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EFFECT OF THIOCARBAMATE HERBICIDES ON FATTY ACID SYNTHESIS BY POTATO

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; aged slices; fatty acid synthesis; elongation; desaturation; thiocarbamates.

Abstract—*S*-ethyldipropylthiocarbamate (EPTC), *S*-(2,3-dichloroallyl)diisopropylthiocarbamate (diallate) and *S*-(2,3,3-trichloroallyl)diisopropylthiocarbamate (triallate) inhibited the formation of very long chain fatty acids by aged potato discs. Incorporation of acetate-[^{14}C] into total fatty acids was inhibited 24% by EPTC, 50% by triallate and 55% by diallate at 10^{-4} M. The relative sensitivity of very long chain fatty acid synthesis to thiocarbamates in potato tuber provides further evidence that these herbicides reduce cuticular wax by inhibiting fatty acid elongation.

INTRODUCTION

Synthesis of very long chain saturated fatty acids has been demonstrated *in vivo* and *in vitro* using a number of higher plant systems [1–6]. Results from inhibitor and labelling studies indicated that a separate elongase was involved in forming stearate from palmitate and another for elongating stearate [see 7]. This second elongase was sensitive, in germinating peas, to inhibition by various thiocarbamate weed-killers at low concentrations [4] and similar results have recently been obtained with leaf slices [8]. This was of interest, since it has been proposed that elongation of fatty acids, followed by decarboxylation, is a likely route for alkane synthesis [8] and that secondary alcohols may be derived from alkanes [9]. Thus inhibition of fatty acid synthesis would have a severe effect on the formation of typical wax components since elongation is such a critical process [9, 10]. The inhibition of wax production in foliage by certain thiocarbamates [11, 12] thus has a logical explanation in that the key precursors, the very long chain fatty acids, are not made [4, 8].

If elongation of acyl chains is an important basic mechanism in thiocarbamate action, then one might expect that inhibition by them would also occur in a system producing very long chain fatty acids but not cutin. Such a system is the aged potato slice which synthesises suberin. The comparative structures of suberin and cutin have been elucidated by Kolatukkudy and coworkers [see 13] and suberin shown to contain relatively high amounts of dicarboxylic acids, phenolics, very long chain fatty acids and very long chain alcohols. Moreover, the aged potato slice provides a preparation with high rates of fatty acid synthesis including very long chain (C_{20}–C_{24}) fatty acids [14–16]. We have, therefore, tested the effects of a number of thiocarbamate herbicides on fatty acid synthesis by these preparations and the results are now reported.

RESULTS AND DISCUSSION

EPTC is a commonly used herbicide which has been shown to affect wax deposition [11, 12]. In germinating peas, it had a pronounced inhibitory effect on elongation of stearic acid [4] and this observation was confirmed in leaf slices [8]. The labelling of fatty acids from acetate-[^{14}C] in aged potato discs was similarly affected (Table 1). Total incorporation was slightly reduced but

Table 1. Labelling of fatty acids in the presence of EPTC

Inhibitor conc. (M)	Total fatty acids (dpm × 10^{-3})	% Distribution of counts 16:0	16:1	18:0	18:1	18:2	20:0	22:0	24:0	Other
O	253	30.9	1.2	6.6	36.1	6.4	8.9	7.1	2.5	0.3
	±8	±2.4	±0.2	±1.2	±3.5	±0.3	±1.0	±1.2	±0.4	±0.2
10^{-5}	218	33.4	0.7	6.6	41.6	3.7	11.6	1.1	tr	1.3
	±9	±2.7	±0.4	±1.1	±3.6	±0.3	±2.4	±0.6		±0.3
10^{-4}	193	34.7	1.0	2.9	58.0	1.3	2.1	tr	n.d.	tr
	±16	±4.0	±0.3	±1.1	±2.7	±0.1	±0.3			

Results are expressed as mean ± s.d. (four experiments). Total counts were corrected for quenching. Fatty acids were indicated with the number before the colon indicating the chain length and the figure after denoting the number of double bonds. Degradation (see Experimental) showed that 16:1 was palmitoleic, 18:1 was oleic and 18:2 was linoleic acid. nd = not detected, tr = trace (<0.2%).

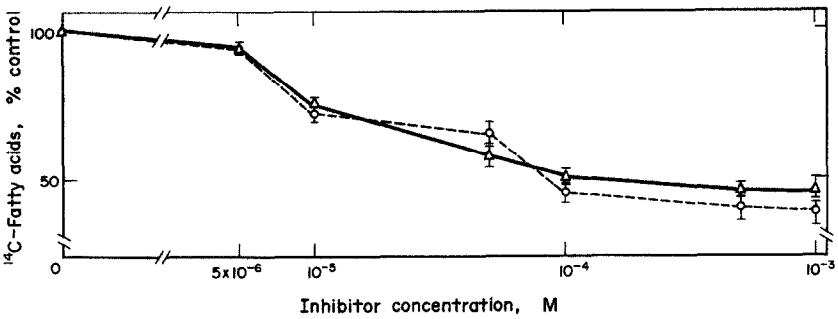

Fig. 1. Effect of diallate and triallate concentration on fatty acid synthesis by aged potato slices. O——O Diallate concentrations; △——△ triallate concentrations. Means ± S.D. (3 experiments) are given.

there was an almost complete absence of very long chain fatty acid products when the herbicide was used at 10^{-5} M. A progressive decrease in the chain length of products was seen with increasing herbicide concentration. Although the aged potato is slightly less sensitive to the herbicide than germinating pea [4], the successive reduction of tetracosanoic, docosanoic and eicosanoic acids is similar to its effect on surface waxes [8]. It is possible that the various very long chain fatty acids are produced by separate elongating enzymes or, alternatively, that partially elongated products are seen on increasing herbicide concentrations.

While EPTC produced only a small reduction in total fatty acid synthesis, the effects of diallate and triallate were more marked (Fig. 1). These herbicides produced up to 60% inhibition of acetate-[^{14}C] incorporation into total fatty acids. It has been clearly demonstrated previously that thiocarbamates inhibit surface lipid formation without affecting internal lipids [4, 8] so that, in aged potato where lipid synthesis is associated with the cut surface [13, 17], it is not surprising to find such high inhibition. The two thiocarbamates produced rather similar amounts of inhibition, a maximal figure, apparently, being approached at 5×10^{-4} M. Typical concentrations likely to be present in the field during spraying would be *ca* 10^{-3} M, a concentration which produced 60% inhibition of fatty acid synthesis (Fig. 1).

When the individual fatty acids produced by aged potato discs, in the presence of diallate (Table 2) or triallate (Table 3), were examined, two obvious effects were noted. Firstly, there was a decline in the proportion of very long chain fatty acids produced and, secondly, a corresponding increase in the percentage of palmitic acid. The strong inhibition of eicosanoic and docosanoic acid formation, taken with similar results by EPTC, is highly significant since it shows that a primary effect of these herbicides is associated with fatty acid elongation. There is thus excellent agreement, in this respect, between the aged potato and the pea seed or leaf systems [4, 8]. Diallate (Table 2) seemed to be somewhat more potent than triallate (Table 3) in so far as inhibition of very long chain fatty acid synthesis was concerned.

A rise in the proportion of label in hexadecanoic acid was noted for both diallate and triallate. This can be explained, either by a general inhibition of elongation reactions such as is observed with arsenite or $CdCl_2$ cf. [7], or that elongation of stearic acid is preferentially reduced but that the latter is present in two compartments—one used for desaturation and the other for elongation. If the pool of stearic acid used for very long chain fatty acids was small then it is unlikely that an increase in stearic acid [^{14}C] would be seen on inhibiting its elongation. Also, if the thiol ester substrates for desaturation and elongation were different then two 'pools' of stearic acid could be easily envisaged. However, it must be remembered that significant reduction in the total amounts of all [^{14}C] fatty acids with a chain length greater than C16 was observed (Fig. 1). Synthesis of palmitic acid was only slightly affected.

The reported results demonstrate quite clearly that elongation of fatty acids is very sensitive to thiocarbamate herbicides. The spectacular action of the latter on

Table 2. Labelling of fatty acids in the presence of diallate

Inhibitor conc. (M)	No. of expts.	Fatty acid labelling (% total) 16:0	16:1	18:0	18:1	18:2	20:0	22:0	Other
0	7	26.6	1.0	6.6	49.4	2.5	9.8	3.6	0.5
		±3.1	±0.4	±1.6	±3.3	±0.5	±1.2	±1.5	±0.2
5×10^{-6}	2	33.5	2.5	4.5	46.5	3.0	8.0	2.0	tr
		±0.5	±0.5	±0.4	±1.5	±1.5	±0.1	±1.0	
1×10^{-5}	3	43.0	2.0	6.1	42.9	2.5	2.1	0.2	1.2
		±1.2	±1.0	±1.0	±2.7	±1.3	±0.9	±0.1	±0.2
5×10^{-5}	2	43.5	1.7	4.5	45.9	2.6	0.7	tr	1.1
		±4.5	±0.4	±0.5	±3.5	±0.6	±0.2		±0.3
1×10^{-4}	5	44.2	0.3	6.5	45.0	2.8	0.2	0.3	0.7
		±0.4	±0.1	±1.4	±1.4	±0.7	±0.1	±0.1	±0.1
5×10^{-4}	3	46.3	0.8	5.0	44.3	3.0	n.d.	n.d.	0.6
		±3.3	±0.3	±1.2	±6.0	±0.1			±0.2

Results are means ± s.d. For details see Table 1.

Table 3. Labelling of fatty acids in the presence of triallate

Inhibitor conc. (M)	No. of expts.	Fatty acid labelling (% total) 16:0	16:1	18:0	18:1	18:2	20:0	22:0	Other
0	4	31.0 ±1.1	0.8 ±0.3	6.3 ±1.2	40.2 ±4.2	5.4 ±0.9	10.0 ±2.0	5.5 ±1.1	0.8 ±0.2
5×10^{-6}	1	27.7 ±6.5	0.8 ±0.4	7.1 ±1.3	43.3 ±5.0	6.1 ±1.5	9.6 ±3.0	1.5 ±1.3	0.9 ±0.4
1×10^{-5}	4	39.8 ±2.1	0.9 ±0.2	4.8 ±0.7	36.4 ±0.9	7.3 ±2.5	9.4 ±1.0	1.0 ±0.2	0.4 ±0.1
5×10^{-5}	1	40.8 ±4.1	1.0 ±0.2	6.4 ±0.7	38.2 ±3.0	6.6 ±2.6	7.1 ±0.5	tr.	0.5 ±0.2
1×10^{-4}	4	41.8 ±2.6	0.5 ±0.2	7.1 ±0.6	36.6 ±2.1	6.6 ±0.9	5.3 ±0.9	1.0 ±0.3	0.6 ±0.2

wax formation can now be definitely stated to be on acyl chain elongation rather than any specific reaction involved in cutin synthesis and assembly. The postulated reactions of the herbicides with thiol groups generally [18] does not explain why fatty acid synthetase is relatively unaffected. The exact nature of the interaction of the herbicides will be most easily determined when isolated elongation enzymes have been prepared. In addition, the relative sensitivity of fatty acid elongation enzymes to inhibition [cf. 7] may allow the development of more potent herbicides for agricultural use.

EXPERIMENTAL

Ageing and incubation conditions. Potatoes (*Solanum tuberosum* var. King Edward) were aged for 18 hr, incubated with acetate-[^{14}C] and the lipids extracted as previously described [16].

Analysis of lipids. Lipids were separated by TLC on Si gel G using Me_2CO–HOAC–H_2O [98:2:1] and $CHCl_3$–MeOH–HOAc–H_2O [170:30:20:7]. Methods for revealing, eluting and identifying the individual components have been previously given [3, 19, 20]. Alcohols were separated using a solvent system of hexane–Et_2O–HOAc [4]. Fatty acid Me esters were separated by GLC on 10% DEGS, 3% SE-30 or 10% SP-2340 (Supelco) columns using both isothermal and temp programmed operation. Identification was based on Rt with authentic standards on at least two different columns. Confirmation was provided by hydrogenation [21] and oxidation [22] of individual fractions. Identification and quantitation of fatty acids and scintillation counting were carried out as previously described [16].

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