Effects of EPTC and Dichlormid on Membrane Lipid Composition of Maize Leaves and Roots*

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The changes in fatty acid composition of maize leaf lipids caused by EPTC were generally similar to known effects of this herbicide in other plants: decreasing of linolenic acid content and increasing of its precursors, palmitic, stearic, oleic and linoleic acids. However, novel effects were detected in roots where the proportion of minor fatty acid palmitoleic acid was increased from 2.1 to 7.6 and 16.6% by EPTC and EPTC + dichlormid treatments, respectively. Simultaneously, the phospholipid content of root lipids was increased by both EPTC as well as EPTC + dichlormid treatments. The possible effects of EPTC and dichlormid on lipid biosynthesis of maize are discussed.

Introduction

Biosynthesis of surface and membrane lipids is a possible target site for the action of the thiocarbamate herbicide EPTC. Though EPTC was shown to modify epicuticular wax structure and composition of pea (*Pisum sativum* L.) [1] and cabbage (*Brassica oleracea* L.) [2], as well as membrane lipid formation of wheat (*Triticum aestivum* L.) [3], spinach (*Spinacia oleracea* L.) [4] and soybean (*Glycine max* L.) [5], there are only limited and contradictory data from maize (*Zea mays* L.). Some of the authors have disputed the involvement of lipid biosynthesis in EPTC mode of action [6], while others demonstrated a fast and strong inhibition of acetate incorporation into cultured maize cells what was reversed by the safener dichlormid [7].

According to detailed investigations, EPTC modified the composition of epicuticular wax of maize [8], thereby resulting in enhanced cuticular transpiration [9]. In addition, strong changes were shown in the incorporation of the label from [1-^{14}C]acetate into membrane lipid classes of maize seedlings treated with EPTC [10]. These data clearly demonstrated the influence of EPTC on lipid biosynthesis of maize, which is not only the economically most important crop treated with EPTC, but also is favoured among monocotyledons because of its moderate EPTC sensitivity and inducible detoxifying system *via* safeners [11].

Only a limited knowledge exists on the effect of safeners on lipid biosynthesis of plants. In previous experiments some safeners reversed the changes of composition or biosynthesis of maize lipids caused by EPTC [7, 8]. However, detailed investigations of EPTC and dichlormid effects on lipid composition of maize membrane lipids have not been published.

Materials and Methods

Chemicals

A commercial sample of EPTC (Nitrokémia, Füzfögyártelep, Hungary) was purified by vacuum distillation (b.p. 112 °C/2.1 kPa). Dichlormid was synthesized in this laboratory (b.p. 96 °C/0.4 kPa). Redistilled analytical grade solvents (Reanal, Budapest, Hungary) were used throughout these experiments. Thin layer plates for lipid separations (Art. 11845), 2-thiobarbituric acid for malondialdehyde tests, and sodium were from Merck (Darmstadt, F.R.G.). Fatty acid methyl ester kits, margaric acid and polar lipid standards were purchased from Supelco (Bellefonte, U.S.A.).

Plant material

Maize (JX-SC 92, Martonvásár, Hungary) grains were pregerminated for two days in dark at 24 °C, then transferred to water culture and main-

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tained to tap water for four days under controlled conditions (illumination 16 h, 10 klux; temperature 22 °C; humidity 60–70%). On the sixth day, 6 plants per pot, were treated with 400 ml of half strength Hoagland's nutrient solution containing 0.05 mM dichlormid and/or 0.1 mM EPTC. Treatments were repeated every second day. Plants were harvested on the 13th day and subjected to lipid analysis after determination of fresh weight and height of roots and shoots.

Lipid analysis

Roots and shoots of 30 plants were extracted with chloroform:methanol mixtures by the modified method of Folch [12]. The extracts were weighed and separated to lipid classes on thin layer plates [13]. The neutral lipid (NL), galactolipid (GL) and phospholipid (PL) fractions were scrapped off and *trans*-esterified directly on the silica with 2 N sodium methoxide in methanol [14]. Fatty acid methyl esters were further purified on thin layer plates and were analyzed by gas liquid chromatography (GLC).

GLC was carried out using a Chrom 4 gas chromatograph (Laboratorni Pristroje, Prague, Czechoslovakia) with a flame ionization detector, attached to a Digint 60 μ integrator (Chinoin, Budapest, Hungary). The glass column (2.5 m × 2.6 mm i.d.) was packed with 80–90 mesh Diatomite C DMCS (Pye Unicam, Cambridge, U.K.) coated with 15% Silar 5-CP (Analabs, Karlsruhe, F.R.G.). The column temperature was 195 °C, the injector and detector temperatures were 250 °C. The carrier gas was nitrogen with a flow rate of 65 ml/min.

Fatty acid methyl esters were identified by comparing their retention times to those of known standards. Margaric acid methyl ester was used as internal standard for quantitative determination of fatty acids.

Each analysis was repeated five times from independent lipid extracts.

Spectrophotometry

Malondialdehyde [15], total chlorophyll [16] and phosphorus content of lipids [17] were determined by standard methods with a Spektromom 204 spectrophotometer (MOM, Budapest, Hungary).

Results

The JX-SC 92 hybrid was sensitive to EPTC treatment under the conditions used. EPTC caused a strong shoot growth inhibition but it did not influence the root (Table I), while EPTC + dichlormid was far less toxic to shoot but decreased the root weight. The latter effect was visible because of decreased root length and weight and was repeatedly detected in several experiments. The total lipid content changed in response to shoot weight but a decreased lipid level was found in root after EPTC treatment. Surprisingly, significant lipid overproduction was measured in roots of plants treated by EPTC + dichlormid.

The fatty acid profile of maize shoot and root lipid classes showed significant changes as a result of herbicide treatments (Fig. 1). The effects of EPTC in maize shoot were similar to those in wheat [3] and soybean [5]: the linolenic acid content of NL and PL fractions decreased, while the proportions of its precursors, palmitic, stearic, oleic, and linoleic acids increased. Just minor changes were detected in GL fraction. EPTC + dichlormid treatment both in NL and PL fractions

Table I. Growth and total lipid content of maize root and shoot*.

Treatment**	Root		Shoot		
	Weight [mg]	Lipid [mg/g]	Height [cm]	Weight [mg]	Lipid [mg/g]
Control	602[a]	2.0[a]	36[a]	869[a]	10[a]
EPTC	719[b]	1.4[a]	16[b]	364[b]	9.9[b]
EPTC + dichlormid	417[c]	2.4[b]	28[c]	676[c]	12[a]

* Values marked by the same letter within a column are not significantly different at the 5% level.
** Doses: EPTC 0.1 mM, dichlormid 0.05 mM.

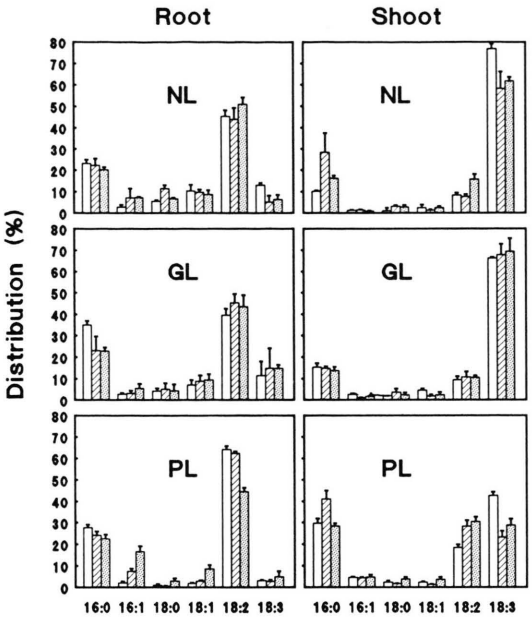

Fig. 1. Effects of EPTC (0.1 mM) and dichlormid (0.05 mM) on composition of major fatty acids of maize root and shoot neutral lipids (NL), phospholipids (PL) and galactolipids (GL). Error bars mean standard deviation of five replicates from different lipid extracts. Abbreviations of fatty acids (carbon number:desaturation number): 16:0 palmitic, 16:1 palmitoleic, 18:0 stearic, 18:1 oleic, 18:2 linoleic, 18:3 linolenic. Detected, but uncalculated minor acids were 12, 14, 16:2 and 20:0. Treatments: □ control, ▨ EPTC, ⊡ EPTC + dichlormid.

decreased the linolenic acid content without changing the proportion of its major precursor palmitic acid.

Surprising and novel results were obtained from investigation of root fatty acids where no linolenic but linoleic acid was the major component. In this case EPTC elevated the proportion of the minor component palmitoleic acid. Moreover, EPTC + dichlormid did not decrease but further elevated the concentration of this acid, *e.g.* up to 18% in PL fraction.

From the above fatty acid composition data distributions of lipid classes were calculated (Table II). The modification of fatty acid profile was accompanied with strong changes of the proportions of NL, GL and PL fractions. The proportions of GL and PL fractions increased to the detriment of the pool of neutral lipids. The most evident changes were again detected in the root where the proportion of phospholipids was elevated from 31.8% to 90.5% by EPTC and to 76.3% by EPTC + dichlormid treatments.

Considering, that increasing of plant root phospholipid content caused by herbicide treatment is a novel observation, different and independent methods were applied to corroborate or dispute this finding. These experiments provided further evidence that the above data are correct (Table II). Each method showed a significant, at least 70% increase of PL content as a result of EPTC treatment and smaller but considerable changes after EPTC + dichlormid application.

The GLC data are considered the most reliable since in other methods of root phospholipid determinations the total lipid basis contained no-lipid materials *e.g.* pigments. The radioactive method detected only the newly synthesized lipids, there-

Table II. Proportions of neutral lipid (NL), galactolipid (GL) and phospholipid (PL) fractions in lipids of maize treated with 0.05 mM dichlormid and/or 0.1 mM EPTC*.

Treatment	Shoot			Root					
	NL	GL	PL	NL	GL	PL	PL		
							GR	UV	AC
	[% of total lipids**]								
Control	59.4[a]	8.9[a]	31.7[a]	63.3[a]	5.2[a]	31.8[a]	33[a]	30[a]	38[a]
EPTC	54.6[a]	21.9[b]	23.5[b]	8.2[b]	1.2[b]	90.6[b]	56[b]	66[b]	65[b]
EPTC + dichlormid	39.9[b]	16.5[b]	43.6[c]	19.5[c]	4.2[c]	76.3[c]	–	41[c]	54[b]

* Values marked by the same letter within a column are not significantly different at the 5% level.

** With the exception of root PL data were calculated from GLC of fatty acids using the following lipid:fatty acid conversion factors: NL 1.00, GL 1.45, PL 1.37. Root PL was determined by gravimetry (GR), spectrophotometric analysis of lipid phosphorus content (UV) and investigation of [1-^{14}C]acetate incorporation into lipids (AC, from [10]) besides GLC.

fore these results indicate the effects of treatments on phospholipid biosynthesis.

One of the possible explanations for the decreased shoot linolenic acid content may be enhanced lipid peroxidation [15] that leads to malondialdehyde (MDA) formation. Literature studies showed, that peroxidation of 1 mol linolenic acid was accompanied with formation of 0.024 mol of MDA [15]. This ratio in maize shoot treated by EPTC was only 0.0037 (from data of Table III) indicating that the decreased linolenic acid content was not a consequence of enhanced lipid peroxidation. The increased MDA content of treated maize may have been derived from higher chlorophyll levels of maize leaves [18] (Table III). Because of modification of total lipid content and proportions of lipid classes the shoot linolenic acid level was only changed to a lesser extent by EPTC.

Table III. Lipid peroxidation in maize shoot. Data were calculated on the basis of fresh weight*.

EPTC [mM]	Linolenic acid** [mg/g]	Malondialdehyde [μg/g]	Chlorophyll [mg/g]
0	5.4[a]	1.5[a]	1.53[a]
0.1	4.7[a]	2.2[b]	1.72[b]

* Values marked by the same letter within a column are not significantly different at the 5% level.
** Calculated from fatty acid and lipid composition data.

Discussion

The changes of fatty acid composition of maize lipids caused by EPTC were generally similar to known effects of this herbicide: decreasing of linolenic acid content and increasing of its precursors, palmitic, stearic, oleic and linoleic acids [3–5]. From these findings it would have been concluded that EPTC is an inhibitor of fatty acid elongase and desaturase enzymes.

However, some of the above results are at least partially contradictory to this simple explanation. The total linolenic acid content of shoot was only slightly decreased by EPTC treatment (Table III) because of changes of lipid content and distribution (Tables I, II). The safener dichlormid did not prevent the modification of shoot fatty acid profile in spite of effective safening action against phytotoxic symptoms. Even in some cases the safener enhanced the changes in lipid composition caused by EPTC, *e.g.* further increases in the proportion of the minor lipid constituent palmitoleic acid in root (Fig. 1) and leaf phospholipid content (Table II). Finally, the strongest changes were detected in the distribution of lipid classes (Table II) indicating that biosynthesis of unsaturated fatty acids is coupled to special phospholipid or galactolipid molecules [19]. Thus a separate evaluation of fatty acid composition data is not correct.

One of the possible explanations of the EPTC effect on maize lipids may be the lack of an essential cofactor of lipid biosynthesis. Such a cofactor may play a role in EPTC detoxication and lipid desaturation simultaneously. If this is the case the safener-induced increase of the rate of EPTC detoxication may further decrease the level of this cofactor causing stronger changes of lipid biosynthesis. This cofactor may be NADPH [20] which is involved in both sulfoxidation of EPTC by monooxygenase enzymes [11] and desaturation of lipids [21]. According to preliminary experiments EPTC really decreased the NADPH level of maize leaves [22].

The other possible explanation is based on earlier experiments of Hilton and Christiansen [23] who demonstrated that the sensitivity of plants to trifluralin depends on lipid content of plant seed and externally applied lipids. In both cases the lipid content decreased the herbicide toxicity. It is suggested, that increased phospholipid content of maize roots (Table II) may serve as internal lipid pool to protect the plants from high EPTC uptake and toxicity. Though, this idea is not proved, it could explain the overproduction of phospholipids by plants treated by EPTC and EPTC + dichlormid.

What is the possible mechanism of enhanced phospholipid and palmitoleic acid production of maize? According to detailed investigations, biosynthesis of unsaturated fatty acids proceeds simultaneously in chloroplasts and extraplastidic systems [19]. The last membrane-bound system was identified as endoplasmic reticulum (e.r.) and showed eight times higher unsaturated fatty acid-synthesizing activity than plastids. This activity was reconstituted by incubation of microsomes (the isolated form of e.r.) in appropriate medium [19]. Considering, that the microsomal cytochrome P-450 monooxygenase inhibitor 1-aminobenzotriazole influenced the toxicity of EPTC to

maize [24], moreover, dichlormid and mainly EPTC + dichlormid treatments elevated the level of the enzyme mentioned [25], the direct effect of EPTC and/or safeners on similar type microsomal desaturases can not be excluded. This hypothesis would explain the origin of phospholipid overproduction as well as the increasing capacity of palmitoleic acid synthesis because of less selectivity of extraplastidic enzymes [19].

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