Chloroacetamide Mode of Action, II: Inhibition of Very Long Chain Fatty Acid Synthesis in Higher Plants

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Herbicide, Chloroacetamide, VLCFA, *Cucumis sativus*, *Hordeum vulgare*, *Zea mays*

In short-term-experiments [^{14}C]-labelled malonic acid, stearic acid and acetate have been incorporated into leaf fatty acids of seedlings of *Cucumis sativus*, *Hordeum vulgare* and *Zea mays*. The pattern of labelled fatty acids changed markedly by treatment with the chloroacetamide herbicides metazachlor, metolachlor or butachlor. During a 2-h incubation time, 1 µM chloroacetamide specifically inhibited up to 100% the formation of the saturated very long chain fatty acids (VLCFAs) with a carbon number of 20, 22 and 24. In cucumber and barley a 50% inhibition of VLCFA formation is achieved with 10 to 100 nM metazachlor representing the most sensitive effect of inhibitors on fatty acid elongation reported as yet. Sensitivity of fatty acid elongation depends on the amide structure present in the compound and on its stereochemistry. Inhibition of oleic acid incorporation correlates with growth inhibition by chloroacetamides of the intact cell (comp. Pestic. Sci. **52**, 381–387, 1998). The present study extends this correlation to inhibition of VLCFA synthesis in higher plants. Obviously the primary mode of action of chloroacetamides and related herbicidal substances is involved in the enzymic four-step fatty acid elongation system.

Introduction

Although chloroacetamides have been used in chemical weed control for about forty years, their primary mode of action has not yet been elucidated (for review see e.g. Fuerst, 1987). In recent years the algal model system *Scenedesmus acutus* was used to focus on lipid metabolism (see accompanying paper Schmalfuß *et al.*, 1998 for a brief summary). Metazachlor could be shown to decrease desaturation of endogenous oleic and linoleic acid at micromolar concentrations (Couderchet and Böger, 1993). The most sensitive chloroacetamide effect found until now, with I_{50} values of 50 nM and lower, however, is the decreased incorporation of exogenously applied [^{14}C]oleic acid into an *insoluble non-lipid* fraction of *S. acutus* cells containing sporopollenin (Couderchet *et al.*, 1996). As has been shown in the accompanying paper, metazachlor markedly reduced algal monounsaturated VLCFAs ranging from 20 to 24 carbon atoms produced from [^{14}C]oleic acid. A metazachlor-resistant cell line selected from *S. acutus* (Couderchet *et al.*, 1995) formed no VLCFAs in favour of C16 and C18 fatty acids, and Schmalfuß *et al.* (1998) assumed this replacement to be the basis of resistance.

In higher plants VLCFAs are structurally important constituents of seed storage lipids, plant membranes and epicuticular waxes, the latter including different long chain components like acids, aldehydes, alcohols and esters, all biochemically deriving from VLCFA precursors (Cassagne *et al.*, 1994; von Wettstein-Knowles, 1993). The chloroacetamide metolachlor has been reported to reduce the wax formation with its C28-C32 components in *Sorghum bicolor* (Ebert and Ramsteiner, 1984). Möllers and Albrecht (1994) showed both the inhibition of 18:2 desaturation and 20:1 elongation in *Brassica napus* embryoids in the presence of chloroacetamides. In the wax fraction obtained from cotyledons of cucumber seedlings treated with metolachlor, decreased amounts of alcohols and long chain alkanes were found (Tevini and Steinmüller, 1987). These data reported required more than 20 µM herbicide.

Our studies do not deal with waxes but with lipids inside the cell. Less than 1 µM chloroacetamide was required for substantial inhibition in *Scenedes-*

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mus. This study should verify a corresponding inhibition of VLCFA formation in higher plants.

Material and Methods

Plants and chemicals

Seeds of *Cucumis sativus var. Delikatess* were obtained from Gawaz, Bergheim, Germany, *Hordeum vulgare var. Colombo* from Küpper, Eschwege, Germany, and *Zea mays var. Anjou* from Saaten-Union, Rastatt, Germany.

[^{14}C]methanol (46 kBq/mol), [2-^{14}C]malonic acid and [1-^{14}C]stearic acid (each 2.1 MBq/mol) were from Hartmann Analytics, Hannover, Germany. [1-^{14}C]oleic acid, [1-^{14}C]linoleic acid and [1-^{14}C]linolenic acid (each 2.1 MBq/mol) were from Amersham, Braunschweig, Germany. Unlabelled fatty acids and 14% BF_3/MeOH were purchased from Sigma, Deisenhofen, Germany.

Incubation procedure

Cucumber, barley, and maize seeds were germinated and grown in plastic pots containing vermiculite and tap water for a period of five days in a 16 h/8 h day/night cycle at 25 °C and 80 $\mu Em^{-2}s^{-1}$. Leaf discs of cucumber cotyledons with a diameter of 5 mm and 5 mm slices of the primary leaf of barley or maize were immersed in nutrient medium containing 20 mM KNO_3, 0.2 mM $CaSO_4$ and 1 µM Na_2MoO_4 (Durner and Böger, 1990). For incorporation experiments with [^{14}C]malonic acid 15 leaf discs of cucumber were placed in petri dishes containing 2 ml nutrient medium and approximately 5 µM [^{14}C]malonic acid (ca. 23 kBq) or 15 µM (70 kBq) acetate. 400 mg of fresh weight from barley or maize slices were incubated in 8 ml nutrient medium giving a final concentration of 2 µM [^{14}C]malonic acid (36 kBq). Herbicide was added as an ethanolic stock solution giving a final concentration of 0.1 vol% ethanol and 10^{-6} to 10^{-8} M herbicide. The samples were incubated for 6 h (or 20 h; data of Table I) at 25 °C and 80 $\mu Em^{-2}s^{-1}$. For stearic acid incorporation, [^{14}C]stearic acid was dissolved in DMSO (with a 0.5 vol% final concentration) and sonicated after addition of nutrient medium. Samples of 20 cotyledon discs were incubated in 6 ml nutrient medium as described above with a standardized final concentration of 1 µM herbicide and approximately 2 µM labelled stearic acid (ca. 25 kBq). In some cases the number of leaf discs and the incubation volume was increased to 50 leaf discs and 20 ml; the concentration of labelled stearic acid remained the same. Uptake and incorporation of radioactivity into fatty acid fractions was monitored by liquid scintillation counting.

Analysis of fatty acids from leaves

After incubation the fatty acids were immediately extracted using a method (Fig. 1) modified from Couderchet and Böger (1993). Leaf tissue was washed 30 sec with 5 ml $CHCl_3$ to remove the cuticular waxes and excess [^{14}C]stearic acid. The lipids of the leaf residue were alkaline-hydrolysed with 4 ml 10% KOH/MeOH at 65 °C for 60 min. The mixture was cooled, acidified with 3 ml 12% HCl and extracted twice with 2 ml *n*-hexane after gentle centrifugation. Using acetate for incorporation labelled carotenoids were extracted twice in to *n*-hexane before acidification and extraction of fatty acids. The hexane phases were combined, dried under nitrogen, and fatty acids methylated with 500 µl 14% BF_3/methanol at 100 °C for 20 min. After addition of 500 µl distilled water, fatty acid methyl esters were extracted twice with 500 µl *n*-hexane and concentrated. ^{14}C-labelled metabolites were determined using a radio-HPLC system as described (Schmalfuß *et al.*, 1998). The fatty acid methyl esters were identified by compar-

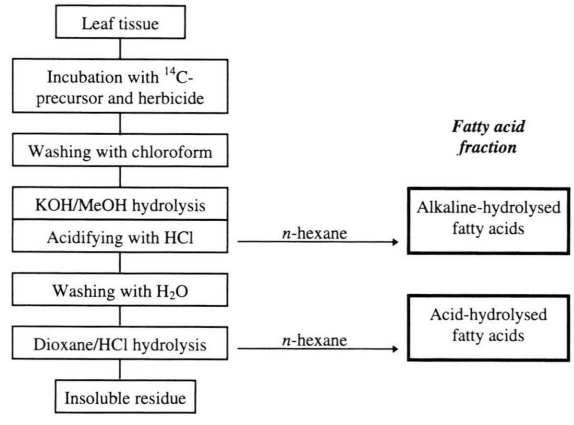

Fig. 1. Flow diagram showing the procedure of fatty acid solubilization from leaf tissue. Fractions of labelled fatty acids analysed by radio-HPLC after methylation are marked by a thick frame.

ison of the retention times with [^{14}C]labelled standards.

The data of Table I, B were obtained by a subsequent *acid hydrolysis* which yielded additional amounts of VLCFAs. For this purpose the remaining leaf discs from the alkaline hydrolysis were subsequently washed with 5 ml of distilled water followed by hydrolysis with 4 ml dioxane/ 1 M HCl (7:1 v/v) at 70 °C for 5 h. After addition of 3 ml of distilled water the sample was extracted twice with 2 ml *n*-hexane and treated in the same way as the hexane phases of the alkaline hydrolysis.

Synthesis of ^{14}C-labelled standard fatty acid methyl esters

Methyl esters of 1-^{14}C-labelled palmitic acid, oleic acid, linoleic acid and linolenic acid were obtained after methylation as described in the preceeding section. We had to develop a special procedure for ^{14}C-methylation of unlabelled fatty acids. 6 mg arachidic acid, 6 mg behenic acid and 6 mg lignoceric acid were placed together into a capped vial and suspended at −70 °C in 300 µl 14% BF_3/[^{14}C]methanol with a final specific activity of 4.6 Bq/mol. The mixture was heated to 80 °C for 45 min until complete dissolution. The solution was cooled and the methyl esters were separated and analysed by radio-HPLC as described above. As shown by Fig. 2 the retention times agreed with the methylated samples of leaf fatty acids which carried the ^{14}C-label in the carbon chain as incorporated from labeled stearic or malonic acid.

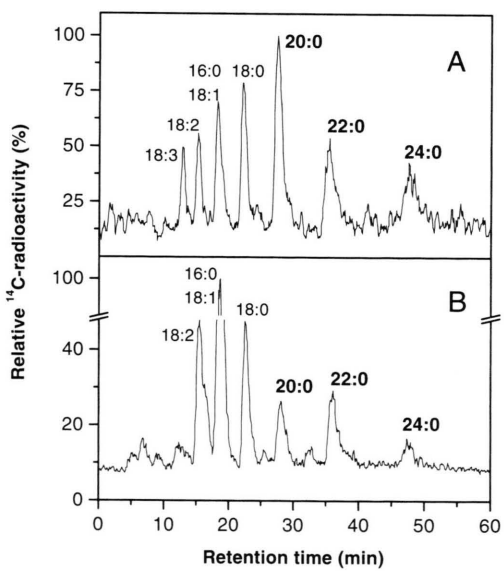

Fig. 2. Radio-HPLC chromatogram of (**A**) fatty acid methyl ester standard which is ^{14}C-labelled at the methyl position and (**B**) sample of methylated chain-^{14}C-labelled fatty acids derived from cucumber tissue.

Data reliability

The experiments were repeated several times. The deviation from the mean within the repetitions did not exceed 10%. In different experiments the incorporation of the ^{14}C-precursor into the leaf tissue was found between 4 and 13% of the applied label, and the radioactivity detected in the plant extracts varied correspondingly. Generally data of a representative experiment are shown.

Results

Fatty acid pattern

Cucumber cotyledons incorporated up to 13% of label into lipids when [^{14}C]malonic acid and [^{14}C]stearic acid were supplied with the nutrient medium. Hexane-extractable lipids were found in the two fractions obtained consecutively by alkaline hydrolysis with KOH/methanol and acidic hydrolysis with dioxane/HCl.

The label from [^{14}C]stearic acid was found in *alkaline-hydrolysed* lipids ranging from linoleic acid (18:2) to lignoceric acid (24:0). About half of the exogenously applied stearic acid was transformed to unsaturated fatty acids as well as to VLCFAs (20:0, 22:0, 24:0) within 20 hours. Applied [^{14}C]malonic acid labelled a higher proportion of VLCFAs while [^{14}C]acetate was poorly incorporated into VLCFAs. Palmitic acid (16:0) and oleic acid (18:1) coeluted under these separation conditions (Table I, A). Applying 1 µM metazachlor, the VLCFAs disappeared almost completely while 16:0, 18:1 and 18:2 remained unchanged. This result could be obtained with fatty acids labelled by [^{14}C]malonic acid as well as by [^{14}C]stearic acid. When the latter fatty acid was applied, reduced VLCFA formation corresponded to an increase of remaining stearic acid. Incorporation of radioactivity from [^{14}C]malonic acid was 47% of control which was due to the decrease of VLCFAs. Using malonic acid as precursor formation of stearic acid (18:0) was diminished with meta-

Table I. Incorporation of radioactivity from ^{14}C-labelled stearic acid, malonic acid and acetate into fatty acids of cucumber cotyledons within 20 h of incubation. Lipids were alkaline-hydrolysed (**A**) followed by acid hydrolysis (**B**). Labelled fatty acids were determined by radio-HPLC, palmitic acid (16:0) and oleic acid (18:1) coelute under these separation conditions. The metazachlor-induced loss of VLCFAs (20:0, 22:0, 24:0) corresponds to a reduced uptake of total label from [^{14}C]malonic acid and to a higher level of remaining stearic acid (18:0) when [^{14}C]stearic acid was applied.

	[^{14}C]		Fatty acid labelling (Bq/ leaf disc)								
	Precursor	Metazachlor	Total	18:2	16:0/18:1	18:0	**20:0**	**22:0**	**24:0**	**VLCFA**	Not id.
A	Stearic acid	none	143.1	19.0	30.7	68.8	7.4	10.1	1.9	19.4	5.2
		1 µM	152.8	17.8	29.0	97.5	1.8	–	–	1.8	6.7
	Malonic acid	none	204.5	14.9	48.0	18.0	17.1	56.3	21.6	95.0	28.6
		1 µM	95.7	15.1	45.5	7.8	3.1	1.1	0.4	4.6	22.6
	Acetate	none	205.7	35.7	118.6	15.3	1.7	2.0	0.3	4.0	32.2
		1 µM	196.6	38.8	109.5	16.1	0.4	–	–	0.4	31.7
B	Stearic acid	none	44.5	–	–	16.0	2.6	6.7	2.6	11.9	16.5
		1 µM	40.9	–	–	28.5	0.5	0.4	–	0.9	11.5
	Malonic acid	none	40.5	–	3.6	2.9	3.9	13.7	8.5	26.1	7.9
		1 µM	10.8	–	3.9	1.4	1.0	–	–	1.0	4.5
	Acetate	none	40.4	–	18.4	4.9	2.3	6.3	2.9	11.5	5.4
		1 µM	48.6	–	30.7	6.3	–	1.9	–	1.9	9.7

zachlor present (Table I, A) by about 50% only, but no inhibition of stearic acid formation was observed with acetate incorporation.

Less label from the applied precursors was found in the *acid-hydrolysed* lipid fraction (20–30% of label of the alkaline-hydrolysed acids), but the label was present almost exclusively in stearic acid, VLCFAs, and in a comparatively higher proportion of non-identified components (Table I, B). With metazachlor present less or no label was found in VLCFAs as it has been shown in Table I, A. The loss of VLCFA synthesis showed up by reduced radioactivity in total lipids labelled by [^{14}C]malonic acid but not by [^{14}C]stearic acid (comp. both Table parts).

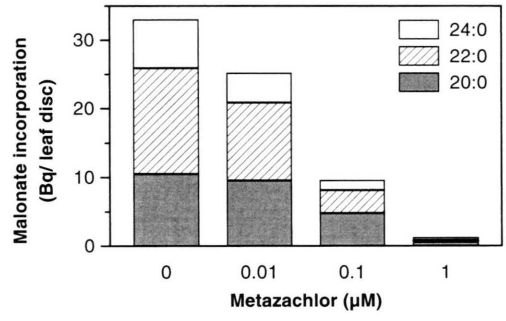

Fig. 3. Metazachlor-induced inhibition of very long chain fatty acid synthesis reflected by incorporation of [^{14}C]malonic acid. VLCFAs are quantified by HPLC after a 6-h incubation of leaf discs from five day old cucumber seedlings with 5 µM [^{14}C]malonic acid treated with increasing metazachlor concentrations.

Herbicide sensitivity, other species

The reduced level of VLCFAs of *Cucumis sativus* could be correlated with the chloroacetamide concentration using different exogenous precursors such as stearic acid, malonic acid and acetate. A 50% reduction of VLCFAs labelled by [^{14}C]malonic acid was achieved with 10 to 100 nM metazachlor. The sensitivity increased in the series 20:0, 22:0, 24:0 (Fig. 3; see Discussion). The time course of fatty acid labelling was studied using [^{14}C]stearic acid as precursor. Label was found in VLCFAs within 2 h which increased slowly within the following hours until saturation. In cotyledons treated with 1 µM metazachlor the VLCFA level decreased immediately and strongly, no more than 6% VLCFAs of the control level was found (Fig. 4, A). The formation of oleic acid and linoleic acid

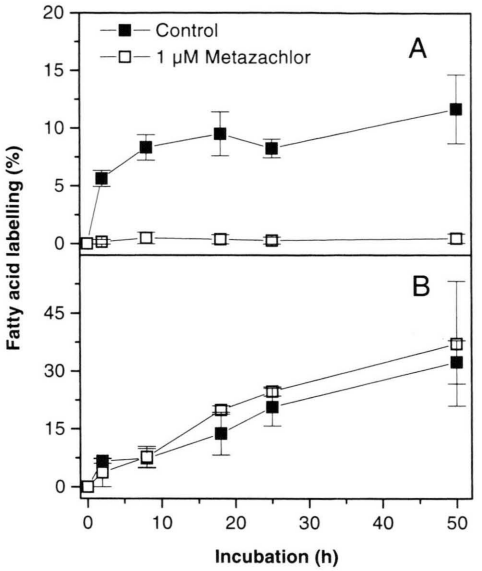

Fig. 4. Time course of [^{14}C]stearic acid metabolism in cucumber cotyledons. Cotyledon discs of five day old cucumber seedlings incorporated 2 µM [^{14}C]stearic acid for 2 to 50 h with or without 1 µM metazachlor. Metabolites determined by radio-HPLC are distinguished in very long chain fatty acids (**A**) and unsaturated C18 fatty acids (**B**).

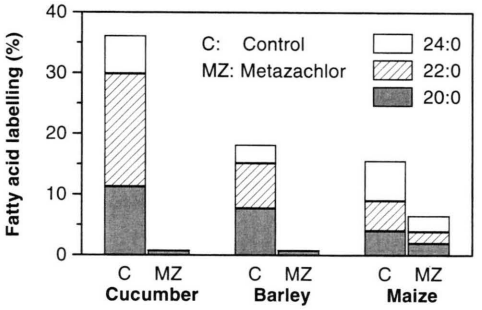

Fig. 5. Very long chain fatty acid synthesis reflected by incorporation of malonic acid into monocotyledonous (*Hordeum vulgare*, *Zea mays*) and dicotyledonous (*Cucumis sativus*) seedlings which were untreated and treated with 1 µM metazachlor. VLCFAs are quantified by radio-HPLC after a 6-h incubation of leaf tissue from five day old seedlings incubated with [^{14}C]malonic acid using concentrations of 5 µM (cucumber) and 2 µM (barley, maize), respectively.

from [^{14}C]stearic acid was about linear during the incubation time yielding unsaturated fatty acids 3-fold as much as VLCFAs within 50 hours. Metazachlor did not change significantly the content of unsaturated C18 fatty acids synthesized at any time (Fig. 4, B).

In parallel experiments the incorporation procedure was performed with *Hordeum vulgare* and *Zea mays*. Five day old primary leaves of barley yielded fatty acids ranging from 16 to 24 carbons similar to the fatty acid pattern in cucumber. The barley primary leaves formed about half as much total VLCFAs as cucumber with a less relative amount of behenic acid (22:0). The VLCFA synthesis in both cucumber and barley leaves was inhibited more than 95% by 1 µM metazachlor (Fig. 5). In maize the decrease of VLCFAs was 58% only with no significant preference to a certain chain length. Labelling with [^{14}C]malonic acid the main VLCFA component was lignoceric acid (24:0). In each tissue C16 and C18 fatty acids were the main components of alkaline-hydrolysed lipids.

Other chloroacetamide herbicides applied to cucumber, namely butachlor and metolachlor, as well as the oxyacetamide flufenacet reduced the formation of arachidic acid (20:0) about 90%, while the formation of behenic acid (22:0) and lignoceric acid (24:0) was not detectable when 1 µM herbicide was applied to cucumber cotyledons. Efficiency of metolachlor depended on the enantiomer. *R*-metolachlor did not show a significant influence upon VLCFA synthesis, but a decrease of 20:0, 22:0 and 24:0 species was obtained with *S*-metolachlor (64%) and, sligthly less effective (60%), with the racemate (Table II). Two phytotoxic phosphinosulfonates RH-4641 and RH-4642 reduced the VLCFA level of cucumber cotyledons as effective as the chloroacetamides, and corresponding results were obtained with cafenstrole, fentrazamide and, less effective, with ethofumesate. Little or no reduction of VLCFA synthesis was detected with EPTC.

Discussion

Methylation of fatty acids in [^{14}C]methanol has been proven to be a suitable method to prepare detectable amounts of ^{14}C-labelled standard fatty acid methyl esters. Thus, identification of labelled very long chain fatty acid metabolites became possible with radio-HPLC (Fig. 2). This means an substantial simplification of sample processing compared to the methods used up to now, GC/MS and HPLC/MS (see Schmalfuß *et al.*, 1998).

The major part of fatty acids was found after alkaline hydrolysis of leaf lipids yielding mainly

Table II. Inhibition of VLCFA synthesis from exogenous [^{14}C]stearic acid with different herbicides. Metabolites are determined by radio-HPLC after a 6-h incubation of cucumber cotyledons with 20 kBq [^{14}C]stearic acid and 1 µM herbicide.

Herbicide class	Herbicide applied (1µM)	% Inhibition of VLCFA-synthesis
Oxyacetamide	Flufenacet(=fluthiamid)	95 ± 3
Chloroacetamide	Metazachlor	89 ± 1
	Butachlor	87 ± 4
	S-Metolachlor	64 ± 3
	rac-Metolachlor	60 ± 2
	R-Metolachlor	1 ± 6
Phosphinosulfonate	RH-4642 (−)	87 ± 1
	RH-4641 (+)	82 ± 1
Triazole	Cafenstrole (CH-900)	84 ± 4
Tetrazolinone	Fentrazamide	83 ± 3
Benzosulfonate	Ethofumesate	63 ± 4
Thiocarbamate	EPTC	18 ± 4

Flufenacet	*N*-(4-fluorophenyl)-*N*-(1-methylethyl)-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl)oxy]acetamid (FOE-5043); Fedtke *et al.* (1998)
Metazachlor	2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl)acetamide
Butachlor	*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide
Metolachlor	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl) acetamide
RH-4641/-4642	(R/S)-*O*-isopropyl-*P*-methyl[[(2-ethyl-6-(trifluoromethyl)phenyl) sulfonyloxy]-methyl]phosphinate; Rosen *et al.* (1996)
Cafenstrole	1-(diethylcarbamoyl)-3-(2,4,6-trimethylphenylsulfonyl)1,2,4-triazole (CH-900); Kanzaki *et al.* (1991)
Fentrazamide	1-(ethylcyclohexylcarbamoyl)-4-(2-chlorophenyl)tetrazolinone (YRC 2388); Yasui *et al.* (1997)
Ethofumesate	2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranylmethanesulfonate
EPTC	*S*-ethyldipropylcarbamothioate

the fatty acids of triacylglycerides, shown by the large content of labelled 16:0 and 18:1 in this fatty acid fraction. As demonstrated by data on *de-novo* synthesized fatty acids by malonate incorporation (Table I), VLCFAs are no minor components but essential membrane constituents, which can especially be found in phospholipids (Bertho *et al.*, 1991; Cook, 1996). The higher proportion of radioactivity in malonate-labelled than in stearate-labelled VLCFAs is probably a result of better metabolism of the water-soluble malonate and reflects the multiple labelling of fatty acids with several C_2-units during their biosynthesis.

About half of all obtained VLCFAs labelled by [^{14}C]stearic acid derive from lipids which could not be alkaline hydrolysed. The dioxane/HCl fraction included exclusively stearic acid, VLCFAs and a significant part of polar components that could not yet be identified. The latter are probably modified fatty acids also found in cutin and suberin (von Wettstein-Knowles, 1993; Schmutz *et al.*, 1996). Additionally, it is to be assumed that oxidized by-products are present due to chemical treatment of the sample, especially after the harsh dioxane/HCl-solubilization (see Methods). Since dioxane/HCl was originally used to isolate cell wall constituents (Langebartels and Harms, 1985) and to solubilize sporopollenin (Couderchet *et al.*, 1996), we assume the VLCFAs hydrolysed by this method originate from the plasma membrane and the cell wall. Accordingly, metolachlor increased the membrane permeability of cucumber roots as reported by Mellis *et al.* (1982). This may be related to the synthesis of VLCFAs assumed to be crucial structure parameters for the stability of membranes (Cook, 1996; Ho *et al.*, 1995).

Chloroacetamides reduce the incorporation of label from stearic acid and the C_2-donors acetate and malonic acid into VLCFAs only. It is safe to assume that the enzyme complex of the elongation reaction, a sequence of four succesive steps localized in the microsomes (Cassagne *et al.* 1994), is inhibited by chloroacetamide action. A 50% reduction of VLCFA synthesis (20:0, 22:0, 24:0) was found with 10 to 100 nM chloroacetamides. In growth experiments a change of the morphology of seedlings became visible with 0.1 µM metazachlor, 1 to 10 µM were found lethal (not documented). Retarded growth was reported from elongation-defect yeast mutants (Dittrich *et al.*, 1998), and from a metazachlor-resistant *Scenedesmus acutus* mutant poor in VLCFAs (Schmalfuß *et al.*, 1998). In wildtype *Scenedesmus acutus* inhibition of VLCFA formation by chloroacetamides (Schmalfuß *et al.*, 1998) correlates with growth inhibition of the intact cell (Couderchet *et al.*, 1998) confirming that VLCFAs are a crucial factor for cell development.

The metazachlor sensitivity of elongation increased with the chain length of the 20:0, 22:0 and 24:0 very long chain fatty acids species (Fig. 3). Inhibition of stearic acid formation (18:0) produced by malonic acid is much less effective,

and is not observed with acetate incorporation (Table I, A, B). These findings indicate that there may be different elongases or elongation steps involved exhibiting different sensitivity to chloroacetamides (for a review on biosynthesis of VLCFAs see Cassagne *et al.*, 1994). In contrast to the effect on fatty acid elongation, metazachlor caused no decrease of desaturated products formed from stearic acid (Fig. 4, B) during the same experimental period. This result, no impact on fatty acid desaturation as found in cucumber cotyledons agrees with findings of soybean cells, but not with those of *Scenedesmus acutus* (Couderchet and Böger, 1993), and with microspore-derived embryoids of *Brassica napus* (Möllers and Albrecht, 1994). The authors reported a reduced level of 18:2 and 18:3 when higher concentrations of chloroacetamides were applied. In comparison to well-known inhibitors of plant elongases, i.e. ethofumesate (Abulnaja *et al.*, 1991) and EPTC (Schmutz *et al.*, 1996) chloroacetamides provide the most potent inhibition of VLCFA synthesis in cucumber (Table II).

Our study deals with lipids and fatty acids inside the cell. Chloroacetamides applied in higher concentrations (more than 20 µM) have been discussed to impair formation of epicuticular waxes. Ebert and Ramsteiner (1984) found a decrease of very long chain wax components in favour of increasing shorter chain homologues in *Sorghum bicolor*. In *Cucumis sativus* similar findings were reported with alkane components of cotyledon wax making Tevini and Steinmüller (1987) to assume that there were two distinct elongases specific for certain chain lengths one of them is preferably inhibited by metolachlor. Since wax components, namely esters, alcohols and alkanes, derive from VLCFAs (Post-Beittenmüller, 1996) it can be assumed that these effects are consequences of a primary inhibition of fatty acid elongation by the applied chloroacetamide herbicide, metolachlor. The mechanism of ethofumesate and thiocarbamate action is not known in detail, and it remains to be clarified, whether the mechanism of action of these herbicides (or derivatives thereof) is comparable to chloroacetamides.

As has been shown previously by Moser *et al.* (1982) the *S*-enantiomer of metolachlor was the efficient phytotoxic isomer in plants. Similar findings with the enantiomers of metolachlor and dimethenamid in *S. acutus* (Couderchet *et al.*, 1997) give evidence of an *enzyme* target for chloroacetamide attack. As shown in Table II, representatives of herbicide classes different from chloro- and oxyacetamides also affected VLCFA synthesis. The triazole herbicide cafenstrole and the new tetrazolinone herbicide fentrazamide showed a loss of VLCFAs as strong as flufenacet, metazachlor and butachlor. This corroborates the assumption of Couderchet *et al.* (1998), that an *N,N*-disubstituted amide function is the essential structural element. The phosphinosulfonates RH-4641/-4642, however, lack this structural feature. In contrast to the different activity of enantiomers of chloroacetamides both enantiomeres of the phosphinosulfonates strongly inhibit fatty acid elongation, possibly indicating that their chiral center is not the potential binding site.

The phytotoxic effect towards saturated VLCFAs of the dicot cucumber was also found with the monocots barley and maize (Fig. 5). Moreover, our findings are in agreement with the chloroacetamide effect on mono-unsaturated VLCFAs of the green algae *S. acutus* (Schmalfuß *et al.*, 1998). In the plants checked a 50% reduction of the VLCFA synthesis is achieved with 10 to 100 nM chloroacetamide suggesting fatty acid elongation to be a general target of chloroacetamide attack in plant cells. In maize, however, metazachlor induced a smaller inhibition of fatty acid elongation than found with cucumber and barley, which may be due to either fast detoxification of the herbicide or to a more resistant elongase complex. At the moment we develop a cell free system to clarify this point.

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