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Effects of Butachlor on Cell Division and Cell Enlargement in Oat (*Avena sativa* L.)

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Butachlor가 귀리(*Avena sativa* L.)의 세포분열 및 신장에 미치는 영향

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ABSTRACT

The effects of varying concentrations and durations of butachlor [N-(bytoxymethyl)-2-chlor-2′, 6′-diethylacetanilide] treatment on oat (*Avena sativa* L.) root cell division were studied. Oats were treated from 0 to 48h with concentration ranging from 1×10^{-6}M to 1×10^{-3}M of butachlor. The highest concentration (1×10^{-3}M) of butachlor caused significant inhibition of cell division after 6h treatment. After 18h treatment, 49% and 66% inhibition of cell division occurred at 1×10^{-5}M and 1×10^{-4}M, respectively, while 16% inhibition of cell division occurred at 1×10^{-6}M concentration at same exposure period. Oat treated with 1×10^{-5}M and 1×10^{-6}M showed 69% and 38% inhibition of cell division after 36h. Increasing herbicide concentration at a specific time increased inhibition of cell division, and increasing the duration of treatment at a specific concentration also increased inhibition of cell division. In most instances the greatest inhibition of cell division occurred between 0 to 18h during 48h treatment. A range of concentration of 1×10^{-5}M to 1×10^{-3}M reduced cell enlargement significantly during 24h incubation period. The 1×10^{-5}M and 1×10^{-3}M caused 34% and 75% inhibition of cell enlargement. It was concluded that butachlor caused the growth inhibition of oats by inhibiting both cell division and cell enlargement.

INTRODUCTION

Butachlor, a widely used herbicide of chloracetamide group, has been developed for preemergence control of annual grases and certain broadleaf weeds in transplant and seeded rice in many asian countries. Especially in Korea, over the 55% of herbicides used was the butachlor last year (Kang *et al.*, 1985). Butachlor is absorbed mainly by the germinating shoots, secondarily by roots of susceptible plants. Butachlor is translocated throught the plant with higher concentrations in vegetative parts than reproductive parts and also is

metabolized very rapidly in plants (Beste, 1983).

Since butachlor has been widly used to control many aquatic weeds of rice in Korea, the total cost of rice production was reduced and the yield of rice may be increased. However, the crop injury was often reported. This crop demage may be due to short knowlege of herbicide usage or characteristics of herbicide mechanism.

Many studies suggested chloracedamide herbicides such as alachlor, metolachlor and propachlor, inhibit plant growth due to inhibition of cell division and cell enlargement (Carlson *et al.*, 1975; Deal and Hess, 1980; Dhillon and Anderson, 1972). However, the mechanism of butachlor is not studied intensively. Cell division and cell enlargement should be required for plant growth. If one of these processes is inhibited or disrupted, plant growth would be affected seriously. Primary study of butachlor showed that this herbicide inhibit plant growth (Hwang, 1786). Thus inhibition of growth treated with by butachlor must be a result of inhibition of cell division, cell enlargement, ATP formation or a combination of these.

The objective of our work was to determine the influence of different exposure times and concentrations of butachlor on cell division and cell enlargement.

MATERIALS AND METHODS

Cell division studies. Oats were germinated on wet filter paper in petridishes. Twenty five oat seedling (2 days old) were transferred to each petri dish containing 5 ml of 2×10^{-4}M $CaSO_4$-solution with herbicides (Harkes, 1973). Two petri dishes were then placed in the dark at 22 ± 2°C. The effect of butachlor on cell division was tested at 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3}M concentrations. After 6, 12, 18, 24, 36, and 48h, samples were taken from each treatment.

For mitotic index analysis, six root tips were sampled at each sample time. Roots were fixed in absolute ethanol and acetic acid (3:1) for one hour at room temperature, then stored in the refrigerator (about 4 C). Root tips were rinesd in distilled water, hydrolyzed in 1 N HCl at 60 C for 10 min, then rinsed again and placed in Schiff regent for 25 min in the dark and transferred to 2ml of a 5% aqueous pectinase solution for at least 8hr (Setterfield *et al.*, 1954). The 2mm root tips were removed and squashed on a microscope slide. The number of dividing cells differentiated into prophase, metaphase, anaphase, and telophase or interphase in 1,000 total cells was determined for mitotic index. In all case three replicates were counted.

Cell enlargement studies. Oat seeds were germinated in wet vermiculite in the dark at 22 ± 2°C for 5 days. All manipulations of seedling were conducted in a dark room illuminated with a green safelight to eliminate phytochrome effect (Deal and Hess, 1980). The 3 to 4cm oat coleoptiles were uniformly cut with a razor. The 5mm sections were cut 3mm below the tip and placed into sterile petridishes containing 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5},

10^{-4}, and 10^{-3}M concentrations of butacblor in 10 ml of 10 mM potassium phosphate medium (pH 5.2). The petri dishes were randomly grouped, wrapped in aluminum foil to exclude light, and placed on a shaking water bath at 25°C for 24h. This treatment time was necessary to obtain measurable cell enlargement in nontreated coleoptiles. The sections were measuredto the nearest 0.5 mm with a ruler under dissecting microscope (Deal and Hess, 1980). Ten sections were measured in each treatment. Data represent the average of four replicate samples.

RESULTS AND DISCUSSION

Cell division studies. The effects of concentration and duration of butachlor treatment on the kinetics of cell division was investigated. In addition, durations and concentrations of the treatmnets where inhibitions of growth first occurred were determined. A comparison of cell division frequencies determined at different herbicide concentrations and durations is shown in Table 1. After 6h treatment, only the highest concentration (1×10^{-3}M) of butachlor caused significant inhibition of cell division. Cell division in oat treated for 12h was also inhibited significantly by 1×10^{-4}M and 1×10^{-5}M butachlor. After 18h treatment, 49% and 66% inhibition of cell division occurred at 1×10^{-5}M to 1×10^{-4}M, respectively, while 16% inhibition of cell division occurred at lower concentration (1×10^{-6}M). After 18h treatment, all treatments caused significant inhibition of cell division. The lower concentration (1×10^{-5}M) required more time to inhibit the appoximately same rate of cell division as higher concentrations (1×10^{-4}M and 1×10^{-3}M). After 6h, butachlor treatment of oat with 1×10^{-5}M inhibited 1% cell division, while butachlor treatment with 1×10^{-5}M caused 34% inhibition after 12h and 49% inhibition after 18h, respectively. These result showed that increasing herbicide concentration at a specific time increased inhibition of cell division, and the duration of treatment at a specific concentration also increased inhibition of cell division. This may be partially because of absorption, translocation, or metabolism of herbicide (Kim, 1983). To demonstrate herbicidal activity, a given herbicide must be absorbed by target plant and sufficient amount of herbicide should be translocated to the sensitive site or sites in its toxic form.

In general, effects at high concentration (higher than 1×10^{-4}M) and 24h or longer treatment may be secondary or due to toxic effects not related to the mechanism causing initial growth inhibition. Mode of action studies conducted at concentrations of 1×10^{-4}M and higher should be conducted at very short time intervals. Lower concentrations should be used for treatment times of 6h and longer (Deal and Hess, 1980). In this experiment, the steep nagative slopes of 1×10^{-4}M and 1×10^{-5}M between 6h to 18h period occurred, while that of 1×10^{-3}M treatment occurred earier than 6h (Fig. 1). Kinetic analysis of the cell cycle showed butachlor treatment caused an inhibition in the G_1, S or G_2 phase. All dividing cells were normal but there was a uniform decrease in prophase, metaphase, and

Table 1. Effect of exposure times and concentrations of butachlor on mitotic index and distribution of mitotic stages in oat root tips

Incubation times (h)	Concentrations (M)	Dividing cells (No./1000 cells)		Distribution of mitotic stages (No./1000 cells)			
		mitotic index	% of control	Prophase	Metaphase	Anaphase	Telophase
6	Control	109	100a	45a	30a	18a	16a
	10^{-6}	105	96a	40a	31a	20a	14a
	10^{-5}	108	99a	44a	33a	17a	14a
	10^{-4}	106	99a	42a	33a	17a	14a
	10^{-3}	71	65b	33b	25b	9b	4b
12	Control	100	100a	47a	26a	15a	12a
	10^{-6}	95	95a	45a	25a	14a	11a
	10^{-5}	66	66b	38ab	15b	6b	7b
	10^{-4}	47	48b	30b	10c	4b	3c
	10^{-3}	46	46c	28b	11a	4b	3c
18	Control	102	100a	45a	29a	14a	14a
	10^{-6}	80	84b	38b	21b	11ab	10ab
	10^{-5}	52	51c	24c	14bc	7bc	7b
	10^{-4}	35	34d	17d	9cd	3cd	6bc
	10^{-3}	14	13e	8e	2d	2d	2c
24	Control	105	100a	48a	31a	15a	11a
	10^{-6}	74	70b	35b	22b	9b	8a
	10^{-5}	40	38c	20c	15c	3c	2b
	10^{-4}	26	25d	12cd	10c	2c	2b
	10^{-3}	14	13e	8d	2d	2c	2b
36	Control	121	100a	58a	30a	15a	18a
	10^{-6}	75	62b	35b	21b	10b	9b
	10^{-5}	38	31c	22c	12c	2c	2c
	10^{-4}	26	21d	12d	10c	1c	3c
	10^{-3}	15	12e	9d	3d	1c	2c
48	Control	114	100a	50a	35a	17a	12a
	10^{-6}	72	63b	32b	20b	13b	7b
	10^{-5}	31	28c	19c	9c	2c	1c
	10^{-4}	24	20cd	12cd	10c	1c	1c
	10^{-3}	13	11d	8d	3d	1c	1c

In a column within each exposure time, means followed by a same letter are not significantly different at the 5% level by Duncan's multiple range test.

anaphase as treatment time increased. Lignowski and Scott (1972) reported that triflualin arrested metaphases. Propachlor produced contraction of chromosomes and disorganization of anaphase seperations in onion root tips (Dhillon and Anderson, 1972). Carlson *et al.* (1975) reported that pronamide treatment of oat root tips produced arrest of metaphase, C-pairs,

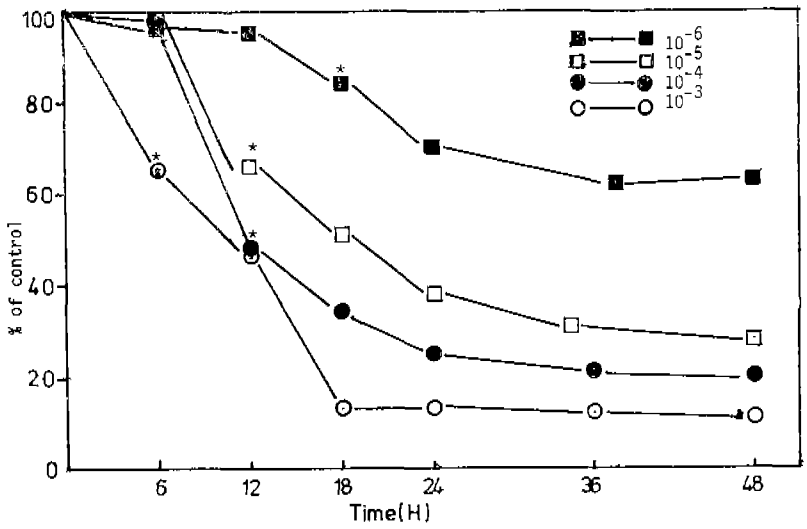

Fig. 1. Cell division in oat root tips, as a percent of the control, exposed to various concentrations of butachlor for specific periods of times.
* indicates the first significant difference from the control as determined by a Duncan's multiple range test at the 5% level.

multinucleate and polyploidy. However, these were not observed for butachlor. Similar results were observed in alachlor, metolachlor, holoxyfop and chlorsulfuron (Deal and Hess, 1980; Ray, 1982; Kim, 1983).

Cell enlargement studies. Oat coleoptile growth tests were used for cell enlargement determination because the first internode growth of oat is due to entirely cell enlargement (Deal and Hess, 1980). The effects of butachlor treatment on cell enlargement in oat coleoptiles is shown in Table 2. The total growth of untreated oat coleoptile during 24h was 1.2mm. A range of lower butachlor concentrations (1×10^{-8} to 1×10^{-6}M) treatment on oat coleoptile did not affect cell enlargement significantly. However, the 1×10^{-5}M concentration reduced 44% cell enlargement. Measured inhibitions of oat coleoptiles at 1×10^{-4}M

Table 2. Effect of butachlor on cell enlargement of oat coleoptile

Concentration (M)	Incremental coleoptile length (mm/24h)	Percent of control (%)
Control	1.2a	100
10^{-7}	1.1a	92
10^{-6}	1.0ab	83
10^{-5}	0.8b	66
10^{-4}	0.5c	42
10^{-3}	0.3c	25

In a colmn, means followed by a same letter are not significantly different at the 5% level by Duncan's multiple range test.

and 1×10^{-3}M butachlor were 58% and 75%, respectively, when compared to normal growth. As the concentrations of butachlor increased, the percent of inhibitions of cell enlargement increased after the first significant inhibition. The percent inhibition of cell enlargement in oat coleoptile growth was less than that of cell division in oat tips after 24h treatment (Tables 1 and 2).

It was concluded from these studies that the inhibition of growth caused by butachlor results from both an inhibition of cell division and cell enlergement. At present, the exact primary site of action of butachlor is not known. Several herbicides are known to inhibit cell division (Moreland, 1982). Although amides which include alachlor, metolachlor, and proamide do not disrupt the mitotic stage in treated plant tissue, they inhibit cell division. These herbicides have been suggested to block some required process which occurs prior to the actural cell division step of the cell division cycle (Moreland, 1982). Butachlor which belongs to amide-type herbicides also showed similar results in cell division to others of amides. This suggests that butachlor affects plant metabolism, which can arrest the cell cycle in G_1, S, or G_2.

摘 要

Butachlor를 귀리의 뿌리에 농도별로 처리한 후 0시간에서 48시간까지의 세포분열에 미치는 영향을 조사하였다. 또한 Oat coleoptile growth test를 이용하여 24시간 incubation한 후 제초제의 농도별 세포신장에 미치는 영향을 조사하였다.

6시간 처리 후에는 1×10^{-3}M에서만 세포분열을 억제하는 것으로 나타났다. 50%이상의 세포분열 억제는 18시간 처리후 1×10^{-5}M에서 1×10^{-3}M까지 나타났으며, 저농도구인 1×10^{-6}M에서는 48시간처리 후에도 40%미만의 세포분열 억제를 보여주었다. 동일 처리시간에서는 Butachlor의 농도가 증가함에 따라 세포분열의 억제정도가 증가되었고 동일 농도처리도 처리시간이 경과함에 따라 억제 정도도 증가되었다. 48시간 처리기간중에 가장 심하게 세포분열 억제효과를 보여준 기간은 대부분 처리구에서 처리 후 18시간이내로 나타났다. 세포분열억제 실험에서는 1×10^{-5}M이상 농도에서 세포신장억제 효과를 보여주었으며 대조구와 비교하여 34%에서 75% 세포신장을 억제하였다. 이상의 결과로 미루어보아 Butachlor의 식물생장억제기구는 세포분열과 세포신장의 억제가 주 원인으로 사료된다.

REFERENCES

Beste, C.E. 1983. Herbicide handbook. Weed Sci. Society of America.

Carlson, W.C., E.M. Lignowski and H.J. Hopen. 1975. The mode of action pronamide. *Weed Sci.* 23(2):155-161.

Deal, L.M. and F.D. Hess. 1980. An analysis of growth inhibitory characteristics of alachlor and metachlor. *Weed Sci.* 28:168-175.

Dhillon, N.S. and J.L. Anderson. 1972. Morphological, anatomical and biochemical effects of propachlor on seedling growth. *Weed Res.* 12:182-189.

Harkes, P.A.A. 1973. Structure and dynamics of the root cap of *Avena sativa* L. *Acta Bot. Neerl.* 22(4):321-328.

Hwang, I.T. 1986. The mode of action of herbicide butachlor in germination and growth of rice. M.S. Thesis. Chŏnbuk National Univ. Chŏnju, Korea.

Kang, J.I., S.I. Oh, J.S. Choi and C.Y. Kang. 1985. A study of supply demand of pesticide. K.R.E.I. 144pp.

Kim, J.C. 1983. The mode of action of CGA82725 and DOWCO453. Ph.D. Dissertation. Ohio State Univ., Columbus, Ohio. 103pp.

Lignowski, E.M. and E.G. Scott. 1972. Effect of Triflualin on mitosis. *Weed Sci.* 20(3): 267-270.

Moreland, D.E. 1980. Mechanisms of action of herbicides. *Ann. Rev. Plant Physiol.* 31: 597-638.

Moreland, D.E. 1982. Biochemical responses induced by herbicides. Washington, D.C.

Ray, T.B. 1982. The mode of action of chlorsulfuron. *Pesticide Biochem. Physiol.* 17:10-17.

Setterfiled, J., R. Schreifer and J. Woodard. 1954. Mitotic frequency determination and microphotometric feulgen dye measurements in root tips. *Stain Technology* 29(3): 113-120.

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