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THE EFFECT OF DIMETHOATE ON LIPID BIOSYNTHESIS IN OLIVE (*OLEA EUROPAEA*) CALLUS CULTURES

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Key Word Index—*Olea. europaea*; olive; callus culture; glycerolipid; microsomal fraction; dimethoate.

Abstract—Intact olive callus cultures were used to investigate any possible effects which dimethoate, a commonly used organophosphorus insecticide in olive agriculture, may have on lipid synthesis. Dimethoate was applied to the surface of callus cultures and the incorporation of radioactivity from [^{14}C]acetate into acyl lipids monitored. The effect of the insecticide depended very much on its concentration. High levels (up to 500 μM) resulted in overall inhibition of lipid synthesis and in changed patterns of both lipid class and fatty acid labelling. At 10 μM on the other hand, dimethoate had relatively little effect on normal labelling patterns except for a reduction in incorporation into unsaturated fatty acids. The possible relevance of these findings to the agricultural use of dimethoate are discussed.

INTRODUCTION

There have been several recent reviews of the use of plant cell cultures in the study of metabolic pathways [1, 2]. In relation to studies of lipid biochemistry, callus cultures of several oil-producing plant species have been investigated. These include *Arabidopsis thaliana* [3], *Theobroma cacao* [4], oil palm [5] and *Brassica napus* [6]. In comparison to the level of research using cultures derived from oilseed rape and palm, other important oil-producing crops such as olive have been neglected. This has been redressed somewhat with a recent study of lipid metabolism in tissue cultures of *Olea europaea* [7–10]. This work established tissue culture systems for olive and investigated a number of aspects of lipid synthesis including triacylglycerol formation and its regulation.

Alterations in the environment are known to cause several changes in lipid metabolism affecting surface, membrane and storage lipids [11]. The effects of temperature have been well documented and can cause short-term as well as chronic changes [12]. Alterations in temperature can bring about changes in the unsaturation of fatty acids in membrane lipids in order to tolerate the effects of chilling [13, 14]. Xenobiotics can interact with plants, for example, through hydrocarbon pollution or through agriculture where insecticides, herbicides and fungicides are used for control. Several classes of herbicides are known to affect lipid metabolism in plants and some such as thiocarbamates, cyclohexanediones and aryloxyphenoxypropionates have their mode of action based on lipid synthetic enzymes [15].

The insect pest complex threatening *O. europaea* causes losses of some 15% of the harvest, which is equivalent to a cost of about £450 million a year. European growers spend approximately £55 million a year on pest control, half of which relates to pesticide use. In the Mediterranean region there are 18 insect pests known to attack and damage olive trees. The main pests are the olive fruit fly, *Bactrocera* (*Dacus*) *oleae*, the olive moth *Prays oleae* and the olive scale, *Sassieta oleae* [16]. Damage caused by these pests can limit the number and/or size of olive fruits and cause a subsequent reduction in the yield and quality of the resulting fruit and oil.

Organophosphorus insecticides have a wide spectrum of physiological and biological properties [17]. Some are used as fumigants, others as contact poisons and others as systemic compounds. Several organophosphorus insecticides, including dimethoate, are registered for use on olive trees. Dimethoate belongs to the carbamate family of systemic insecticides which are compounds with moderate to high chemical stability. Their oil/water partition coefficients are such that they are able to enter plants and be translocated within them [17]. Dimethoate is used to protect crops such as apples, pears and other top fruits against aphids

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and also with tobacco against aphids and leaf miners. The metabolism of dimethoate in bean plants has been investigated using radiolabelled *N*-hydroxymethyl dimethoate and *N*-desmethyl dimethoate [18]. These compounds have been shown to be formed via the oxidative metabolism of dimethoate [19, 20].

Since dimethoate is chemically related to herbicidal compounds which have known effects on lipid metabolism [15], we decided to investigate whether this widely-used insecticide for olive agriculture could have any effect on olive oil formation. Our test systems were the olive callus cultures which show low levels of polyploid nuclei and good rates of triacylglycerol formation [7]. They, therefore, provided a suitable test system to examine the direct effects of dimethoate *in vitro*.

RESULTS AND DISCUSSION

In a recent study on lipid biosynthesis in olive callus cultures, heterotrophic callus was characterised by its ability to accumulate triacylglycerol, rich in oleate, a situation similar to that in olive fruit [9]. Our studies revealed that the lipid composition of the callus cultures was influenced by both the state of differentiation of the tissue and also by temperature. Moreover, we also incubated callus cultures of both *B. napus* and *O. europeae* with [^{14}C]acetate and found that radiolabel was incorporated into all major lipid classes and that calli grown at a higher temperature could accumulate a greater proportion of triacylglycerol. Palmitate and oleate, the main products of *de novo* fatty acid synthesis were the most highly labelled fatty acids with smaller amounts of radiolabel in linolenate.

In this study, we used the incorporation of radiolabel from [^{14}C]acetate into lipids as a means of investigating possible effects of dimethoate on lipid metabolism. The appropriateness of this precursor for the study of lipid synthesis has been thoroughly discussed by Roughan and Slack [21]. Examination of the cultures used here showed that they differed in lipid composition from those used by Williams *et al.* [9]. The calli used by Williams *et al.* in their experiments were characterised by an ability to accumulate a large proportion of triacylglycerol, namely 32% of the total lipid content. Triacylglycerol made up only 16% of the total lipid of the cultures used in this study, but there was a concomitant doubling in the proportion of diacylglycerol (Table 1). There were also differences in the acyl composition of the two sets of cultures (Table 2) with less oleate and a greater percentage of linolenate in the cultures used in this study. These differences could also be seen in the acyl composition of individual lipid classes (Table 2). These facts imply that there was less accumulation of storage lipids in the cultures used here, a phenomenon seen in other ageing plant tissue cultures [11]. The relatively high proportions of α-linolenic acid in these calli indicate Δ15-desaturase activity, whereas this was apparently very low in the calli prepared by Williams *et al.* [9].

Table 1. The lipid composition of olive callus

Lipid class	% composition (g/g)
PC	16.0±0.4
PG	3.1±0.3
PE	8.0±0.3
PA	14.1±1.8
MAG	1.7±0.4
DAG	23.3±0.4
NEFA	8.4±0.6
TAG	16.1±0.9
Others	9.4±2.7

Data are expressed as means ± standard deviation ($n = 6$).
Abbreviations: PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PA, phosphatidate; MAG, monoacylglycerol; DAG, diacylglycerol; NEFA, non-esterified fatty acids; TAG, triacylglycerol.

Nevertheless, the cultures used for our experiments contained all the typical lipids of plant tissue cultures [22] and showed good viability. Because they also showed significant neutral acylglycerol levels, they were clearly also suitable for studies of fat accumulation. The labelling patterns produced using [^{14}C]acetate are known to reflect the tissue composition of the olive callus cultures, i.e., their development [8]. There was significant labelling of α-linolenic acid (e.g. Table 4), not found in the previous work (8), but that was not surprising given that a large percentage of endogenous α-linolenate (Table 2) was present. In previous experiments (7), triacylglycerol was the most labelled lipid with only relatively low labelling of diacylglycerol. In this study, however, diacylglycerol was the most labelled lipid (see later and Table 6) with less accumulation in triacylglycerol during the incubation period. This again reflected the endogenous lipid composition of the olive calli (Table 1).

Dimethoate has a moderate aqueous solubility, so it was applied to the surface of the callus cultures as a solution in isotonic (330 mM) sorbitol. Initially, 100 μM dimethoate was applied to the surface of the callus cultures and then 48 h later [^{14}C]acetate was added for periods of 24 h (Table 3) or longer. Excellent levels of incorporation of radiolabel into chloroform-soluble material were achieved but there was no statistically significant difference in the total labelling of lipids between the control and insecticide-treated calli. There was, however, a difference in the appearance of the calli following treatment in that there was a distinct browning of the tissue in dimethoate-treated cultures. Moreover, there was a difference in the pattern of labelling of fatty acids on treatment with dimethoate (Table 3) in that there was a statistically significant decrease in the relative labelling of oleate. This change was mainly associated with an increase in linoleate suggesting that Δ12-desaturation could

Table 2. The acyl composition of the major lipid classes of olive callus

Lipid class	Acyl composition (%)				
	16:0	18:0	18:1	18:2	α-18:3
Total	23.0±2.2	5.0±2.1	30.1±2.7	12.2±2.1	29.6±3.6
PC	25.2±3.1	3.9±1.0	36.8±4.3	9.6±2.1	24.7±3.3
PG	38.8±3.6	2.5±1.0	31.4±2.3	5.3±1.5	22.0±3.0
PE	27.3±3.1	3.7±1.4	28.7±1.0	11.2±1.2	29.0±4.0
PA	20.2±1.4	4.1±2.0	34.0±3.4	12.2±2.5	29.6±1.1
MAG	32.6±5.0	5.4±1.5	35.4±1.3	6.7±3.2	13.0±3.9
DAG	30.7±2.5	3.9±1.9	29.7±3.1	8.9±2.6	26.9±4.6
NEFA	31.1±6.2	6.4±3.3	45.5±6.7	5.0±0.9	12.2±3.6
TAG	30.9±3.6	6.1±1.5	35.1±3.1	6.1±1.8	21.9±2.3

Data are expressed as means ± standard deviation ($n = 7$).
Abbreviations: see legend to Table 1 and 16:0, palmitate; 18:0, stearate; 18:1, oleate; 18:2, linoleate; 18:3, α-linolenate.

Table 3. The effect of dimethoate on the incorporation of radioactivity from [^{14}C]acetate and the relative labelling of fatty acids in olive callus cultures

Dimethoate	Incorporation (% uptake)	Fatty acid labelling (% total)				
		16:0	18:0	18:1	18:2	α-18:3
0 μM	57.0±8.5	29.1±0.5	4.7±1.3	44.9±1.9	13.2±2.4	8.2±0.2
100 μM	89.5±18.5	34.9±6.6	3.8±0.3	36.1±0.7*	20.4±5.7	4.9±1.8

Olive callus cultures (approximately 1.0 g fr wt) were incubated with dimethoate for 48 h and followed by 1 μCi [^{14}C]acetate for 24 h.
Data are expressed as means ± standard deviation ($n = 3$).
Statistical significance (Student's t-test) * $P < 0.05$.
For fatty acid abbreviations see Table 2.

have been increased. On the other hand, an increase in palmitate labelling suggested that dimethoate may be affecting the activity of KAS II, the condensing enzyme responsible for the chain-lengthening of palmitate to stearate. However, because of variability in individual callus samples, the effects of fatty acids other than oleate were not statistically significant in the experiment shown (Table 3).

Olive calli were then incubated with a higher concentration of dimethoate (500 μM) simultaneously with [^{14}C]acetate. The result was very dramatic in that the treated calli rapidly turned brown in colour after only 24 h, as if senescence was taking place. The incubation was stopped at this point and analysis showed that the level of incorporation of radiolabel into lipids was severely reduced (Table 4). The pattern of fatty acid labelling was drastically affected (Table 4) with a decrease in the amount of label in oleate as found above (Table 3). In this case, however, the biggest differences were not in the relative labelling of palmitate or linolenate, but the appearance of substantial labelling of normally minor hexadecenoate (provisionally identified by GLC as palmitoleic acid) (16:1). This data indicates that 500 μM dimethoate may be inhibiting the β-ketoacyl-ACP-synthase II (KAS II) of fatty acid synthase. Such an action would increase the availability of palmitoyl-ACP for chloroplast Δ9-desaturation and, hence, increase the radiolabel in palmitoleate.

Lower concentrations of dimethoate (10 μM and 50 μM) were tested to see whether the above effects were also seen at lower concentrations. These concentrations were applied simultaneously with [^{14}C]acetate for up to 96 h. As expected from the results with 100 μM dimethoate, there were no differences on the total incorporation of radiolabel into lipids (Table 5). As a result of treatment, however, there were some differences in the relative labelling of lipid classes. There was a relative decrease in the percentage labelling of phosphatidylcholine (Table 6), although this was only statistically significant at 10 μM dimethoate. In addition, there was an increase in the relative labelling of diacylglycerol (Table 6) together with a decrease in the labelling of non-esterified fatty acids. Diacylglycerol is used for the biosynthesis of phosphatidylcholine and the change in their relative labelling implies that dimethoate may in someway impair this process. The build-up of diacylglycerol did not

Table 4. The effect of a high concentration of dimethoate on the simultaneous incorporation of radioactivity from [^{14}C]acetate and the relative labelling of fatty acids in olive callus cultures

Dimethoate	Incorporation (% uptake)	Fatty acid labelling (% total)					
		16:0	16:1	18:0	18:1	18:2	α-18:3
0 μM	82.0±1.	46.0±3.1	n.d.	15.6±4.3	29.9±3.5	5.7±0.9	2.6±0.3
500 μM	4.0±0.4†	42.9±2.8	19.8±4.5†	6.2±4.2	16.0±6.2	10.8±6.5	4.4±0.4*

Olive callus cultures (approximately 1.0 g fr wt) were incubated with dimethoate and 1 μCi [^{14}C]acetate simultaneously for 24 h.

Data are expressed as means ± standard deviation ($n = 3$).

Statistical significance (Student's t-test) * $P < 0.05$, † $P < 0.01$.

For fatty acid abbreviations see Table 2.

Table 5. The effect of dimethoate on the simultaneous incorporation of radioactivity from [^{14}C]acetate and the relative labelling of fatty acids in olive callus cultures during extended incubations

Dimethoate	Incorporation (% uptake)	Fatty acid labelling (% total)				
		16:0	18:0	18:1	18:2	α-18:3
0 μM	60.0±0.1	32.4±1.0	4.4±0.6	31.3±0.9	16.3±0.7	15.8±0.1
10 μM	59.2±0.1	32.5±3.5	9.5±2.3*	28.9±11.2	15.7±5.7	13.4±8.6
50 μM	58.4±0.1	34.9±0.2	10.5±2.4*	26.9±3.9	14.2±3.0	13.5±3.5

Olive callus cultures (approximately 1.0 g fr wt) were incubated with dimethoate and 1 μCi [^{14}C]acetate simultaneously for 96 h.

Data are expressed as means ± standard deviation ($n = 3$).

Statistical significance (Student's t-test) * $P < 0.05$.

For fatty acid abbreviations see Table 2.

Table 6. The effect of dimethoate on the relative labelling of lipid classes from [^{14}C]acetate in olive callus cultures

Lipid class	Control	Dimethoate (10 μM)	Dimethoate (50 μM)
PC	16.9±3.7	8.9±0.6*	10.1±2.7
PG	1.6±0.5	2.7±1.0	2.8±0.7
PE	8.6±1.9	5.8±1.2	7.1±1.0
PA	11.0±2.4	12.2±1.3	14.7±1.5
MAG	1.0±0.4	1.8±1.5	1.0±0.7
DAG	23.1±2.8	33.1±5.6	31.2±1.9†
NEFA	12.3±0.3	9.1±5.2	7.0±2.5*
TAG	13.7±0.3	13.5±2.4	12.9±1.0
Others	11.9±2.4	12.9±1.6	13.2±3.2

Data are expressed as means ± standard deviation ($n = 3$).

Statistical significance (Student's t-test) * $P < 0.05$, † $P < 0.02$.

For labelling conditions see Table 5.

For lipid abbreviations see Table 1.

result in increased triacylglycerol labelling, again agreeing with the conclusions made above that, in the cultures used for the present work, diacylglycerol acyltransferase was somewhat limiting.

The pattern of total fatty acid labelling (Table 5) showed little change caused by the lower concentration of dimethoate. A change in desaturation at the C-18 level was again seen but, in this case, a significant rise in stearate labelling was found. With regard to the fatty acid labelling pattern in the major lipid classes, there were several significant differences in the labelling pattern of phospholipids and diacylglycerol (Table 7). There were significant decreases in the percentage labelling of linolenate in all three

Table 7. The effect of dimethoate on the fatty acid labelling patterns of major lipid classes in olive callus

Lipid class	Dimethoate	Fatty acid labelling (%)				
		16:0	18:0	18:1	18:2	α-18:3
PC	0 μM	32.3±2.7	9.0±4.3	32.3±3.5	9.4±2.4	17.1±2.6
	10 μM	33.8±3.3	11.6±0.5	32.1±3.7	9.7±4.6	12.8±2.7
	50 μM	38.8±2.5*	8.0±2.3	35.5±1.7	7.8±3.2	9.9±3.4*
PE	0 μM	26.5±1.9	6.6±2.4	37.8±1.4	9.7±1.2	19.5±2.1
	10 μM	32.6±5.5	10.3±1.7	29.3±4.2*	11.3±0.8	16.5±2.9
	50 μM	30.2±3.0	6.7±0.4	33.3±3.0	15.8±4.0	14.0±2.1*
PA	0 μM	25.8±2.4	9.2±1.8	38.3±1.8	12.2±3.1	14.5±2.3
	10 μM	33.8±1.3§	12.5±0.7*	34.7±5.8	9.6±2.4	9.1±2.4*
	50 μM	45.9±2.2‡	8.7±3.4	24.9±3.0§	10.7±2.6	9.8±3.1
DAG	0 μM	27.1±1.1	6.1±1.9	29.3±1.6	19.2±2.4	18.3±1.7
	10 μM	39.6±1.7‡	9.4±3.1	26.9±9.0	1.6±6.1	10.5±6.3
	50 μM	38.2±1.7‡	8.2±0.3	28.0±2.7	9.6±0.3‡	16.0±0.6
NEFA	0 μM	35.0±2.3	21.5±8.1	34.0±6.8	4.7±2.9	4.9±3.2
	10 μM	32.9±3.7	23.3±6.3	33.0±8.5	6.5±3.0	4.3±3.5
	50 μM	36.7±4.2	32.4±1.8	21.7±4.7	4.0±1.5	5.2±1.9
TAG	0 μM	32.8±9.6	18.3±5.5	28.2±8.4	11.7±8.0	9.1±5.6
	10 μM	26.7±4.1	24.4±4.0	29.1±3.5	9.1±2.5	11.0±2.2
	50 μM	40.6±5.0	22.2±6.2	21.9±1.3	8.6±5.8	6.8±4.6

Data are expressed as means ± standard deviation ($n = 3$).
Statistical significance (Student's t-test) * $P < 0.05$, § $P < 0.01$, ‡ $P < 0.001$.
For labelling conditions see Table 5.
For abbreviations see Tables 1 and 2.

phospholipids shown and also in the proportion of labelling of oleate in phosphatidylethanolamine and phosphatidate. In diacylglycerol, the only statistically significant decrease was in the labelling of linolenate at 50 μM. These changes were accompanied by an increase in the relative labelling of palmitate in most cases, especially for treatment with 50 μM dimethoate.

These data, taken together with results for fatty acid labelling from other experiments (Tables 3–5), indicate that dimethoate is capable of altering patterns of labelling in the callus cultures. Changes in the relative labelling of unsaturated acids and the proportion of radioactivity in C_{16} products indicate that fatty acid desaturases and β-ketoacyl-ACP synthase II are the usual targets.

Previous work [23] has established that microsomal fractions from olive callus cultures are an excellent *in vitro* system for studying glycerolipid synthesis. Investigating the effect of dimethoate on such microsomal incubations revealed that the insecticide had little direct effect on the enzymes responsible for the Kennedy pathway to triacylglycerol or other associated activities (results not shown).

The results described here indicate that dimethoate can affect lipid biosynthesis in various ways. At very high concentrations, lipid synthesis is severely reduced, while at the 10–100 μM level there are changes in the quality of lipids made. The alterations in total fatty acid patterns, especially the reduction in the percentage of oleate, seems especially relevant in view of the high level of this acid in olive oil [24]. Thus, in theory, it is possible that dimethoate applications could lead to reductions in olive oil production and also its quality, although pesticide residues in the mature olives or their oil do not seem to be detectable [25]. However, it is important to consider whether concentrations of dimethoate ever reach the levels used in the above experiments when the insecticide is utilised in olive groves. Evidence on this point is equivocal. In one study [26], dimethoate treatment appeared to affect olive development and olive oil quality. However, in a more careful follow-up experiment, no significant effects were observed [27]. Furthermore, residue levels of insecticides rapidly decline on olive [28] such that it seems doubtful that they could cause lasting disturbance to olive oil accumulation. Nevertheless, the fact that a commonly-used insecticide can cause changes in the metabolism of the relevant crop, suggests that choice of a pesticide may need to be made not only with regard to efficacy against the target pest(s) but also any unanticipated side-effects. Where such side-effects are found *in vitro*, they will have to be evaluated carefully with regard to their relevance to field situations.

EXPERIMENTAL

Materials

Olive (*O. europea* L., Picual) fruits were obtained from twenty-year-old trees growing near Seville, Spain. During the growing season olive fruits were

picked and sent by air to arrive in Cardiff 3–4 days later.

Generation of olive callus cultures

Olive fruits were obtained approximately 120 days after flowering. The pericarp was removed from the fruit and broken. The kernel was surface-sterilized with 4% (w/v) sodium hypochlorite for 20 min and allowed to imbibe in cold running water for 6 h. The kernels were then rinsed in sterile water and germinated aseptically at 25°C. Etiolated embryos were dissected so that the cotyledons were cut transversely into two basal and two apical pieces. The individual segments were incubated on Murashige and Skoog medium [29] supplemented with 12 μM 2,4-dichlorophenoxyacetic acid and 0.56 μM benzylaminopurine riboside. The cultures were incubated at 25°C or 35°C with illumination (20 μE m^{-2} s^{-1}) and a 12 h light/dark cycle. The resultant callus cultures were routinely sub-cultured at 28 day intervals.

Incubation of olive callus cultures with [^{14}C]acetate

[$^{1\text{-}14}$C]Acetate (37kBq/100 μl) (in 330 mM sorbitol) was aseptically added to the surface of callus cultures. Incubation conditions are detailed in the legends of Tables for individual experiments. At the end of the incubation period, the calli were washed in 300 mM sorbitol to remove excess radiolabel.

Lipid extraction

Olive callus tissue was rinsed to remove unincorporated radiolabel and then homogenised in 1.25 ml hot *iso*-PrOH (to inactivate lipases) using a pestle and mortar. After heating at 70°C for 30 mins, the homogenate was cooled and 1 ml distilled water, 1.25 ml MeOH and 1.25 ml $CHCl_3$ were added. The mixture was allowed to stand at room temperature for 15 min and lipid extraction continued by the addition of a further 1.25 ml $CHCl_3$. Non-lipid contaminants were removed by vigorously shaking the mixture with 1.25 ml Garbus solution (2M KCl in 0.5M phosphate buffer pH 7.4) [30]. The upper phase was carefuly removed and the lower chloroform phase, was filtered through glass wool which was then washed with chloroform : methanol (2 : 1, v/v). An aliquot of the upper phase was retained for the determination of total incorporation of radioactivity from the precursor. The combined lower phase and washings, containing lipids, was used for further analysis.

Lipid analysis

Pre-coated 0.25 mm thick silica gel G plates (Merck) were activated before use for 1 h at 110°C. Polar lipids were separated using $CHCl_3$/MeOH/HOAc/H_2O (170 : 30 : 20 : 7, by volume) as the solvent. Neutral lipids were separated using petrol (60–80 bp)/Et_2O/HOAc (80 : 20 : 2, by volume) as the solvent. For lipid extracts obtained from microsomal incubations, both polar and neutral lipids were separated on a single plate. Initial separation was carried out in the polar system when the solvent front was taken to 5 cm from the top of the plate. The separation was completed in the neutral solvent system. Fatty acid methyl esters (FAMEs) were analysed using a Pye Unicam GCD gas chromatograph connected via an effluent splitter to a LabLogic RAGA (Sheffield, U.K.) gas flow proportional counter. An internal standard of heptadecanoic acid was used for quantitation. The effluent splitter ratio between the flame ionisation detector (f.i.d.) and the radio-detector was approximately 1 : 6. The FAMEs were separated using 10% SP-2330 on 100/120 mesh Supelcoport (Supelco), at 180°C. Individual radiolabelled and mass peaks were integrated using the Rachel software package (LabLogic, Sheffield).

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REFERENCES

1. Rhodes, M. J. C. and Robins, R. J., in *The Biochemistry of Plants* Vol. 13 ed. D. D. Davies. Academic Press, New York, 1987, pp. 65–125.
2. Weber, N., Taylor, D. C. and Underhill, E. W., *Adv. Bioch. Engin. Biotech.*, 1992, **45**, 99.
3. Brockman, J. A., *Phytochemistry*, 1990, **29**, 1447.
4. Tsai, C. H. and Kinsella, J. E., *Ann. Bot.*, 1981, **48**, 549.
5. Jones, L. H. and Hughes, W. A., in *Biotechnology in Agriculture & Forestry* 10 *Trees II*, ed. Y. P. S. Bajaj. Springer-Verlag, Berlin, 1989, pp. 176–202.
6. Radwan, S. S., Gross-Oetringhaus, S. and Mangold, H. K., *Chem. Phys. Lipids*, 1978, **22**, 177.
7. Williams, M., PhD Thesis, University of Wales, 1992.
8. Williams, M., Sanchez, J. and Harwood, J. L., *Biochem. Soc. Trans.*, 1991, **19**, 268.
9. Williams, M., Sanchez, J., Hann. A. C. and Harwood, J. L., *J. Exp. Bot.*, 1993, **268**, 1717.
10. Williams, M. and Harwood, J. L., *Biochem. J.*, 1995, **304**, 463.
11. Harwood, J. L., *Prog. Lip. Res.*, 1994, **33**, 193.
12. Harwood, J. L., Jones, A. L., Perry, H. J., Rutter, A. J., Smith, K. L. and Williams, M., *Temperature Adaptation of Biological Membranes*, ed. A. R. Cossins. Portland Press, London, 1994, pp. 107–118.
13. Jones, A. L., Lloyd, D. and Harwood, J. L., *Biochem. J.*, 1993, **296**, 183.
14. Avery, S. V., Lloyd, D. and Harwood, J. L., *J. Euk. Microbiol.*, 1994, **41**, 396.
15. Harwood, J. L., in *Target Sites for Herbicide Action*, ed. R. C. Kirkwood. Plenum Press, London, 1991, pp. 57–91.
16. Claridge, M. F. and Walton, M. P., in *Research*

Collaboration in European IPM Systems, ed. P. T. Haskell. BCPC, Farnham, 1992, pp. 3–12.
17. Hassall, K. A., *The Biochemistry and Uses of Pesticides*, 2nd Edition, Macmillan, London, 1990.
18. Garner, W. Y. and Menzer, R. E., *Pestic. Biochem. Physiol.*, 1986, **25**, 218.
19. Lucier, G. W. and Menzer, R. E., *J. Agric. Food Chem.*, 1968, **16**, 936.
20. Lucier, G. W. and Menzer, R. E., *J. Agric. Food Chem.*, 1970, **18**, 698.
21. Roughan, P. G. and Slack, C. R., *Annu. Rev. Plant Physiol.*, 1982, **33**, 97.
22. Radwan, S. S. and Mangold, H. K., *Advances in Lipid Research*, 1976, **14**, 171.
23. Rutter, J. L., Sanchez, J. and Harwood, J. L., *Phytochem.*, 1997, **46**, 855.
24. Gunstone, F. D., Harwood, J. L. and Padley, F. B. (eds), *The Lipid Handbook*, 2nd Edition. Chapman and Hall, London, 1994.
25. Lentza-Rizos, C., *J. AOAC. Int.*, 1994, **77**, 1096.
26. de la Vega, M., Harwood, J. L. and Sanchez, J., in *Metabolism, Structure and Utilisation of Plant Lipids*, ed. A. Cherif, D. B. Miled-Daoud, B. Marzouk, A. Smaoui and M. Zarrouk. Centre Pédagogique, Tunis, 1992, pp. 368–371.
27. Sanchez, J., Eclair 209 Final Report, 1994.
28. Montiel Bueno, A., Eclair 209 Final Report, 1994.
29. Murashige, T. and Skoog, F., *Physiol. Planta.*, 1962, **15**, 473.
30. Garbus, J., DeLuca, H. F., Loomans, M. E. and Strong, F. M., *J. Biol. Chem.*, 1963, **238**, 59.