Biochem. J. (1988) 254, 811–817 (Printed in Great Britain)

Effects of the selective herbicide fluazifop on fatty acid synthesis in pea (*Pisum sativum*) and barley (*Hordeum vulgare*)

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Concentrations of fluazifop-butyl sprayed on intact plants caused large decreases in the incorporation of radioactivity from [1-^{14}C]acetate into lipids of barley (*Hordeum vulgare*) leaves and stems, but did not affect leaves or stems of pea (*Pisum sativum*). Labelling of all acyl lipids, but not pigments, was reduced. The effects of the active acid form, fluazifop, were also determined in leaf pieces and chloroplasts. Concentrations of (*R,S*)-fluazifop up to 100 μM had no affect upon quality or quantity of fatty acids produced from [1-^{14}C]acetate in pea. In barley, however, 100 μM-(*R,S*)-fluazifop caused 89% (leaf) or 100% (chloroplasts) inhibition in labelling of fatty acids from [1-^{14}C]acetate. Lower concentrations of fluazifop (< 25 μM) caused incomplete inhibition and significant decreases in the proportion of C_{18} fatty acids synthesized, particularly by isolated chloroplasts. Synthesis of fatty acids from [2-^{14}C]malonate was also inhibited (59%) in barley leaf tissue by 100 μM-(*R,S*)-fluazifop. The labelling pattern of products showed that elongation reactions were unaffected by the herbicide, but synthesis *de novo* was specifically diminished. By using resolved stereoisomers, it was found that the (*R*) isomer was the form which inhibited fatty acid synthesis, a finding that is in agreement with its herbicidal activity. These results suggest that inhibition of fatty acid synthesis *de novo* forms the basis for the selective mode of action of fluazifop.

INTRODUCTION

Several types of herbicides have been shown to affect the synthesis of lipids (Harwood *et al.*, 1987, 1988). Some of these effects are secondary and can be attributed to a diminution in necessary cofactors. However, a few classes of compounds seem to be particularly toxic towards lipid synthesis. Examples of these chemicals would be substituted thiocarbamates such as EPTC (*S*-ethyl dipropylthiocarbamate) (Harwood & Stumpf, 1971; Wilkinson & Smith, 1975), diallate [(*S*)-2,3-dichloroallyl di-isopropyl(thiocarbamate)] and triallate [(*S*-2,3,3-trichloroallyl di-isopropyl (thiocarbamate)] (Bolton & Harwood, 1976*a*,*b*) or the substituted pyridazinones such as San 9785 [4-chloro-5(dimethylamino)-2-phenyl-3(2*H*)-pyridazinone] (St. John, 1976; Willemot *et al.*, 1982; Murphy *et al.*, 1985).

The phenoxyphenoxypropionic acid-derivatized herbicides are a new grass-selective group of compounds. These chemicals cause necrosis in meristematic tissue and necrosis or chlorosis in developing leaf tissues of sensitive plant species (Cho *et al.*, 1986; Duke & Kenyon, 1987). Associated biochemical effects include increases in membrane permeability (Crowley & Prendeville, 1979) an inhibition of auxin-induced reactions (Shimabukuro *et al.*, 1982) and a breakdown of the membranes in different tissues (Brezeanu *et al.*, 1976). Further studies with several of the herbicides have shown that lipid synthesis is inhibited directly rather than secondarily by a decrease in photosynthesis (e.g. Hatzios, 1982; Hoppe, 1981). The most thoroughly examined herbicide is diclofop {2-[4(2′,4′-dichlorophenoxy)phenoxy]propionic acid}. This compound has been used by Hoppe and his associates, who have demonstrated (*a*) that lipid synthesis was affected in root tips and leaves of sensitive plant species (Hoppe, 1981; Hoppe & Zacher, 1982), (*b*) that the lipid effect was due to the inhibition of fatty acid synthesis *de novo* (Hoppe & Zacher, 1982) and (*c*) that other metabolic reactions such as carbohydrate, nucleic acid and protein syntheses were not inhibited significantly (Hoppe, 1981). Fatty acid synthesis by isolated chloroplasts from sensitive, but not from resistant, species was inhibited by diclofop and by its methyl ester, but hardly at all by the non-herbicidal 5′-hydroxy metabolite (Hoppe & Zacher, 1985). Significantly, chloroplast fatty acid synthesis was much more sensitive to the D enantiomer (Hoppe & Zacher, 1985), a finding that agrees with the low herbicidal activity of the L enantiomer *in vivo* (Nestler, 1982).

Fluazifop-butyl {butyl 2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionate} is a post-emergence grass-selective herbicide marketed under the formulation 'Fusilade'. This compound shows high selective activity against monocotyledons and a very low toxicity towards mammals (Plowman *et al.*, 1980). No convincing evidence has been found for major differences in distribution and metabolism between sensitive and resistant plants (B. G. White, unpublished work). Because of the similar physiological and morphological effects of fluazifop compared with diclofop, we have investigated its action on lipid metabolism. In addition, we have examined whether differential effects on lipid synthesis form a basis for its selectivity *in vivo*.

EXPERIMENTAL

Materials

Pea (*Pisum sativum* L., cv. Feltham First) seeds were obtained from Asmer Seeds, Leicester, U. K. Barley

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(*Hordeum vulgare* cv. Maris Otter) seeds were kindly provided by Dr. T. Galliard, RHM Research Ltd., High Wycombe, Bucks., U.K. Seeds were germinated in soilless compost and grown at 20 °C with 650 $\mu E \cdot s^{-1} \cdot m^{-2}$ illumination and a 12 h-day/12 h-night cycle. For chloroplast isolation or leaf-slice experiments the plants were 12 or 14 days old respectively. (*R,S*)-Fluazifop, the racemic mixture, and the resolved (*R*) and (*S*) stereoisomers, were obtained from ICI Agrochemicals.

Sodium [1-^{14}C]acetate (56 mCi/mmol), sodium [2-^{14}C]malonate (1.7 mCi/mmol) and L-[3,4-^{3}H]leucine-(120 mCi/nmol) were purchased from Amersham International, Bucks., U.K., and $NaH^{14}CO_3$ (7 mCi/mmol) from NEN Research Products, [du Pont (U.K.), Stevenage, Herts., U.K.]. Phospholipids, sterol and sterol esters were purchased from Sigma, Poole, Dorset, U.K., and checked for purity by t.l.c. before use. Fatty acid standards were obtained from Nu-Check-Prep, Elysian, MN 56028, U.S.A.

Chloroplast isolation

Chloroplasts were isolated from the leaves of barley and pea plants by the procedure of Mills & Joy (1980), except that bovine serum albumin was omitted from the Percoll solution. The Percoll solution was used at a concentration of 35% (v/v) (barley) or 40% (v/v) (pea). The yield for barley was low, but of good intactness (80–90%). The intactness for pea chloroplasts was 85–90%, both as determined by phase-contrast microscopy.

Incubations

(*a*) Whole tissue. Healthy barley, wheat (*Triticum aestivum*) or pea plants were sprayed with an aqueous suspension of Fusilade (the commercial formulation of fluazifop) corresponding to a 1 mM solution of fluazifop-butyl. Untreated plants were sprayed with water only. At intervals from 3–7 days after treatment, stems and leaves were detached from pea plants. Primary leaves of barley plants were used, these being cut as close to soil level as possible. These tissues were allowed to take up [1-^{14}C]-acetate (1 μCi/leaf or blade) through the cut surface. Uptake was facilitated by a draught of air (15.5×10^3 l/min). Illumination (800 $\mu E \cdot s^{-1} \cdot m^{-2}$) was provided by 'Warm White' lights. At the end of the incubation period the tissues were rinsed briefly and heated in propan-2-ol at 70 °C for 30 min. Extraction and analysis were performed as below.

(*b*) Leaf sections. In the case of barley, 4 cm sections of primary leaves were cut under water to maintain the transpiration stream intact. A section of at least 2 cm at the base of the leaf was rejected in order to avoid taking rapidly differentiating tissue, and leaves of at least 8 cm were chosen to give more uniform width sections. Careful note was made of the polarity of the leaf section so that this polarity of transpiration could be maintained during experiments. One leaf section was used per sample. Leaf sections of pea were cut under water with a 6–5 mm cork borer, and three discs were used per sample. Before incubation, pea leaf discs were vacuum-infiltrated for 10 min at room temperature with the appropriate solutions of substrates, inhibitors etc. During incubations, leaf discs were floated on these solutions, adaxial surface uppermost. For barley, the lower cut surface was placed in a small trough of the appropriate solution and stood vertically, adaxial surface towards the light. Incubations were carried out at room temperature (18–21 °C) for 6 h under constant illumination in a swept sealed chamber. For $^{14}CO_2$ fixation, 1 h before termination, $NaH^{14}CO_3$ solution was injected into the bathing solution of pea discs. In the case of barley, 2 M-formic acid injected through a septum into a vial of $NaH^{14}CO_3$ solution released $^{14}CO_2$ into the sealed non-swept chamber.

(*c*) Chloroplasts. For acetate incubations, intact chloroplast preparations were resuspended in 25 mM-potassium phosphate (pH 7.9) / 330 mM-sorbitol / 1 mM-$MgCl_2$ / 10 mM-$NaHCO_3$ ± fluazifop. The 1 ml incubations were started immediately by the addition of 10 μl (1 μCi) of [1-^{14}C]acetate and incubated for 1 h at 25 °C under constant illumination (800 $\mu E \cdot s^{-1} \cdot m^{-2}$).

In general, all chloroplast and whole-tissue experiments were carried out with triplicate samples for control and each treatment, or in duplicate in the case of leaf-piece experiments.

Lipid extraction and analysis

***(a)* Total lipids.** For the analysis of acyl lipids, pigments and other chloroform-soluble material, incubations were stopped by the addition of propan-2-ol. Samples were then heated at 70 °C for 30 min in order to inactivate any endogenous lipases. Extraction was then continued by the method of Garbus *et al.* (1963), with propan-2-ol substituting for methanol. This procedure has previously been shown to give quantitative extraction of all major plant lipids, including polar components (cf. Harwood, 1980). Neutral lipids were separated by t.l.c. on prepared silica-gel G plates (E. Merck, Darmstadt, Germany) using light petroleum (b.p. 60–80 °C)/diethyl ether/acetic acid (90:10:1, by vol.) and polar lipids by using chloroform/methanol/acetic acid/water (170:30:20:7, by vol.) as solvent. Lipid bands were revealed by spraying with 0.02% (w/v) ethanolic 8-anilino-4-naphthosulphonic acid and exposing to u.v. light. The identity of individual lipid bands was routinely made by reference to standards run simultaneously, but all major lipids in barley, wheat and pea had been fully identified previously (Bolton & Harwood, 1977; Wharfe & Harwood, 1978). Radiolabelled bands were revealed by photography in a spark chamber (Birchover Instruments, Hitchin, Herts., U.K.). Individual bands were either scraped off and counted for radioactivity or methylated for fatty acid analysis as described below.

(*b*) Fatty acids. When only fatty acids were to be analysed, chloroplast incubations were terminated by the addition of aq. 60% (w/v) KOH to give a final concentration of 6% (w/v). Extractions were continued as described by Walker & Harwood (1985). Incubations with leaf sections were stopped and extracted as described for lipid extraction, except that lipid extracts were transmethylated. Transmethylations were performed with 2.5% (v/v) H_2SO_4 in dry methanol at 70 °C for 2 h. Analysis of the fatty acid methyl esters was by g.l.c. in glass columns (1.5 m × 4 mm internal diameter) containing 15% EGSS-X on 100–120-mesh Chromosorb W-AW (Supelco, Bellefonte, PA, U.S.A.) with isothermal runs at 190 °C and either a Pye 104 or Pye GCD gas chromatograph connected to a Panax or Ray-test RAGA gas-flow proportional counter.

Table 1. Effect of spraying barley or pea plants with fluazifop-butyl on their subsequent ability to incorporate radioactivity from [1-^{14}C]acetate into lipids

'†' indicates that plants were sprayed twice, with a second spraying after 3 days. Treatment with fluazifop did not affect the uptake of [1-^{14}C]acetate into the tissues. Results are means ± S.D. An asterisk (*) indicates that the result was significant by Student's t test ($P < 0.05$). Abbreviation used: tr, trace (< 0.5%).

Species and tissue	Time after treatment (days)	Fluazifop-butyl	10^{-5} Total lipid radioactivity (c.p.m.)	Distribution of radioactivity (% of total lipid)			
				Pigments	Neutral lipids	Glycolipids	Phospho-lipids
Barley							
Leaves	3	−	7.6±1.9	33±2	18±1	24±2	25±3
	3	+	2.1±0.7*	81±6*	9±4*	6±3*	4±2*
Stems	3	−	11.6±0.5	16±2	13±2	18±2	53±5
	3	+	11.9±0.3	17±3	19±5	19±2	45±8
Leaves	4	−	4.7±0.3	22±2	15±1	27±6	36±3
	4	+	3.3±0.3*	33±3*	14±1	22±3	31±1
Leaves	6†	−	4.3±0.2	24±2	14±1	34±4	48±4
	6†	+	2.3±tr*	49±4*	14±1	22±3*	15±3*
Stems	6†	−	10.9±2.2	18±1	16±1	26±5	40±5
	6†	+	4.1±1.1*	38±5*	13±3	19±1	30±4
Pea							
Leaves	4	−	1.5±0.7	14±2	5±1	26±1	55±4
	4	+	1.2±0.1	14±1	6±1	27±2	53±3
Stems	4	−	5.2±tr	19±4	6±1	20±1	55±5
	4	+	7.7±3.4	13±1	6±1	22±1	59±3

Other analyses

$NaH^{14}CO_3$ incubations were terminated by washing briefly then freezing in liquid N_2. Frozen samples were then ground and extracted, and water-soluble products were further separated by Sephadex ion-exchange chromatography as described by Redgewell (1980).

[^{3}H]Leucine incubations were terminated and extracted by the method of Willemot & Stumpf (1967) to produce cleared leaf samples for radioactivity counting.

Radioactivity counting

Cleared leaf samples were incubated for 2 h at 50 °C in 1 ml of Soluene-350 (Packard, IL, U.S.A.). Portions of these solubilized extracts and lipid samples were counted in a scintillant consisting of PCS(Amersham–Searle)/xylene (2:1, v/v) using an Intertechnique SL4000 liquid-scintillation counter. Quench corrections were made by the external-standard method. Efficiencies were never less than 68% for any aqueous or non-aqueous sample.

RESULTS AND DISCUSSION

Previous experiments had shown that fluazifop-butyl treatment of susceptible plants produced physiological symptoms similar to those caused by diclofop-methyl treatment. Since the latter had been shown to inhibit lipid synthesis, we carried out experiments with whole plants to determine whether fluazifop-butyl caused comparable inhibition. The results in Table 1 show clearly that treatment of barley with fluazifop-butyl caused a significant decrease in the incorporation of radioactivity from [1-^{14}C]acetate into total leaf lipids. Similar results were also obtained with another monocotyledon, wheat (results not shown). Longer treatment periods also decreased the labelling of stem lipids. Analysis of the whole-leaf lipid fraction by t.l.c. revealed that, whereas the total radioactivity in the pigment fraction was virtually unchanged, that in acyl lipid fractions was considerably diminished. Previous experiments (e.g. those of Wharfe & Harwood, 1978) had shown that almost all (> 90%) of the radioactivity from [^{14}C]acetate is found in the acyl chains of phospholipids, whereas the glycolipid fraction contained a significant proportion in the sugar residues. Therefore, it was significant that the percentage decrease in total radioactivity in the phospholipid fraction tended to be the greatest and was much more than the neutral-lipid fraction, which also contains sterols and their derivatives. These data together suggested that fluazifop-butyl decreased lipid synthesis at the level of fatty acid formation or acylation.

In contrast with the data for barley, no significant effect was seen on lipid synthesis in pea plants, which represent a species physiologically resistant to fluazifop-butyl. Thus neither the total lipid radioactivity nor the distribution of radioactivity between lipid classes was altered (Table 1).

The specificity of the actions of fluazifop-butyl on lipid synthesis was examined further by the use of different precursors and by the analysis of other compounds. Whereas the incorporation of radioactivity from [1-^{14}C]-acetate into the total lipids of barley leaf pieces was severely diminished, the water-soluble compounds were

Table 2. Effect of 100 μM-*(R,S)*-fluazifop (acid form) on incorporation of radioactivity from different labelled precursors

Incubations were carried out in triplicate with leaf pieces as described in the Experimental section. Abbreviation: n.a., not analysed separately.

Precursor	Tissue	Treatment	10^{-3} × Radioactivity in fractions (d.p.m.) Lipid	Water-soluble	Protein
[1-^{14}C]Acetate (5 μCi/sample)	Barley	Control	537.4	53.4	n.a.
		Treated	63.0	144.4	n.a.
	Pea	Control	667.5	56.8	n.a.
		Treated	735.8	49.5	n.a.
$NaH^{14}CO_3$ (6.6 μCi/sample)	Barley	Control	38.0	374.7	n.a.
		Treated	13.0	421.0	n.a.
	Pea	Control	89.0	1012.9	n.a.
		Treated	92.0	1046.1	n.a.
[^{3}H]Leucine (9 μCi/sample)	Barley	Control	n.a.	n.a.	18.0
		Treated	n.a.	n.a.	19.4
	Pea	Control	n.a.	n.a.	33.3
		Treated	n.a.	n.a.	31.9

Table 3. Influence of *(R,S)*-fluazifop on fatty acid biosynthesis from [1-^{14}C]acetate by barley or pea leaf pieces

Abbreviations: tr, trace (< 0.5%); n.d., none detected. Results are means ± S.D. Statistical analysis was by Student's *t* test for paired samples, with the number of experiments being five.

Species	Treatment	Total incorporation (% of control)	Fatty acid...	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids) $C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$†	$C_{22:0}$	$C_{24:0}$
Barley	None	100		tr	13±4	tr	2±tr	26±8	49±9	8±2	1±1	n.d.
	100 μM-Fluazifop	11±5*		3±1	18±1*	2±1*	12±2*	12±3*	18±5*	9±2	22±5*	4±6*
Pea	None	100		tr	21±4	tr	5±2	27±2	40±5	7±2	3±1	tr
	100 μM-Fluazifop	105±15		tr	17±4	tr	5±2	26±3	42±2	7±2	3±1	n.d.

* Significantly different from controls ($P < 0.05$).
† Includes $C_{20:0}$.

increasingly labelled (Table 2), presumably because of feedback from lipid precursors. Again, there was no effect on the labelling of pea fractions from [1-^{14}C]acetate (Table 2). The water-soluble fraction, which included organic acids and carbohydrates, was also labelled from photosynthetically assimilated $^{14}CO_2$. Fluazifop-butyl did not affect the labelling of this fraction in either barley or pea, nor did it alter the labelling of proteins from [^{3}H]leucine (Table 2). Thus fluazifop-butyl appeared to resemble diclofop-methyl (Hoppe, 1981; Hoppe & Zacher, 1982) in that its only effect on synthesis in susceptible plants appeared to be to decrease (acyl) lipid formation.

The affect of fluazifop on fatty acid synthesis was examined further by using leaf pieces from barley and pea (Table 3). Interestingly, the inhibition of labelling from [^{14}C]acetate was not the same for all fatty acids of barley. Thus the labelling of total C_{18} fatty acids was particularly decreased. In fact, the total labelling of very-long-chain fatty acids (C_{20}–C_{24}) was not significantly diminished. These acids [as well as a portion of the stearate (see Harwood, 1988)] are synthesized by elongation systems outside the chloroplast (Cassagne *et al.*, 1987), whereas the plastid is responsible for synthesis *de novo* of C_{16} and C_{18} products (see Stumpf, 1980; Harwood, 1988). Therefore one can conclude that fluazifop has a primary effect on fatty acid synthesis *de novo*. As mentioned above, the control (herbicide-resistant) tissue, namely that of pea, was not affected by fluazifop (Table 3). In all these experiments we used the free acid, fluazifop, because this is known to be the active form of the herbicide (Hendley *et al.*, 1985) and so that we could compare the results with later data for subcellular fractions, which may have lacked the esterase needed to activate the butylform. However, fluazifop-butyl also produced exactly comparable results with tissue pieces (results not shown).

Two enzyme systems are, of course, required for fatty

Table 4. Influence of (*R,S*)-fluazifop on fatty acid biosynthesis from [2-^{14}C]malonate by barley or pea leaf pieces

Results are means ± S.D. for three experiments performed in duplicate. Statistical analysis was by Student's *t* test for paired samples. Abbreviation: n.d., none detected.

Species	Treatment	Total incorporation (% of control)	Fatty acid...	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids)							
				$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$†	$C_{22:0}$	$C_{24:0}$
Barley	Control	100		tr	4±1	8±1	5±2	8±4	8±2	31±5	36±6
	100 μM-Fluazifop	41±5*		tr	n.d.*	16±2*	n.d.*	n.d.*	12±3*	40±5*	32±3*
Pea	None	100		tr	23±3	6±1	25±2	5±1	7±1	7±2	6±2
	100 μM-Fluazifop	81±15		1±tr	22±5	6±2	26±3	5±2	8±2	8±2	6±5

* Significantly different ($P < 0.10$).
† Confirmed by argentation t.l.c.

Table 5. Concentration effects of fluazifop on the pattern of fatty acids made from [1-^{14}C]acetate by barley leaf tissue or isolated chloroplasts

Results are shown as means ± S.D. for one (tissue) or three (chloroplasts) experiments performed in triplicate. Abbreviations: as for Table 3, and: n.a., insufficient incorporation for analysis; n.d., none detected.

Tissue or organelle	Fluazifop acid stereo-isomer	Concn. (μM)	Total synthesis (% of control)	Fatty acid...	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids)							
					$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$ + $C_{20:0}$	$C_{22:0}$
Leaf	(*R*)	0	100		tr	11±1	n.d.	2±tr	21±2	57±tr	10±tr	n.d.
		5	24		3±1	26±4	n.d.	6±1	11±1	31±3	10±3	13±3
		25	18		3±3	22±2	n.d.	12±tr	10±3	17±tr	15±6	21±5
		50	16		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Chloroplasts	(*R,S*)	0	100		4±2	32±4	12±4	7±3	43±5	n.d.	n.d.	n.d.
		5	43±9		6±4	36±8	22±6	n.d.	35±10	n.d.	n.d.	n.d.
		15	27±2		6±3	33±4	30±3	n.d.	32±7	n.d.	n.d.	n.d.
		25	22±7		10±4	52±7	36±11	n.d.	n.d.	n.d.	n.d.	n.d.
		100	n.d.		n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

acid formation *de novo*: acetyl-CoA carboxylase and fatty acid synthetase. When [2-^{14}C]malonate was used as precursor, fluazifop was still found to inhibit fatty acid labelling (Table 4). Bearing in mind the much higher rates of elongation which are seen with [^{14}C]malonate as precursor, the inhibition of synthesis *de novo* by fluazifop was at least as great with [2-^{14}C]malonate (Table 4) as with [1-^{14}C]acetate (Table 3). Hoppe & Zacher (1982) concluded, from similar experiments, that fatty acid synthetase was the target for diclofop-methyl. However, decarboxylation of [2-^{14}C]malonate to [2-^{14}C]acetate in barley and pea tissues (results not shown) does not allow such a conclusion to be made in our experiments.

It is accepted generally that fatty acid synthesis *de novo* in leaves is concentrated in the chloroplast (Stumpf, 1980; Harwood, 1988). Accordingly, these organelles were challenged directly with fluazifop. Whereas fatty acid synthesis in chloroplasts isolated from barley was greatly decreased (Table 5), that in chloroplasts from pea leaves was unaffected (results not shown). Isolated barley chloroplasts form mainly a mixture of saturated and monoenoic C_{16} and C_{18} acids, and it is noticeable that increasing concentrations of fluazifop altered the pattern of products, with C_{18} acids representing a smaller percentage of the total. Thus the production of C_{18} acids seemed to be particularly susceptible to fluazifop inhibition in isolated chloroplasts, as well as in tissue pieces (Tables 3 and 5).

This modification of the proportion of C_{16} and C_{18} and products by fluazifop can be explained in two ways. If fatty acid synthetase is the target for the herbicide, then the only individual enzymes which have a chain-length specificity are the condensing enzymes (see Harwood, 1988). Two β-oxoacyl-acyl-carrier-protein synthetases have been reported in plants, and the inhibition of β-oxoacyl-acyl-carrier-protein synthetase II leads to palmitate being the end-product of the fatty acid synthetase (Shimakata & Stumpf, 1982). Thus if fluazifop inhibits the condensing enzymes, one would predict that β-oxoacyl-acyl-carrier-protein synthetase II would be particularly susceptible and, hence, account for the change in the relative percentage of the end products.

Table 6. Effect of stereoisomeric forms of fluazifop on fatty acid synthesis by barley leaf tissue or isolated chloroplasts

The (*S*) isomer of fluazifop contained 7% of the (*R*) isomer as contaminant. Results shown are means ± S.D. Abbreviations are as for Table 3; further abbreviation: VLCFA, very-long-chain fatty acids (includes $C_{18:3}$, $C_{20:0}$, $C_{22:0}$ and $C_{24:0}$).

Tissue or organelle	Fluazifop		Total synthesis (% of control)	Fatty acid...	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids)						
	Stereo-isomer	Concn. (μM)			$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	VLCFA
Leaf	–	0	100		tr	14±1	n.d.	2±tr	32±11	45±11	8±1
	(*S*)	50	77±2		2±1	26±3	n.d.	5±2	19±8	33±4	16±7
	(*R*)	50	15±6		2±tr	21±1	n.d.	14±1	15±tr	8±1	38±5
	(*R*,*S*)	100	13±7		2±tr	18±1	n.d.	12±2	9±2	16±5	43±6
Chloroplasts	–	0	100		1±tr	33±5	10±3	6±2	46±5	n.d.	n.d.
	(*S*)	17.5	43		2±tr	38±3	14±4	4±1	40±3	n.d.	n.d.
	(*R*)	17.5	13		4±tr	56±4	28±5	n.d.	11±4	n.d.	n.d.
	(*R*,*S*)	35	14		5±tr	55±5	26±4	n.d.	12±6	n.d.	n.d.

Alternatively, if fluazifop inhibits acetyl-CoA carboxylase, then the increased ratio of primer (acetyl-CoA) to malonyl-CoA would also be expected to lower the chain length of the fatty acid synthetase products (see Harwood, 1988). Direct measurement of fatty acid synthetase and acetyl-CoA carboxylase activities showed that only the latter is inhibited by fluazifop (see below).

For inhibition of fatty acid synthesis *de novo* to be a primary target for fluazifop, then it was important to see whether such inhibition showed the same stereochemical requirements as the herbicidal activity. Data obtained with both leaf pieces and isolated chloroplasts clearly showed that the (*R*) isomer, which is 100 times as herbicidally active as the (*S*) isomer (Dicks *et al.*, 1985), was by far the most effective stereoisomer at inhibiting fatty acid labelling from [1-^{14}C]acetate (Table 6). Unfortunately, no sample of the (*S*) isomer could be completely resolved from the (*R*) isomer, so that residual activity was still found with the preparations used (Table 6). It was, however, noticeable that inhibition of fatty acid synthesis by the (*R*) isomer was equal to the value obtained by exactly twice the concentration of the (*R*,*S*) isomer mixture. Again, control experiments with pea leaf pieces or chloroplasts showed no action by fluazifop (results not shown). Therefore the effect on fatty acid synthesis by fluazifop shows all the specificity requirements for its herbicidal action on plants. Another phenoxyphenoxypropionic acid derivative, diclofop-methyl, also shows stereochemical specificity in its inhibition of fatty acid labelling (Hoppe & Zacher, 1985), which agrees with its herbicidal activity (Nestler, 1982).

The above experiments have demonstrated that physiologically relevant concentrations of fluazifop prevent acyl lipid formation by inhibiting plastid-localized synthesis of fatty acids *de novo*. This mode of action may be general for the phenoxyphenoxypropionic acid-derived herbicides (see Harwood *et al.*, 1988). Of particular interest is that the herbicidal specificity is also maintained throughout the fatty acid inhibition studies. Taken together with the lack of convincing differences in the uptake or metabolism of [^{14}C]fluazifop by susceptible or resistant plants, this points to the site of action as confirming specificity.

It is also noteworthy that Burton *et al.* (1987) have recently reported that sethoxydim and haloxyfop (two structurally unrelated herbicides which produce similar symptoms to fluazifop) inhibit acetyl-CoA carboxylase in susceptible plants. Our observation (Tables 3 and 5) that a shift in pattern of fatty acid labelling towards shorter chains is seen with intermediate concentrations of fluazifop would be in agreement with acetyl-CoA carboxylase being the target enzyme. Assays *in vitro* of the carboxylase and fatty acid synthetase show that the former is inhibited by fluazifop (K. A. Walker, S. M. Ridley & J. L. Harwood, unpublished work). Further experiments are required to demonstrate clearly that acetyl-CoA carboxylase is the target enzyme for fluazifop in susceptible plants.

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Received 5 February 1988/28 March 1988; accepted 21 April 1988