A Study of Mode of Action of Fluazifop-butyl[1)]

II. Fluazifop-butyl Effect on Cell Division, Cell Enlargement, and Protein Synthesis in Oat(*Avena sativa* L.) Roots

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Fluazifop-butyl의 除草機構에 관한 硏究[1)]

第II報 Fluazifop-butyl이 귀리뿌리의 細胞分裂, 細胞神張 및 蛋白質合成에 미치는 影響

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ABSTRACT

The effects of varying concentrations and duration of fluazifop-butyl [(±)-butyl [2- [4- [(5-(trifluoro methyl)-2-pyridinyl] oxy] phenoxy] propanate] treatment on cell division, cell enlargement, and protein synthesis were studied. Oat (*Avena staiva* L.) were treated from 0 to 48 hr with concentration ranging from 1×10^{-6} M to 1×10^{-3}M of fluazifop-butyl in the cell division study. There was a significant reduction in the mitotic indices of oat roots treated with 1×10^{-4}M after 6 hr. After 18 hr treatment, All herbicide treatment inhibited cell division significantly. After 24 hr treatment almost 100% inhibition of cell division occurred at 1×10^{-4}M to 1×10^{-3}M while 20% inhibition of cell division occurred at 1×10^{-6}M concentration at same exposure period. The greatest inhibition of cell division occurred between 0 to 18 hr. The avena coleoptile straight- growth test were used to determine the influence of fluazifop-butyl on coleoptile growth. Significant inhibition of elongation of oat coleoptiles were observed at 1×10^{-7}M to 1×10^{-3}M after 24 hr incubation. Protein incorporation study showed that the 1×10^{-4}M of fluazifop-butyl caused 60% inhibition of protein synthesis. It was concluded that the growth of inhibition of plants caused by fluazifop-butyl results from inhibition of cell division, cell enlargement, and protein synthesis.

Key words: cell division, cell enlargement, avena coleoptile straight-growth test, Avena sativa

INTRODUCTION

Fluazifop-butyl is a herbicide developed for post emergence control of annual and perennial grasses (Peregory and Glenn 1985; Plowman and Stonebridge, 1980). This herbicide has excellent selectivity on cotton, soybean, alfalfa, fruit and ornamental trees, and many vegetable crops. An importent feature of this chemical is its high herbicidal activity at low application rates. Satisfactory control of annual and perennial grasses can be obtained with rates of 0.1 to 0.5 kg ai/ha and 0.3 to 1.1 kg ai/ha, respectively (Beste, 1983). Perennial grasses are more susceptible to fluazifop-butyl at later growth stage, whereas this herbicide effectively controls

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annual grasses at early growth stages (peregory and Glenn, 1985).

Fluazifop-butyl is a phloem-mobile and xylem-mobile herbicide (Monaco et al. 1985). The parent acid is considered to be the herbicidal active form in plants (Hendley et al. 1985). Selectivity among species to postemergence herbicides can be due to differences is site of uptake, spray, retension, herbicide absorption and translocation, site of action, or herbicide metabolism. Differential absorption and translocation did not contribute to the selectivity of fluazifop-butyl (Kells et al. 1985). However, after foliar treatment, fluazifop-butyl is translocated and accumulated in meristem regions. Therefore, the site of action is proposed to be meristematic tissue.

Little research on the mode of action fluazifop-butyl in plants has been published. Peregory and Scott (1985) reported that 50% of protein synthesis in soybean (*Glycine max.* (L.) Merr.) hypocotyl was inhibited by $1x10^{-4}$M, RNA and DNA synthesis was also inhibited by $1x10^{-4}$M in soybean. Haloxyfop-methyl 2- [4- [[3-chloro-5-(trifluoromethyl)-2 pyridinyl[oxy] phenoxy] propanoic acid , a compound chemically similar to fluazifop-butyl also inhibited protein, RNA and DNA synthesis in oat roots (Kim, 1983). Many studies suggest herbicides that inhibit growth are inhibitors of protein synthesis (Ashton and Craft, 1981).

The objectives of this research were to determine the mode of action of fluazifop-butyl. The effect of fluazifop-butyl on cell division, cell enlargement and protein synthesis is oat roots was specifically investigated.

MATERIALS and METHODS

Cell division study. Oats were germinated on wet filter paper in petridishes at 22 ± 2°C in the dark. Twenty five oat seedlings were transfered to each petridish containg 5 ml of $2x10^{-4}$M $CaSO_4$ solution with herbicides (Harkes, 1973). The petridishes were than placed in the dark at 22 ± 2°C. A range of concentrations (10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3}M) of fluazifop-butyl was tested for 48 hrs.

For mitotic index analysis, after treatment, six root tips were sampled at each sample time and fixed in absolute ethanoliacetic acid (3:1) for one hour at room temperature. Oat root tips were rinsed in disitilled water, hydrolyzed in 1 N HCl at 60°C for 10 min., then rinsed again and placed in Schiff reagent for 25 min. in the dark and transfered to 2 ml of a 5% aqueous pectinase solution (Setterfield and Woodard, 1954). Two mm oat root tips were then squashed on a microscope slide. The number of dividing cells in 1000 total cells was determined.

Cell enlargement study. Oats were germinated in moist vermiculite in the dark at 22 ± 2°C for 5 days. All manipulations of seeding were conducted in a dark room illuminated with a green safelight to prevent phytochrome growth response. Five mm sections were cut 3 mm below the coleoptile tip and placed into sterile petridishes with treatment in 10 ml of 10 mM potasium phosphate medium. Fluazifop-butyl was tested at 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3}M concentrations. The pereidishes were randomly grouped, wrapped in aluminum foil, and placed on a shaking (1 cycle/sec) water bath at 25°C for 24 hrs. The sections were measured to the nearest 0.5 mm with a ruler and dissecting microscope (Deal and Hess, 1980). Ten oat sections were measured in each treatment. The data represent the average of four replicate samples.

Protein incorporation study. Oat seeds were surface sterilized with 5% sodium hypochlorite for 3 min., washed three times with sterile disitilled water, and germinated on wet filter paper in strilized petridishes at 22 ± 2°C in the dark for 2 days.

Twenty oat seedlings were suspended in 20 ml of $2x10^{-4}$M $CaSO_4$ solution (Harkes, 1973) on hollow petridishes above a petridishes (doubled petridish). After 12 hr incubation on a shaker (1 cycle / 2 sec.), oat seedlings were transfered to other petridishes containing 20 ml $2x10^{-4}$M $CaSO_4$ solution with herbicide ($1x10^{-4}$M) plus 14 C-leucine (0.5 microcuri/ml) to measure incroporation as indication of protein synthesis.

After 8 hr incubation, forty-five roots were sampled and divided three groups. Fifteenn root tips were ground in 5 ml of cold 80% ethanol. The ground tissue plus the material in solution were poured to on a millipore filter (2.4 cm GF/C) vacuum funnel and washed with 10 ml of cold 80% ethanol.

To measure protein synthesis, the millipore filter was washed with 5 ml of cold 10 trichloroacetic acid (TCA), cold 5% TCA, cold 95% ethanol, cold absolute ethanol-diethyl ether (1:1), and diethyl ether. The filter was dried in air and measured in liquid scintillation system (Kim, 1983).

RESULTS and DISCUSSIONS

Plant grows contineousely by cell division and enlargement. Inhibition of one of these processes can affect plant growth (Kim, 1983). Inhibition of cell division during mitosis is the result of the an abnormal function or an absence of the spindle. If herbicide affects plant metabolism, the cell cycle in G1, S or G2 can be arrested. Enlargement can be inhibited by metabolic changes or an increase in cell wall rigidity prior to enlargement (Moreland et al. 1982). Protein synthesis is essential for the process of cell enlargement to proceed at a normal state and a cell enterance into DNA synthesis or mitosis (Rost, 1976). To determine the mechanism of action of fluazifop-butyl affecting growth, the effects of this herbicide on cell division, enlargement and protein synthesis were investigated respectively.

Cell division studies. The herbicide effect of concentrations and durations of treatment on the cell division was determined. A comparison of cell division frequencies determined at different herbicide concentrations and duration is shown in Table 1. Fluazifop-butyl of 1×10^{-4}M and 1×10^{-3}M caused significant inhibition of cell division in oat roots within 6 hr of treatment. After 12 hr of treatment, oats treated with 1×10^{-4}M and 1×10^{-3}M showed 32 and 66% inhibition of cell division (Fig. 1). However, treatments of 1×10^{-5}M and 1×10^{-6} M did not inhibit cell division significantly. After 18 hr of treatment, significant inhibition of cell division occurred in all treatments (1×10^{-6}M to 1×10^{-3}M). During 36 hr of treatment, oat roots

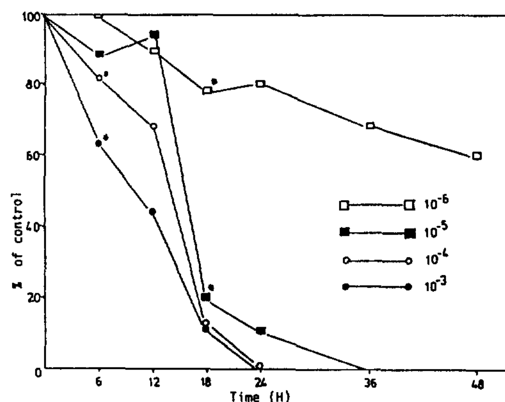

Fig. 1. Cell division in oat root tips, as a percent of the control exposed to various concentrations of fluazifop-butyl for specific periods of times. The * indicates the first significant difference from control determined by a Ducan's multiple range test at 5% level.

Table 1. Effect of fluazifop-butyl, on cell division of oat root-tip

Concentration (M)	Mitotic index (No./1000 cells)					
	Incubation time (hr)					
	6	12	18	24	36	48
control	105 a	94 a	112 a	106 a	103 a	111 a
1 X 10^{-6}	105 a	84 a	87 a	85 a	70 b	66 b
1 X 10^{-5}	92 ab	89 a	89 a	12 b	0 c	0 c
1 X 10^{-4}	86 b	64 b	64 a	1 b	0 c	0 c
1 X 10^{-3}	66 c	41 c	41 b	0 b	0 c	0 c

a 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.

treated with 1×10^{-5} M to 1×10^{-3} M showed 100% inhibition of cell division, while treatment of 1×10^{-6} M, the lowest concentration caused only 30% inhibition of cell division. These results indicated that increasing herbicide concentrations at a specific time increased cell division inhibition, and the duration of treatment at a specific concentrations also increased inhibition of cell division. Many reseachers reported that herbicides of growth inhibition showed very similar results in their concentrations and duration studies (Kim, 1983; Ray, 1982; Deal and Hess, 1980). Ashton et al. (1982) suggested that to be demonstrated effect of herbicide, a given herbicide must be absorbed by the target plant and sufficient amount of herbicide should be translocated to molecular site of action.

The greatest inhibition of cell division of oat roots occurred. During the first 18 hr of treatment oat roots treated with 1×10^{-5} M to 1×10^{-3} M showed approximately 80% inhibition of cell division in this duration. Deal and Hess (1980) reported the 1×10^{-4} M of alachlor and metolachlor caused only 50 to 80% inhibition of cell division while this concentration caused nearly complete inhibition of growth. In this study, almost 80% inhibition of cell division caused nearly complete inhibition of root growth. After 36 hr of treatment, 100% inhibition of cell division induced 100% inhibition of oat root growth (Data in Kim, 1986).

This study also showed that all dividing cells were normal but there was a uniform decrease in prophase, metaphase, and anaphase as treatment time increased. Fluazifop-butyl did not arrest metaphase as trifluralin did (Lignowski and Scott, 1972) and did not produced C-pairs, multinucleate and polyploidy similar to pronamide (Carlson et al. 1974). These results were similar to alachlor, metalachlor, haloxyfop-butyl, and chlorosulfuron (Deal and Hess, 1980; Kim, 1983; Ray, 1982). Therefore, these observation indicated that the inhibition could occur in G1, S, or G2 phase in interphase of cell cycle. This herbicide may affect plant metabolism such as DNA, RNA, and protein synthesis.

Cell enlargement study. The oat coleoptile straight-growth tests were used in this study to investigate inhibition of cell enlargement from fluazifop-butyl treatment. Coleoptile and frist internodes of grasses were best suited for examining enlargement in the absence of cell division (Nitsch and Nitsch, 1956). The average total growth of untreated oat coleoptile during the 24 hr incubation period was 1.4 mm. The effects of fluazifop-butyl

Table 2. Effect of fluazifop-butyl on elongation of oat coleoptile[a)]

Concentration (M)	Incremental coleoptile length (mm/24h)	Percent of control (%)
Control	1.4 a	
10^{-8}	1.1 ab	79
10^{-7}	1.0 bc	71
10^{-6}	0.7 c	50
10^{-5}	0.5 cd	36
10^{-4}	0.5 cd	36
10^{-3}	0.2 d	14

a) In a column, means followed by a same letters are not significantly different by the 5% level of Duncan's multiple range test.

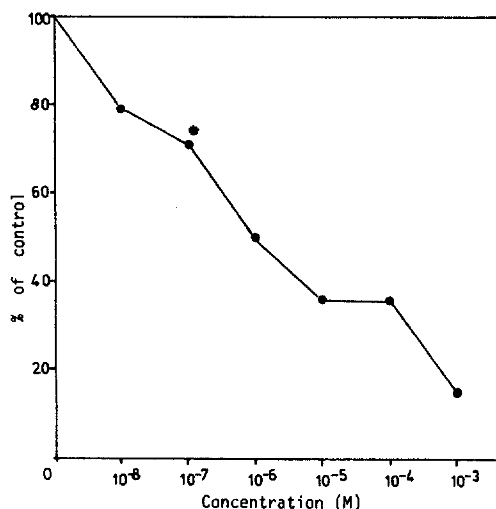

Fig. 2. Cell enlargement in oat coleoptiles, as a percent of the control, exposed to various concentrations of fluazifop-butyl for 24 hours. The * indicates the first significant difference from control determined by a Ducan's multiple range test at 5% level.

treatment on cell enlargement in oat coleoptiles are showen in table 2. Average cell enlargement rates were significantly inhibited at concentration of $1x10^{-7}$ reducing enlargement by 29%. The highest concentration, $1x10^{-3}$M treatment reduced growth by 86% after a 24 hr treatment. As the concentrations of fluazifop-butyl increased, the percent of inhibition of fluazifop-butyl increased. After 24 hr treatment the persent inhibition of cell enlargement at $1x10^{-6}$M to $1x10^{-3}$M was less then that of cell division (Table 1 and 2). These observations indicated that fluazifop-butyl also interfered with the normal cell enlargement. As a result, the herbicide reduced plant growth. However, what causes inhibition of cell enlargement could not be explained easily. Key (1964) suggested that RNA and protein synthesis were essential for the process of cell enlargement to proceed at a normal rate.

Protein incorporation study. The rates of herbicide inhibition of protein synthesis were measured by liquid scintillation. The effect of fluazifop-butyl on protein synthesis is showen in Table 3.

Table 3. Effect of Fluazifop-butyl on protein synthesis as measured by ^{14}C-leucine incorporation after 8 hr.

Treatment	^{14}C-leucine incorporation	
	dpm	% control
control	13162	
Fluazifop-butyl, 1 10^{-4}M	5184	39.4

Values are the mean of three replicates with 15 root tips per replicate.

After a 8 hr treatment with $1x10^{-4}$M fluazifop-butyl, there was over 60% inhibition of protein synthesis. Peregoy and Scott (1985) reported that fluazifop-butyl of $1x10^{-4}$M caused 50% inhibition of protein synthesis in soybean hypocotyl and 30% inhibition in corn coleoptile. Haloxyfop-butyl, a compound chemically similar fluazifop-butyl also inhibited protein synthesis in oat roots (Kim, 1983). There results indicated that fluazifop-butyl inhibited protein synthesis.

It was concluded from the studies of the cell division, cell enlargement, and protein incorporation that growth inhibition could be due to reduction of cell division, cell enlargement, and protein synthesis. Herbicide inhibition of growth can result from interference at a single site action or several sites of action simulataneously. Because herbicides acting by inhibiting growth may have diverse biochemical sites of action (Mereland et al. 1982). These growth inhibited by fluazifop-butyl may resulted from three factors (cell division, cell enlargement and protein synthesis). Protein involves in cell division and cell enlargement. However the role of protein in cell division and cell enlargement are not studied throughfully. Further studies on growth inhibition by fluazifop-butyl should be cell division cycle and some biochemical events related to the cell division and cell enlargement.

摘 要

본 연구는 生長抑制劑인 fluazifop-butyl의 除草作用機構를 구명하기 위하여 본제초제가 생장의 기본요소인 細胞分裂과 伸長 그리고 蛋白質合成에 미치는 영향을 조사하였다. 본제초제를 귀리의 뿌리에 농도별로 처리한 후 0에서 48시간까지의 細胞分裂에 미치는 영향을 조사하였다. 또한 細胞伸長에 미치는 제초제의 영향은 oat coleoptile straight-growth test 방법으로 조사하였다. 蛋白質合成에 미치는 제초제의 영향은 ^{14}C-leucine을 뿌리에 흡수시켜서 合成抑制 정도를 liquid scintillation counter로 측정했다. 16시간 처리 후 1×10^{-3}M과 1×10^{-4}M 구에서 세포분열을 억제하였다. 18시간 처리 후 모든 처리구에서 세포분열이 억제되었다. 24시간 처리 후에는 1×10^{-3}M은 100%, 1×10^{-4}M은 99% 1×10^{-5}M은 89%의 세포분열을 억제시켰으나 적농도구인 1×10^{-6}M은 같은 처리 기간동안에 20%의 세포분열만을 억제시켰다. 농도와 처리시간이 증가함에 따라 抑制效果도 증가하였다. 억제효과가 가장 크게 나타난 기간은 처리후 0에서 18시간 이내에 나타났다. 본 제초제의 세포신장억제 효과는 1×10^{-7}M 이상의 고농도에서 有意性을 나타냈으며 1×10^{-6}M 이상의 고농도에서는 50% 이상의 세포신장억제를 보여 주었다. 단백질합성에 관한 조사에서는 8시간 처리후에 60%의 단백질합성이 억제되

었다. 이상의 결과를 종합하여 볼 때 fluazifop-butyl 의 植物生長 抑制機構는 세포분열과 신장 그리고 단백질합성을 억제함으로써 기인된 것으로 사료된다.

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