Effects and Absorption of Sethoxydim on Excised Root Tips of Corn *(Zea mays)* and Pea *(Pisum sativum)*

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Sethoxydim, Growth Inhibition, Cell Cycle, Fatty Acids

The mechanism of selective herbicidal action of sethoxydim was investigated by using cultured root tips of corn and pea. Sethoxydim inhibited the growth of susceptible corn root at concentrations of 3 nM and above, but did not affect that of pea at 0.1 mM. Meristematic cells in cultured roots were arrested at the G_1 and G_2 stages of the cell division cycle by sucrose starvation, and resumed growth and cell division (proliferation) upon sucrose addition. Corn root growth was not inhibited by sethoxydim even at 0.01 mM when the roots were arrested by sucrose starvation. Corn roots that resumed growth upon sucrose provision were more sensitive to sethoxydim than those kept in constant growth condition. Better absorption of [^{14}C]sethoxydim into the meristematic region of corn roots was observed when cells were in the proliferative condition, which was not observed when cells were arrested by sucrose starvation. No stronger uptake of the herbicide was observed into pea meristems in either growth condition. In the cell cycle study, stronger absorption of [^{14}C]sethoxydim into the corn root meristem took place at a certain limited period prior to the S (DNA synthesis) stage. The physiological effects and the better absorption of sethoxydim clearly depended on cell cycle progression of the corn root meristem, while fatty acid synthesis, as well as its inhibition by sethoxydim, was neither associated with cell cycle progression nor with stronger absorption of the herbicide. The selective herbicidal action of sethoxydim may be caused by interference with selected stages (G_1 and/or the transition step between G_1 and S stages) of the cell division cycle, where affinity sites for the herbicide might be expressed.

Introduction

Sethoxydim (2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) is a selective postemergence herbicide which provides excellent control against several annual and perennial grasses in broad leaf crops [1]. Sethoxydim directly or indirectly inhibits growth and cell division in root and shoot meristems of graminaceous plants following foliar application [2–5]. Visual injury symptoms caused by a high herbicide rate are cessation in plant growth, followed by necrosis, while symptoms by a lower rate are growth inhibition accompanied with chlorosis of expanding leaves and anthocyanin pigments increase [1, 5]. Inhibition of growth and cell division was also observed in primary root meristems of corn seedlings treated hydroponically [5]. Therefore, we tried to obtain useful information to determine the mechanism of selective herbicidal action of sethoxydim, using the excised root tip culture system [6–9].

Many cells in the meristematic regions of plant roots are in a continuous cell division cycle [10]. This cycle is a sequentially progression of steps, consisting of the stages G_1, S, G_2, and M, whereby cells in G_1 and G_2 become metabolically prepared for passing through DNA synthesis (S) and mitosis (M), respectively. Van't Hof and co-workers [11] have developed an experimental system with plant root meristems which is useful in the study of the mitotic cycle. The system consists of culturing roots in sucrose-deficient medium long enough to accumulate meristematic cells in G_1 and G_2 of the cell division cycle (sucrose starvation). Cells in such meristems, referred to as stationary phase cells, will resume growth and cell division (proliferation) upon addition of sucrose (sucrose provision).

Several hypotheses are reported concerning about the mode of action of the graminicides such as sethoxydim. Sethoxydim affected lipid metabolisms in corn seedlings, isolated soybean leaf cells, isolated corn chloroplasts, etc. [12–16]. Recent investigations have indicated that sethoxydim inhib-

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ited ACCase (acetyl-CoA carboxylase) [16–18]. As yet, a correlation between inhibition of growth and the enzyme has not been verified. On the other hand, it was reported that a redox system bound on plasmalemma was inhibited by sethoxydim [19].

The objective of this study is to provide information concerning about the site of action and selectivity mechanisms of sethoxydim using the excised root culture system developped by Van't Hof *et al.*

White's medium, 25 °C, dark, shaking 100 rpm.
1 cm terminal tips of primary roots
Into the medium with 2% sucrose : constantly growing
↓
Into the medium without sucrose : arrested in G_1 and G_2 (sucrose starvation)
↓
Into the medium with 2% sucrose : regrowth synchronously (sucrose provision)

Fig. 1. Scheme of root tip culture system developed by Van't Hof *et al.*

Materials and Methods

Excised root tips of corn (*Zea mays* L. "Golden-crossbantam") and pea (*Pisum sativum* L. "Alaska") were cultured as reported previously (Fig. 1) [6, 9, 20]. Sethoxydim and [4-^{14}C]sethoxydim (10.3 mCi/mmol) were synthesized [21, 22]. Other radioactive chemicals were from Amersham, and other non-radioactive chemicals were obtained from Wako Chemical Co. Ltd. Treatments and analyses were conducted as previously reported [6–9].

Results and Discussion

Growth of excised corn root tips was inhibited by sethoxydim at concentrations of 3 nM and above, while that of pea was not affected even at 0.1 mM (Fig. 2). The degree of the inhibition clearly depended on the applied concentrations. In contrast, IAA-induced elongation of corn coleoptile *versus* pea epicotyl was not very sensitive to sethoxydim, and the degree of inhibition was almost similar [6]. These results indicated that sethoxydim selectively inhibited the root growth by affecting cell division rather than cell enlargement. Sethoxydim caused inhibitions of growth, DNA synthesis, and mitosis of cultured corn roots within periods between 4 and 48 h after treatment (Fig. 3, 4, 5). However, the herbicide inhibited neither DNA synthesis nor mitosis of corn roots directly or immediately. These indicated that the cell division inhibition by sethoxydim did not occur in the S or M phase of the cell division cycle.

Sethoxydim did not affect directly DNA, RNA, protein, cell wall synthesis, or respiration in excised corn roots [7]. It did inhibit lipid metabolism in a shorter time but the degree of inhibition was rather weak compared with growth inhibition (Fig. 6).

On the other hand, stronger absorption of [^{14}C]sethoxydim was observed in proliferating meristematic regions of susceptible corn roots, while the concentration of the absorbed herbicide into other corn tissues or any pea tissues was almost equal to that in the treatment medium (Table I). The better absorption of the herbicide by the corn meristem was not affected by 0.1 mM 2,4-dinitrophenol treatment, whereas active uptake of [^{14}C]glucose was completely inhibited by the treatment [8]. Thus, the stronger absorption is considered to be mediated by a passive rather than an active transport system. The better absorption of [^{14}C]sethoxydim was not observed when the cells in the corn meristem were arrested in G_1 and G_2 stages of the cell division cycle by sucrose starvation (Table II). In addition, corn root growth was not inhibited by sethoxydim even at 0.01 mM when the treated roots were arrested in G_1 and G_2, and roots that resumed growth upon sucrose pro-

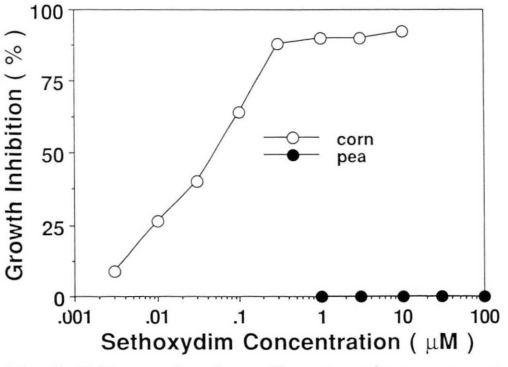

Fig. 2. Effects of sethoxydim at various concentrations on the growth of excised root tips of corn and pea after 48 h of treatment.

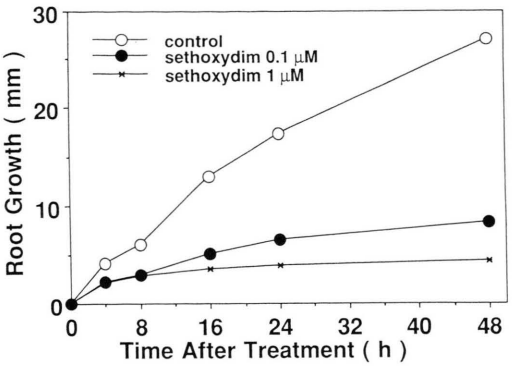

Fig. 3. Growth of excised corn root tips within 48 h after treatment with 0.1 and 1 μM sethoxydim.

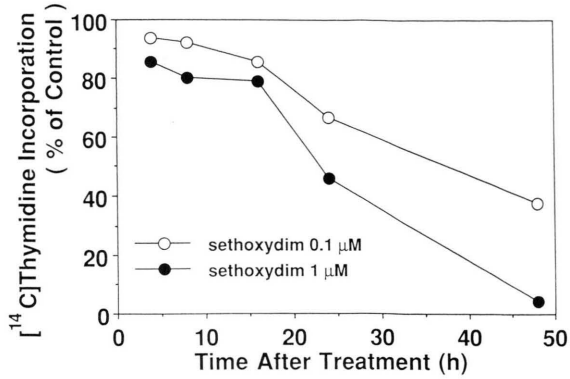

Fig. 4. DNA synthesis of excised corn root tips within 48 h after treatment with 0.1 and 1 μM sethoxydim.

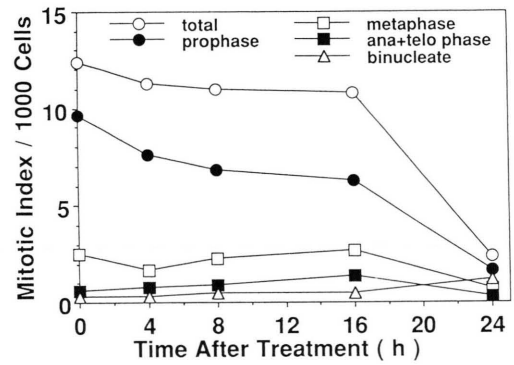

Fig. 5. Mitosis of excised corn root tips within 48 h after treatment with 0.1 and 1 μM sethoxydim.

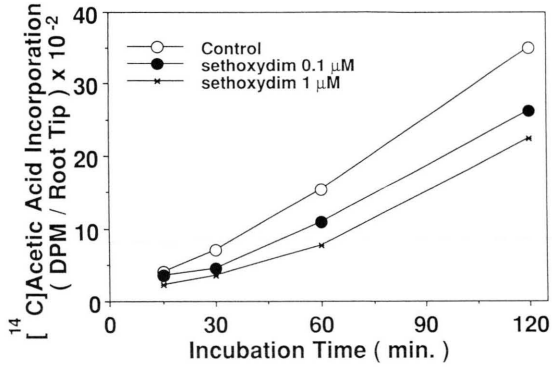

Fig. 6. [^{14}C]Acetic acid incorporation into lipid fraction of excised corn root tips within 2 h after treatment with 0.1 and 1 μM sethoxydim.

Table I. Absorption of [^{14}C]sethoxydim into tissues of corn and pea after 2 h of treatment.

Sethoxydim concentration [μM]	[dpm/μl solution]	Tissue	Corn [dpm/mg fresh weight][a]	Pea	Corn/Pea
1	23	coleoptile	36a	–	
1	23	epicotyl	–	27a	1.3
1	23	leaf disk	22a	30a	0.75
1	23	root (P)[b]	154c	21a	7.4
1	23	root (N)[b]	31a	19a	1.7
5	115	coleoptile	184c	–	
5	115	epicotyl	–	167c	1.1
5	115	leaf disk	126bc	124bc	1.0
5	115	root (P)	754d	102bc	7.4
5	115	root (N)	164c	87b	1.9

[a] Means followed by the same letter are not different at the 5% levelaccording to Duncan's multiple range test.
[b] Proliferative (P) and nonproliferative (N) regions are assayed.

Table II. Absorption of 1 μM [^{14}C]sethoxydim into excised root tips of corn and pea.

Plant	Treatment medium (+/−)[a]	Assayed region (P/N)[b]	Incubation time [min] 15	30	60	120
			[dpm/mg fresh weight]			
Constantly growing roots:						
Corn	+	P	83.1	96.9	152.0	223.4
	+	N	6.4	8.1	12.9	19.4
	−	P	78.2	101.5	148.6	212.7
	−	N	5.9	8.3	13.3	18.5
Pea	+	P	5.4	7.4	12.3	18.0
	+	N	5.2	8.1	10.4	13.5
	−	P	5.5	7.5	12.2	19.0
	−	N	5.4	7.8	10.4	13.7
Arrested roots by sucrose starvation:						
Corn	+	P	7.1	9.1	14.3	19.1
	+	N	5.4	9.2	10.7	13.1
	−	P	7.4	9.1	14.7	19.1
	−	N	5.7	9.0	10.8	13.1
	LSD ($P < 0.05$)[c]		(6.6)	(6.1)	(7.7)	(23.8)

[a] Presence (+) or absence (−) of sucrose in the treatment medium.
[b] Proliferative (P) and nonproliferative (N) regions are assayed.
[c] LSD for comparison between means within a column.

vision were more sensitive to sethoxydim than those kept in constant growth [9]. These results indicate that the stronger absorption and the effects of sethoxydim are closely associated with proliferative conditions of susceptible plants and that sethoxydim did not affect arrested (G_1 or G_2) stages of mitotic cycle.

In the cell cycle study, the first mitosis of the G_2-arrested cells in corn root meristems were observed 4 h after provision of sucrose to the starvation medium (Fig. 7). The number of mitotic cells increased to a peak at 9 h and then decreased until 12 h after sucrose provision. As shown in Fig. 8, the first cells from G_1 began to synthesize DNA after 4 h. The cells in which DNA synthesis increased to the first peak at 9 h, were considered to be the G_1-arrested population. The cells arrested in G_2, passed M and G_1, and initiated DNA synthesis

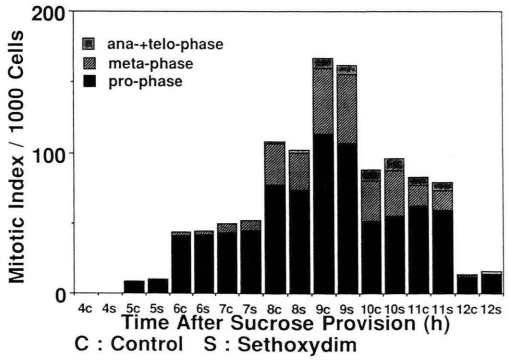

Fig. 7. Effects of 1 μM sethoxydim on M progression in the cultured corn root. Time followed by "c" or "s" means no treatment control or sethoxydim treatment, respectively.

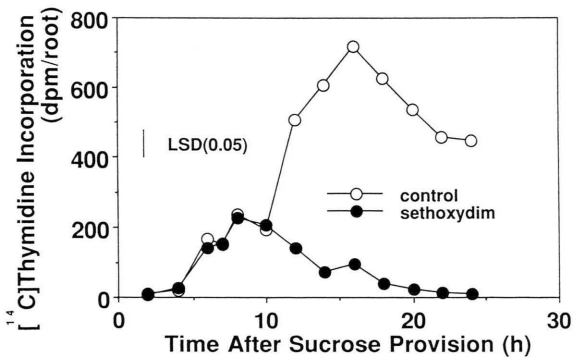

Fig. 8. Effects of 1 μM sethoxydim on S (DNA synthesis) progression in the cultured corn root.

after 10 h or more. It appeared in the second large peak, which increased rapidly until 16 h. 1 μM sethoxydim did not affect the progression from arrested-G_2 to M (Fig. 7), or that from arrested-G_1 to S (Fig. 8), but completely blocked the progression through M and G_1 into S of G_2-arrested population. In addition, the greater absorption into the meristematic regions of corn roots was observed at the certain limited time, prior to DNA synthesis, in the cell division cycle (Fig. 9). These results indicate that there exist possible affinity sites for sethoxydim in the meristematic regions of susceptible corn roots, expressed only in G_1 stage, and/or during the transition step between G_1 and S stages of the cell cycle, when the herbicide exhibits its activity (Fig. 11).

Recent studies have shown that sethoxydim selectively inhibits acetyl-CoA carboxylase (ACCase) in the chloroplast isolated from suceptible plant seedlings, which catalyzes the conversion of acetyl-CoA to malonyl-CoA. It was proposed that ACCase was the primary site of action of sethoxydim, and that the inhibition of the enzyme caused decrease of the *de novo* fatty acid synthesis selectively in graminaceous plant. However, the inhibition concentration of [^{14}C]acetate incorporation into fatty acids was much higher than that of growth in corn root culture system. Moreover, the half-inhibition concentration (IC_{50} = 2.9 μM) in ACCase assay [16] was rather high, compared with growth inhibition activity in the root culture system. As indicated above, both the physiological ef-

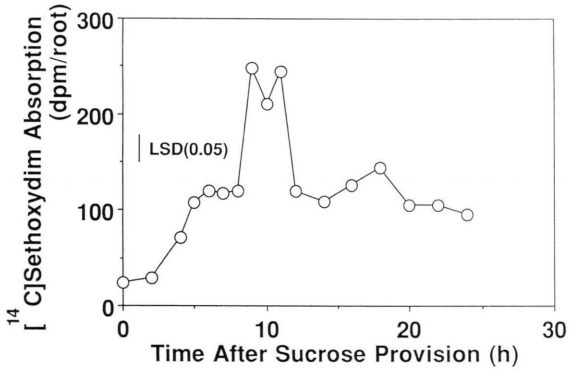

Fig. 9. [^{14}C]Sethoxydim absorption into corn root meristem during cell cycle progression.

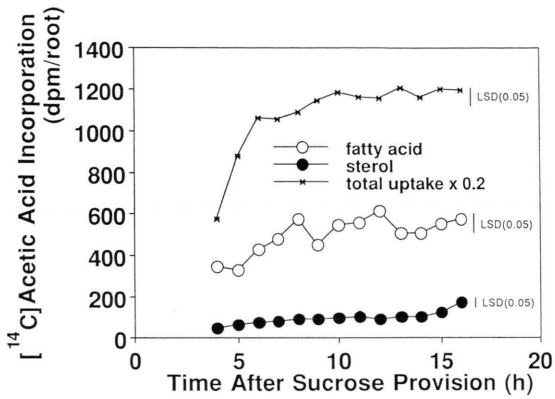

Fig. 10. Fatty acid synthesis during cell cycle progression in the cultured corn root.

Table III. Effects of sethoxydim at various concentrations on fatty acid synthesis during cell cycle progression in the cultured corn root.

Sethoxydim concentration [μM]	Assayed time after sucrose provision [h]	Fatty acid synthesis [dpm/root]	Inhibition [%]
0 (Control)	9	452.67	0
0.01	9	461.00	0
0.03	9	459.33	0
0.1	9	253.50	44
0.3	9	226.33	50
0 (Control)	16	572.00	0
0.01	16	537.67	6
0.03	16	549.00	4
0.1	16	320.33	44
0.3	16	279.67	49
LSD ($P < 0.05$)[a]		(109.115)	

[a] LSD for comparison between means within a column.

fects and the greater absorption of sethoxydim depended on cell cycle progression. In contrast, fatty acid synthesis, as well as its inhibition by sethoxydim, did not associate either with cell cycle progression or greater absorption of the herbicide (Fig. 10 and Table III). Apparently, the inhibition of fatty acid synthesis did not considered to play a major role in the mechanism of action and selectively of sethoxydim in the excised root culture system.

What is the target site that causes selective inhibition of growth and cell division by sethoxydim? From the results of this study, we propose more probable target site of sethoxydim, which has a high affinity to the herbicide, and is expressed in selected stages of the cell division cycle (Fig. 11). In addition, we suggest that the selected stage might be either in G_1, and/or the transition step between G_1 and S stages.

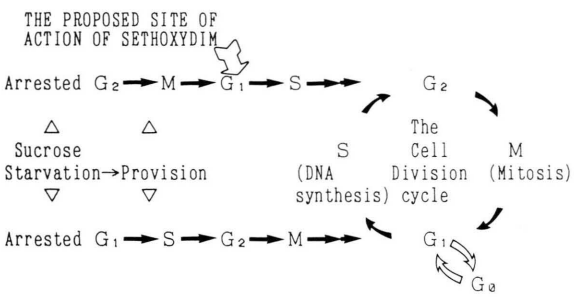

Fig. 11. Diagram showing the proposed site of action of sethoxydim during cell cycle progression.

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