (FAS) and metabolism. We have investigated the cosubstrate and coenzyme reactivity of spinach ACP I, ACP II and E. coli ACP in de novo FAS, malonyl-CoA:ACP transacylase (MCT), oleoyl-ACP thioesterase and glycerol-3-phosphate acyl transferase. Both plant ACP isoforms were active in de novo FAS and MCT with only minor differences in reactivity as compared to E. coli ACP. However, the oleoyl-ACP thioesterase possessed a 10-fold lower Km for spinach ACP I as compared to ACP II. In contrast, the acyl transferase possessed a 5-fold lower Km for oleoyl-ACP II as compared to oleoyl-ACP I. Data will be presented which suggest that the expression of plant ACP isoforms may partially regulate the flow of long-chain fatty acid between the CoA reactions and ACP reactions of plant lipid metabolism.

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OIL SEED RAPE ACYL CARRIER PROTEIN (ACP) - PROTEIN AND GENE STRUCTURE. A.R. Slabas, J. Harding, P. Roberts, A. Hellyer, R. Safford, J. de Silva, C. Lucas, J. Windust, C.M. James and S.G. Hughes. Unilever Research, Colworth House, Sharnbrook, Bedford MK44 1LQ,

ACP has been purified from developing seeds of oil seed rape. The sequence of the first 44 amino acids has been determined. The gene has been cloned and its complete nucleotide sequence determined. The results of this investigation will be presented.

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CLONING AND ANALYSIS OF cDNA TO ACYL CARRIER PROTEIN. D. Scherer, D.K. Shintani and V.C. Knauf, Calgene, Inc., Davis, CA.

A cDNA clone encoding the precursor to acyl carrier protein (ACP) was isolated from a spinach leaf cDNA library using oligonucleotide probes. The primary structure of the precursor protein was determined by nucleotide sequence analysis and compared to published ACP amino acid sequences. The 81 carboxy-terminal residues of the precursor presumably comprise the mature protein, based on a 98% homology with the previously published amino acid sequence of spinach ACP-I. The 57 amino-terminal residues represent a transit peptide which has some features in common with other nuclear-encoded chloroplast proteins. The cDNA clone was used to study expression of ACP mRNAs in various spinach tissues and also to determine homology of the ACP cDNA to mRNA from other plant species.

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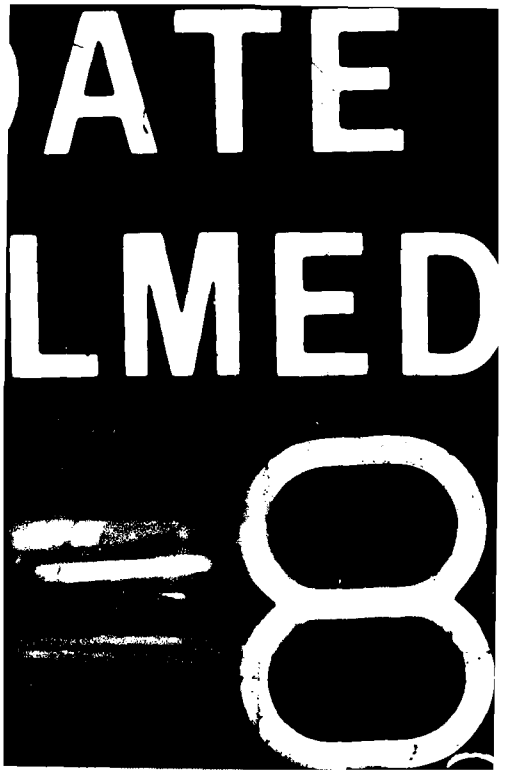
TRANSFORMATIONAL ANALYSIS OF LIPOXYGENASES. D. F. Hildebrand, M. Altschuler, G. Bookjans, G. Benzion, T. Adams, P. Lazzeri, X. Wang, R. A. Andersen, P. D. Fleming, T. R. Hamilton-Kemp, J. G. Rodriguez, C. J. Volden, G. C. Brown, A. G. Hunt, G. B. Collins, and J. C. Polacco, Department of Agronomy, University of Kentucky, Lexington, KY 40546 and Department of Biochemistry, University of Missouri, Columbia, MO 65201.

Lipoxygenase (LOX) is implicated as playing a role in volatile flavor and aroma compound generation and in pest resistance. We have used partial cDNAs for soybean seed LOX-1 and LOX-3 to generate full length cDNAs and to isolate genomic LOX DNAs from soybeans and tobacco. It appears that one 18Kb genomic DNA clone contains part of both LOX-1 and LOX-2. Despite the close linkage, it appears that LOX-1 and LOX-2 mRNA are not under strict coordinate developmental expression. The specific expression of the LOXs in their native state will be presented. Tobacco is being transformed with the cDNA and genomic DNAs in the + and - sense orientation under the control of strong constitutive and inducable promoters using the Ti mediated transformation system. Data for the molecular analysis of the transformants will be presented. Preliminary information on the effects of increased and decreased LOX gene expression of the transformants on volatile compound levels and pest resistance will also be discussed. A transformation/regeneration for soybeans is under development and transfomational analysis of LOXs in a soybean system is also being conducted.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