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DIFFERENTIAL RESPONSES OF A RANGE OF PHOTOSYNTHETIC TISSUES TO A SUBSTITUTED PYRIDAZINONE, SANDOZ 9785. SPECIFIC EFFECTS ON FATTY ACID DESATURATION

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Key Word Index—Lipid metabolism; fatty acid synthesis; desaturation; Sandoz 9785; pyridazinone herbicide.

Abstract—The effect of a substituted pyridazinone (4-chloro-5(dimethylamino)-2-phenyl-3(2H)pyridazinone; Sandoz 9785; BASF 13-338) on the formation of fatty acids from radiolabelled precursors has been studied in a number of angiosperms, bryophytes and algae. The labelling of [^{14}C]linolenic acid was decreased by the herbicide in leaves of barley and rye grass and in cucumber cotyledons regardless of whether [^{14}C]acetate, [^{14}C]oleate or [^{14}C]linoleate was used as precursor. A commensurate increase in the labelling of [^{14}C]linoleic acid was also observed in these species. In contrast, the pattern of fatty acid labelling in maize, pea and spinach leaves was unaffected by 0.1 mM Sandoz 9785. More generalized inhibition of the incorporation of radioactivity from [^{14}C]acetate into the fatty acids of bryophytes and algae was seen. Sandoz 9785 did not alter the distribution of radioactivity in different lipid classes of higher plant leaves, nor did it change the proportions of radioactive fatty acids in phosphatidylcholine. In contrast to phosphatidylcholine, which never contained more than trace amounts of [^{14}C]linolenate, diacylgalactosylglycerol contained high levels of the radioactive acid. The relative labelling of linolenate was severely reduced in diacylgalactosylglycerol by Sandoz 9785 in sensitive angiosperms. Uptake studies, in which [^{3}H]Sandoz 9785 was employed demonstrated that the uptake of Sandoz 9785 was a reflection of water uptake. Following its uptake, Sandoz 9785 was rapidly converted into other compounds in pea but only gradually metabolized in cucumber and ryegrass. The results are interpreted as showing, firstly, that the different sensitivity of higher plants to Sandoz 9785 is due to variations both in uptake and in metabolism. Secondly, Sandoz 9785 specifically inhibits the desaturation of linoleate to linolenate and, thirdly, diacylgalactosylglycerol plays a role in this conversion.

INTRODUCTION

The photosynthetic membranes of plants are notable for their exceptionally high content of glycosylglycerides containing α-linolenic acid [1, 2]. Since α-linolenic acid constitutes up to 70% of the total acyl residues of photosynthetic tissues, the pathway of its biosynthesis has attracted considerable attention recently [3, 4]. Most, if not all, of the α-linolenic acid is formed following the sequential desaturation of stearic acid via oleate and linoleate [5, 6]. Diacylgalactosylglycerol may be involved in the terminal desaturation in some leaf tissues [7–9] but not in other tissues [3]. However, the exact mechanisms of linolenate formation has yet to be elucidated [3].

In an attempt to learn more about α-linolenate biosynthesis we have employed 4-chloro-5(dimethylamino)-2-phenyl-3(2H)pyridazinone (Sandoz 9785, BASF 13-338). This compound has been shown to affect selectively the levels of α-linolenate in several species of higher plants without causing any gross change in leaf development and chloroplast content [10–14]. Labelling studies with fatty acid precursors, such as $^{14}CO_2$ and [^{14}C]acetate, have demonstrated a reduction α-linolenate radiolabelling in the presence of Sandoz 9785 and a concommitant increase in the levels of [^{14}C]linoleate [11, 15–17].

Sandoz 9785 has been suggested to have a direct effect upon the conversion of linoleate to α-linolenate [13]. Although this appears to be the primary effect of Sandoz 9785, effects have also been reported upon photosynthetic oxygen evolution [15, 17], thylakoid ultrastructure [11, 12, 18] and chlorophyll-proteins [12]. It has been reported that, whereas Sandoz 9785 inhibited the incorporation of [^{14}C]acetate into α-linolenate in spinach leaf discs, it had no effect upon this incorporation in either isolated chloroplasts or whole leaves of spinach [14]. The effects of Sandoz 9785 upon photosynthetic oxygen evolution that were reported for both *Vicia faba* leaf discs [14, 15] and isolated spinach chloroplasts were not found in the case of whole leaves of barley [12]. It therefore appears that Sandoz 9785 may be quite variable in its effects upon plants depending upon the species studied and the pretreatment of the tissue.

In this study we examine the uptake and metabolism of Sandoz 9785 and its effects upon fatty acid biosynthesis in a wide variety of both higher and lower plants. The results clearly demonstrate the rapid uptake of Sandoz 9785 into all plants tested. Low concentrations of the herbicide

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selectively inhibited the desaturation of linoleate to α-linolenate, but only in sensitive species of higher plants. More general effects were observed with bryophytes and algae.

RESULTS

Uptake and metabolism of Sandoz 9785

The rates of uptake of [^{3}H]Sandoz 9785 into several higher plant tissues are shown in Table 1. The rates of uptake varied from about 10 nmol. $gFW^{-1}\,hr^{-1}$ for pea to about 85 nmol. $gFW^{-1}\,hr^{-1}$ for ryegrass. These rates apparently reflected the rates of water uptake in each of the various species since no change in cpm ml^{-1} of the solutions in which the plants were placed was observed over a 24 hr uptake period in spite of substantial uptake of solution. The patterns and extent of metabolism of the incorporated [^{3}H]Sandoz 9785, as measured by its conversion into chromatographically-distinguishable products, varied markedly among the tissues used. The products of [^{3}H]Sandoz metabolism in pea leaves were noticably different from those in the other tissues tested. Chromatographically-distinct metabolites of [^{3}H]Sandoz accounted for 97% of the total [^{3}H]-label in pea leaves after 6 hr, while they only accounted for 33–48% of the label in the other three tissues.

Effect on fatty acid synthesis in different plants

Initial experiments on the incorporation of radioactivity from [1-^{14}C]acetate into the fatty acids of spinach, barley and ryegrass leaves indicated considerable variation between different plant species. Accordingly, the studies were extended to include other species of angiosperms, bryophytes and algae. The effect of Sandoz 9785 on fatty acid synthesis from [^{14}C]acetate in higher plants is shown in Table 2. Three monocotyledonous and three dicotyledonous plants were examined. In none of these plants was the total incorporation of radioactivity into lipids affected markedly by the herbicide during the experimental period. However, the pattern of fatty acids

Table 1. Uptake and metabolism of [^{3}H]-Sandoz 9785 by different higher plant tissues

Tissue	Uptake (nmol. g $FW^{-1}\,hr^{-1}$)			Metabolism (nmol. g $FW^{-1}\,hr^{-1}$)		
	Incubation time (hr)					
	3	6	24	3	6	24
Barley leaf	9.85	8.36	6.08	5.25	3.49	3.22
Pea leaf	22.41	33.64	24.47	17.33	32.53	17.72
Ryegrass leaf	84.85	84.19	75.00	28.78	27.95	21.79
Cucumber cotyledon	29.94	22.96	n.m.	7.21	11.02	n.m.

Experiments were carried out as described in the Experimental. 'Metabolism' refers to the conversion of [^{3}H]Sandoz 9785 into other compounds following its uptake into the plants, as monitored by the chromatographic procedures outlined in the Experimental. Sandoz 9785 was present at 10^{-4} M concentration. Abbreviation: n.m., not measured.

Table 2. The effect of 10^{-4} M Sandoz 9785 on the incorporation of [1-^{14}C]acetate into the lipids of higher plant leaves

Species	Treatment	Total incorporation (cpm/leaf or cpm/cotyledon)	Distribution of radioactivity (% total [^{14}C] fatty acids)					
			16:0	16:1	18:0	18:1	18:2	18:3
Barley (monocot leaf)	None	51300	17.0	n.d.	4.1	15.5	41.4	22.0
	San 9785	56500	17.7	n.d.	4.4	14.4	53.6	10.9
Ryegrass (monocot leaf)	None	51900	22.7	n.d.	1.3	22.4	39.2	12.4
	San 9785	44300	22.2	tr	tr	21.9	51.3	3.1
Maize (monocot leaf)	None	55200	19.3	n.d.	n.d.	14.3	53.2	13.3
	San 9785	42000	22.3	n.d.	n.d.	12.9	52.1	11.6
Cucumber (dicot cotyledon)	None	130000	46.7	n.d.	tr	15.1	19.7	18.5
	San 9785	131000	42.3	n.d.	2.7	17.3	24.9	12.6
Pea (dicot leaf)	None	68600	19.4	n.d.	5.3	13.4	52.4	8.7
	San 9785	66700	19.3	n.d.	5.0	13.7	53.5	6.9
Spinach (dicot leaf)	None	128000	22.0	n.d.	3.2	20.8	29.1	24.0
	San 9785	117600	23.1	n.d.	5.0	19.2	28.3	22.7

Experiments were carried out as described in the Experimental with incubations for 13 hr. Abbreviations: nd, not detected; tr, trace; 16:0, palmitic acid; 16:1, hexadecenoic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, α-linolenic acid.

made was altered significantly in some of the plant types. Thus, the proportion of radioactive linolenate in ryegrass was decreased from 12% to 3% with a commensurate rise in [^{14}C]linoleate. In barley the decrease in [^{14}C]linolenate was from 22% to 11% while the third monocotyledon, maize, did not show a change in the proportions of acid products after treatment with Sandoz 9785. Of the dicotyledons, cucumber showed a decrease of [^{14}C]linolenate from 19% to 13%, while pea and spinach did not exhibit any significant change. The percentage of the other principal fatty acid products, palmitate, stearate and oleate, showed little change in any of the angiosperm species studied.

In contrast to angiosperms, the incorporation of radioactivity from [^{14}C]acetate into the fatty acids of bryophytes and algae was severely inhibited by the presence of 10^{-4} M Sandoz 9785 (Table 3). The percentage inhibition of acetate incorporation into acyl residues was 72% for *Chlorella pyrenoidosa*, 95% for *Chlorella vulgaris*, 63% for *Azolla fuliculoides* (a temperate species), 84% for *Azolla mexicana* (a sub-tropical species) and 94% for *Anabaena cylindrica*. Variation was considerable, not only in the pattern of fatty acids made, but also in the effects of Sandoz 9785 on that pattern. The percentage of [^{14}C]palmitate was increased in both *Azolla fuliculoides* and *Chlorella vulgaris* while it was decreased in *Anabaena cylindrica*. The pattern of [^{14}C]fatty acid products in *Azolla mexicans* and *Chlorella pyrenoidosa* were relatively unaffected by the herbicide. Although all five of the species used contained endogenous α-linolenic acid, only *Chlorella vulgaris* synthesized radiolabelled linolenate during the experimental period.

Time-course of [^{14}C]acetate, [^{14}C]oleate and [^{14}C]linoleate incorporation into fatty acids of cucumber cotyledons

Since part of the reason for the different susceptibility of higher plant species towards Sandoz 9785 could have been the variation in uptake of the herbicide or its conversion to a toxic or non-toxic intermediate, the time-course of the effect on a plant of moderate susceptibility was examined (Table 4). The earliest time at which [^{14}C]linolenate was detected in cucumber with [^{14}C]acetate or [^{14}C]oleic acid as substrates was about 6 hr. In the presence of herbicide, little labelled linolenate was detected. At 9 and 13 hr the inhibition of linolenate labelling was 54% and 51% for [^{14}C]acetate as substrate, 68% and 58% for [^{14}C]oleic acid as substrate and 78% and 58% for [^{14}C]linoleic acid as substrate. These results showed that the herbicide rapidly reached a subcellular site where it or a toxic metabolite could inhibit desaturation.

Distribution of radioactive fatty acids in different lipids

Radiolabelled lipids which were obtained from different tissues after incubation with different [^{14}C]substrates were separated by thin layer chromatography (cf. Experimental) and their individual fatty acid compositions determined. This was undertaken because firstly, the effect of Sandoz 9785 was seen particularly in certain lipids [11, 17, 19] and, secondly, because fatty acid exchange between different lipids may be an integral part of the desaturation process [9, 20–22]. The major radiolabelled lipids were the galactosylglycerides and phosphatidylcholine in all six species of angiosperm tested. The actual percentage of radioactivity in each lipid class varied considerably for different plant species, as previously noted [22]. The two most interesting lipids with regard to the above point are phosphatidylcholine and diacylgalactosylglycerol. The proportions of their [^{14}C]fatty acids, which were labelled from [1-^{14}C]oleic and [1-^{14}C]linoleic acids, are shown in Table 5. Sandoz 9785 caused a marked decline in the proportion of linolenate labelled from [1-^{14}C]oleic acid in diacylgalactosylglycerol. A simultaneous increase in the proportion of [^{14}C]linoleate in this lipid was also seen in both barley and cucumber. In contrast, the distribution of radioactivity in the fatty acids of phosphatidylcholine was unaffected. The proportion of [^{14}C]linolenate in diacylgalactosylglycerol formed from [1-^{14}C]linoleic acid was also severely reduced by the herbicide in both plant species.

Several investigators have commented on the high levels of fatty acid and/or complex lipid exchange which may take place in higher plant leaves. Because such exchange reactions may be involved in the overall desatur-

Table 3. The effect of Sandoz 9785 on the incorporation of [1-^{14}C]acetate into the lipids of lower plants

Species	Addition	Total incorporation (cpm)	Distribution of radioactivity (% total [^{14}C] fatty acids)					
			16:0	16:1	18:0	18:1	18:2	18:3
Azolla fuliculoides	None	94230	18.5	n.d.	n.d.	71.0	10.4	n.d.
(6 hr)	+San 9785	35200	31.9	n.d.	n.d.	58.7	9.4	n.d.
Azolla mexicana	None	104400	50.0	n.d.	n.d.	40.0	10.0	n.d.
(6 hr)	+San 9785	17000	46.0	n.d.	tr	34.7	19.3	n.d.
Chlorella vulgaris	None	2346100	6.0	n.d.	n.d.	69.4	18.3	6.4
(8 hr)	+San 9785	118580	46.8	n.d.	n.d.	24.3	22.4	6.5
Chlorella pyrenoidosa	None	658700	85.0	n.d.	n.d.	10.0	5.2	n.d.
(8 hr)	+San 9785	184500	85.0	n.d.	n.d.	10.0	5.0	n.d.
Anabaena cylindrica	None	68000	30.8	n.d.	5.0	28.1	36.0	n.d.
(3 hr)	+San 9785	3840	6.1	n.d.	n.d.	26.1	67.8	n.d.

Incubations were carried out as described in the Experimental in the presence or absence of 10^{-4} M Sandoz 9785. Incubation times were as indicated in parentheses. For abbreviations see Table 2.

Table 4. Time course of the incorporation of different precursors into the fatty acids of cucumber cotyledons in the presence or absence of Sandoz 9785

Precursor	Treatment	Time (hr)	Distribution of radioactivity (% total ^{14}C)				
			16:0	18:0	18:1	18:2	18:3
[1-^{14}C]Acetate	Control	3	44.4	tr	55.6	tr	n.d.
		6	40.0	3.1	32.5	20.0	4.4
		9	41.2	2.9	23.7	22.3	9.6
		13	38.6	1.1	19.1	23.4	17.6
	Treated	3	51.3	n.d.	37.0	11.6	n.d.
		6	42.8	1.7	30.2	23.2	2.0
		9	39.7	3.6	25.5	26.8	4.4
		13	43.4	2.4	16.7	28.9	8.6
[1-^{14}C]Oleate	Control	3	n.d.	n.d.	94.7	5.3	n.d.
		6	n.d.	n.d.	83.9	10.8	5.3
		9	n.d.	n.d.	58.6	22.6	18.8
		13	n.d.	n.d.	42.3	34.7	22.9
	Treated	3	n.d.	n.d.	88.8	11.2	n.d.
		6	n.d.	n.d.	82.8	15.4	1.8
		9	n.d.	n.d.	69.6	24.1	6.0
		13	n.d.	n.d.	44.7	45.7	9.6
[1-^{14}C]Linoleate	Control	3	n.d.	n.d.	n.d.	91.5	8.5
		6	n.d.	n.d.	n.d.	86.3	13.7
		9	n.d.	n.d.	n.d.	75.5	24.5
	Treated	3	n.d.	n.d.	n.d.	100	n.d.
		6	n.d.	n.d.	n.d.	96.9	3.1
		9	n.d.	n.d.	n.d.	89.7	10.3

Table 5. The effect of Sandoz 9785 on the incorporation of [^{14}C]oleate and [^{14}C]linoleate into the fatty acids of phosphatidylcholine and diacylgalactosylglycerol in barley and cucumber

Plant	Precursor	Treatment	Lipid	Distribution of radioactivity (% total ^{14}C fatty acids)		
				18:1	18:2	18:3
Barley	[1-^{14}C]Oleate	None	DGG	23.9	48.7	27.0
		+Sandoz 9785	DGG	31.2	63.8	4.6
	[1-^{14}C]Oleate	None	PC	41.0	58.9	tr
		+Sandoz 9785	PC	45.0	54.8	n.d.
	[1-^{14}C]Linoleate	None	DGG	n.d.	56.1	43.9
		+Sandoz 9785	DGG	n.d.	80.9	19.0
Cucumber	[1-^{14}C]Oleate	None	DGG	44.3	28.1	27.7
		+Sandoz 9785	DGG	45.8	44.1	10.0
	[1-^{14}C]Oleate	None	PC	46.1	52.0	1.9
		+Sandoz 9785	PC	48.5	51.3	tr
	[1-^{14}C]Linoleate	None	DGG	n.d.	22.7	77.3
		+Sandoz 9785	DGG	n.d.	53.7	46.3

Incubations were for 13 hr, and all radiolabelled substrates were added at 1 μCi/leaf or cotyledon at the specific activity given in the Experimental. Abbreviations: PC, phosphatidylcholine; DGG, diacylgalactosylglycerol. For other abbreviations see Table 2.

ation process, we studied the distribution of radioactivity in acyl lipids in the presence and absence of Sandoz 9785 (Table 6). The data show that diacylgalactosylglycerol and, particularly, phosphatidylcholine were highly labelled from all the precursors used. The distribution of the radioactivity between acyl lipids was unaffected by the presence of the herbicide whether [1-^{14}C]acetate, [1-^{14}C]oleate, [1-^{14}C]linoleate or [1-^{14}C]linolenate was employed as the precursor.

DISCUSSION

It has previously been shown that Sandoz 9785 caused decreases in endogenous levels of α-linolenic acid together

Table 6. The distribution of radioactivity in different acyl lipids of barley leaves and cucumber cotyledons in the presence or absence of Sandoz 9785

		Distribution of radioactivity (% total acyl lipids)							
		[1-^{14}C]Acetate		[1-^{14}C]Oleate		[1-^{14}C]Linoleate		[1-^{14}C]Linolenate	
		Treatment (+10^{-4} M Sandoz 9785)							
Plant	Lipid	−	+	−	+	−	+	−	+
(i) Barley	DGG	27	26	13	12	18	21	17	19
	DDG	21	18	6	7	6	10	8	11
	PC	29	31	68	67	53	47	58	57
	PE	10	9						
	PG	4	4	13	12	23	22	17	13
	DSQG	4	6						
	PA+DPG	5	6						
(ii) Cucumber	DGG	16	14	8	7	19	17	16	15
	DDG	13	10	1	2	4	4	2	1
	PC	30	30	39	37	40	35	35	39
	PE	15	17	24	22	17	21	15	15
	PG	10	10	11	10	7	9	4	3
	DSQG	6	7	7	9	6	5	7	5
	PA+DPG	8	11	10	12	6	7	11	17

Abbreviations: DDG, diacyldigalactosylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DSQG, diacylsulphoquinovosylglycerol; PA, phosphatidic acid; DPG, diphosphatidylglycerol. For other abbreviations see Tables 2 and 5.

with simultaneous and comparable increases in linoleic acid in several species of higher plant [10, 11, 17, 19, 23]. Metabolic studies employing $^{14}CO_2$ or [^{14}C]acetate have demonstrated that incorporation of label into α-linolenate in the presence of Sandoz 9785 was inhibited to varying extents depending upon the species used [14, 16]. However, because the herbicide also drastically inhibited photosynthetic O_2 evolution in the *Vicia faba* leaf discs and isolated spinach chloroplasts employed in these studies, the effects of lipid metabolism may have been secondary. More recently, it has been reported that intact barley seedlings were able to grow for at least 6 days after germination in the presence of 10^{-4} M Sandoz 9785 with no discernible effects on overall photosynthetic competence [12]. In the latter case, the usual inhibitory effects of the herbicide on α-linolenate biosynthesis were observed. Therefore, in the present study, we have used intact leaves or whole plants during incubations of up to 13 hr to study effects of Sandoz 9785 on fatty acid biosynthesis, rather than using leaf discs or isolated organelles, which may be more susceptible to other herbicidal effects.

The data presented in this paper provide direct evidence that the site of action of Sandoz 9785 on fatty acid biosynthesis in higher plants is at the level of linoleic acid desaturation. However, the susceptibility of different plant species to this type of herbicide effect did vary considerably (Table 2). In this regard, it is interesting that the species exhibiting the highest tolerance to Sandoz 9785, i.e. pea, was also the one that exhibited the most rapid rate of conversion of absorbed herbicide to other compounds (Table 1). It is possible that, in this species, the metabolism of Sandoz 9785 resulted in its conversion into inactive products. Furthermore, the species which was most sensitive to Sandoz 9785, i.e. ryegrass, had the highest rates of uptake but was only able to metabolize about one third of the herbicide taken up. The time course of the inhibition in a moderately-sensitive species, cucumber (Table 4), also indicated that differences in sensitivity of individual higher plants to the herbicide were probably due not only to the relative rates of uptake of the herbicide but also to its speed of modification to inactive compounds or to its removal from the site of desaturation by sequestration into another compartment.

The variation in susceptibility of lipid synthesis in different species towards Sandoz 9785 is emphasised by the data for the bryophytes and algae (Table 3). Total incorporation of radioactivity from [^{14}C]acetate was severely inhibited in all cases, with the level of inhibition being higher for the less complex organisms. Since the total production of all fatty acids was decreased and only *Chlorella vulgaris* synthesized detectable [^{14}C]linolenic acid during the experimental period even in the absence of Sandoz 9785, the herbicide was obviously affecting reactions other than linoleic acid desaturation in these organisms.

It is generally agreed that oleic acid desaturation normally occurs on a phosphatidylcholine-linked microsomal desaturase complex in such plants as pea, cucumber, barley, wheat, maize and safflower [21, 22, 24–27]. However, less is known about the subsequent desaturation of linoleic to α-linolenic acid. Following time course studies of [^{14}C]acetate or [^{14}C]fatty acid incorporation into complex lipids, it was proposed that diacylgalactosylglycerol is involved in linoleate desaturation [22, 27–29]. Further evidence for this suggestion has come from experiments with isolated chloroplasts where the linoleate desaturation has been found to be associated with diacylgalactosylglycerol [8, 9] and exogenous [^{14}C]linoleoyl-diacylgalactosylglycerol acted as the preferred substrate [7]. In the present experiments,

[^{14}C]linolenate accumulated regardless of the precursor used (Table 5). At the same time linolenate in phosphatidylcholine was scarcely labelled at all, in agreement with other experimental results [22, 28].

If phosphatidylcholine were a substrate for linoleic acid desaturation as suggested for some tissues [3] then it could be argued that the lack of label accumulating in linolenate esterified to the phospholipid was associated with rapid transfer of the newly synthesised acid to diacylgalactosylglycerol. This argument, however, would not easily explain the significant endogenous levels of linolenate in phosphatidylcholine and, moreover, as can be clearly seen (Table 6) exogenous [^{14}C]linolenic acid was rapidly transferred to phosphatidylcholine in the same way as other fatty acids. Table 5 shows that addition of Sandoz 9785 caused a large rise in the proportion of label in the linoleate of diacylgalactosylglycerol regardless of whether [^{14}C]oleate or [^{14}C]linoleate were used as substrate. The same result was found when [^{14}C]acetate was employed as substrate (results not shown). The pattern of label in phosphatidylcholine, and other lipids (data not shown), was affected very little by the herbicide. These results are consistent with a role for diacylgalactosylglycerol in the conversion of linoleate to linolenate [cf. 20].

The present experiments provide direct evidence that the site of action for Sandoz 9785 on fatty acid synthesis in higher plants is at the desaturation of linoleate. The data also emphasise the large variations in sensitivity between different plant species and confirm the probable role for diacylgalactosylglycerol in linoleate desaturation. Further experiments should attempt to localize and purify the desaturase in order to gain some insight into the molecular mechanism of action of substituted pyridazinones.

EXPERIMENTAL

Plant material. Cultures of the blue-green alga *Anabaena cylindrica* grown in the medium of Arnon and Allen [30] were supplied by Dr. J. C. Meeks, Department of Bacteriology, University of California, Davis. Cultures of *Chlorella vulgaris* and *Chlorella pyrenoidosa*, grown in Bold's basal medium [31] were supplied by Dr. N. Lang, Department of Botany, University of California, Davis. Specimens of the freshwater ferns *Azolla mexicana* and *Azolla fuliculoides* were supplied by Dr. D. W. Rains, Department of Agronomy and Range Science, University of California, Davis. Seedlings of *Cucumis sativus*, *Zea mays* [6] and *Pisum sativum* cv. Alaska [22] (5 days old) were grown as previously described. *Hordeum vulgare* cv. Newmar was grown for 5 days under a 15 hr day/9 hr night regime at 25° and 13°, respectively. *Spinacia oleracea* cv. Kyoho was grown for approximately 7 days under an 8 hr day/16 hr night regime at 20° and 18°, respectively. The first true leaves were used. *Lolium perenne* (ryegrass) was grown for approximately 7 days under normal daylight conditions (June).

Reagents and substrates. [1-^{14}C]Acetate (58 Ci.mol^{-1}), [1-^{14}C]oleic acid (56 Ci.mol^{-1}), [1-^{14}C]linoleic acid (60 Ci.mol^{-1}) and [1-^{14}C]linolenic acid (60 Ci.mol^{1}) were purchased from Amersham/Searle. Ethyleneglycol monoethyl ether (EGME) was obtained from Sigma. All solvents and chemicals were of analar grade. [^{3}H]Sandoz 9785 (2 Ci.mol^{-1}) was prepared by custom synthesis by Amersham International, Amersham, HP7 9LL, U.K. It was purified to radiochemical homogeneity by thin layer chromatography before use.

Incubation of tissues. Algal cultures were incubated in their nutrient media, to which both substrate and inhibitor were added as appropriate. Whole plants of the *Azolla* spp. and excised leaves or cotyledons (in the case of cucumber) of the higher plants were incubated as outlined by Wharfe and Harwood [22]. Aerobic conditions at 22° under white light of intensity (quantum flux) 150–200 μE.m^{-2} sec^{-1} were used for all tissues. One μCi of [1-^{14}C]acetate (58 Ci.mol^{-1}) was added to the incubation medium from which it was readily taken up. [^{14}C]Fatty acids (1 μCi) were applied to the leaves or cotyledons in the form of microdroplets of EGME soln which were allowed to dry on the tissue [32]. Control tissues were incubated in water and herbicide treatments contained 10^{-4} M Sandoz 9785.

Lipid extraction and analysis. Reactions were terminated by the addition of hot *iso*-PrOH or by immediate extraction of the tissue into either $CHCl_3$–MeOH (2:1) or hexane–*iso*-PrOH (3:2). Details of the lipid extraction and analysis by TLC and GLC are given elsewhere [22, 32].

Sandoz 9785 uptake experiments. Tissues were incubated for up to 24 hr as described above in the presence of 10^{-4} M [^{3}H]Sandoz 9785 (approx. 10 μCi/ml soln). At the end of the incubation, tissues were removed and rinsed in unlabelled 10^{-4} M Sandoz 9785 before heating in EtOH at 70° for 30 min in sealed tubes. Tissues were homogenized and the EtOH extracts were separated by TLC on silica gel G in Et_2O. The position of Sandoz 9785 was located after spraying with 0.05% (w/v) 8-anilino-1-naphthalenesulphonic acid in MeOH and viewing under UV light. Bands corresponding to Sandoz 9785 and other radiolabelled compounds were scraped from the plates and counted as described in ref. [18].

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