Effects of Sulfonylurea Herbicides on the Seedling Growth of Corn (*Zea mays* L.) Plants

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Abstract

Sulfonylurea herbicides are used primarily for rice weed control in Taiwan, but are seldom used on other land-crops. The study estimates the damage of seedling growth of corn plants caused by three sulfonylureas, i.e. bensulfuron-methyl, pyrazosulfuron-ethyl and imazosulfuron. Corn, cv. Tainung No.1 (TN 1), was used as materials and various rates of the herbicides were applied into soil directly at the 4th leaf stage. The dose-response of plant height was calculated by the log-logistic function and the results indicated that plant height was significantly inhibited by pyrazosulfuron-ethyl with 4.8-7.3 g a.i/ha of GR_{50} but little affected by other two sulfonylureas. In addition, the dose-response curve of plant dry matter production against three sulfonylureas showed the 9.7, 14.9 and 44.3 g a.i/ha of GR_{50} for bensulfuron-methyl, pyrazosulfuron-ethyl and imazosulfuron, respectively. Also the lower limit of the response curve was largely declined for pyrazosulfuron-ethyl by 84% of the upper

limit. Furthermore, the experiment using 20g a.i/ha of sulfonylurea herbicides revealed that dry matter production started to be inhibited after one week and inhibited significantly to 87, 83 and 67% for imazosulfuron, bensulfuron-methyl and pyrazosulfuron-ethyl, respectively, three weeks later. However, together with that free amino acids of leaves were declined to 30, 41 and 77% for imazosulfuron, bensulfuron-methyl and pyrazosulfuron-ethyl within one week, these changes of free amino acids apparently could not reflect the inhibition degree of seedling growth. In this experiment, the valine content was not influenced by sulfonylureas but bensulfuron-methyl declined the isoleucine to 40-55% within one week and even to 25% after three weeks. Besides, leucine was slightly decreased, ca. 20%, by imazosulfuron and was decreased to 40-46% by bensulfuron-methyl or pyrazosulfuron-ethyl. The significant decreases of leucine and isoleucine within one week after treatment may possibly be related to the inhibition of seedling growth.

Key words: Sulfonylurea, Herbicide, Corn, Growth inhibition, Branched-chain amino acids.

硫醯尿素類除草劑對玉米幼苗生長之影響

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摘 要

本研究主要目的在比較台灣地區現行三種硫醯尿素類 (sulfonylureas) 除草劑 bensulfuron-methyl, pyrazosulfuron-ethyl 及 imazosulfuron 對於玉米幼苗生長之劑量反應，並探討其差異性傷害之原因。本試驗以玉米台農一號 (TN 1) 為材料，於四葉期分別在盆缽土壤中施用 0、3.9、7.8、15.6、31.3、62.5、125、250 及 500g a.i/ha 之劑量。於處理

後三週利用劑量與植株反應關係之 log-logistic 分析模式，結果發現玉米幼苗之株高生長受到 pyrazosulfuron-ethyl 顯著抑制，其 GR_{50} 約為 4.8-7.3g a.i/ha，對其他二種除草劑之反應較不敏感。此外，由乾重變化分析顯示，bensulfuron-methyl 與 pyrazosulfuron-ethyl 之 GR_{50} 劑量相近，分別為 9.7 及 14.9g a.i/ha，而 imazosulfuron 則為 44.3 g a.i/ha，但仍以 pyrazosulfuron-ethyl 造成之下限 (lower limit) 值最低，顯示 pyrazosulfuron-ethyl 抑制幼苗乾重之幅度最大，bensulfuron-methyl 次之，imazosulfuron 最小。進一步以 20 g a.i/ha 之劑量處理，於處理後一週起幼苗乾物質生產開始受到抑制，至第三週時 imazosulfuron、bensulfuron-methyl 及 pyrazosulfuron-ethyl 處理者分別降為對照處理之 87、83 及 67%。分析葉部之游離胺基酸，則顯示其在處理後一週內分別降至 30、41 及 77%，隨後逐漸恢復，似乎游離胺基酸之含量變化無法直接反映出三種除草劑抑制玉米生長的程度差異。由於硫醯尿素類除草劑主要係抑制支鏈胺基酸合成，故本試驗進一步分析，但發現 valine 含量並無明顯變化且略有增加，而 isoleucine 則在三種除草劑處理後一週內降至 40-55%，其中 bensulfuron-methyl 處理三週後降至 25%。至於 leucine 則以 imazosulfuron 處理者降低 20%，而 bensulfuron-methyl 及 pyrazosulfuron-ethyl 處理者均降至 40-46%。故推測三種除草劑於處理後一週內 leucine 與 isoleucine 含量下降，可能直接影響生長抑制效果。

關鍵語：硫醯尿素類除草劑、玉米、生長抑制、支鏈胺基酸。

INTRODUCTION

Sulfonylureas herbicides can inhibit the biosynthesis of branched-chain amino acids by inhibiting acetolactate synthase (ALS; EC 4.1.3.18) activity. Due to efficient control of weeds by low-rate of sulfonylureas and the less toxicity to mammals (Levitt, 1991), this herbicide is paid more attentions. In Germany, atrazine is used primarily in weed control of corn field, which easily causes pollution and accumulation of toxic residues; thus sulfonylureas are gradually considered to instead of atrazine (Kees and Lutz, 1991). In addition, weed controls of corn and sorghum field have also been performed by applying sulfonylureas in some areas (Vidrine et al., 1990; Vizantinopoulos, 1989). In Taiwan, three commercialized products of sulfonylurea herbicides are available, bensulfuron-methyl (Londax; DuPont Co.), pyrazosulfuron-ethyl (Sirius) and imazosulfuron. Since the application of sulfonylureas is recommended only in rice weed control in Taiwan, the effects of above-mentioned sulfonylureas on the

growth of corn plants is unclear.

Since 1981, a number of sulfonylurea herbicides such as triasulfuron, beacon, nicosulfuron, primisulfuron, thifensulfuron, bensulfuron, rimsulfuron and chlorsulfuron have been developed. However, due to the herbicides with different structures, different phytotoxicity might exist for the target plants or enzymes. Mekki and Leroux (1994) indicated that the activities of ALS extracted from *Ambrosia artemisiifolia, Panicum miliaceum, Amaranthus retroflexus, Digitaria ischaemum* and *Avena fatua* were all inhibited by nicosulfuron, rimsulfuron or the mixture of both. Mostly, ALS activities were more inhibited by rimsulfuron than nicosulfuron. However, the reason for differential inhibition is unknown.

In general, various analogs of sulfonylureas also cause different injury levels of corn plants, i.e. chlorsulfuron>triasulfuron>nicosulfuron (Kimura *et al.*,1989; Vizantinopoulos,1989; Green and Ulrich, 1993). Of particular interest is to know the reason why the identical type of herbicides causes the different degrees of herbicidal toxicity in corn plants. Therefore, this experiment was performed to estimate the effects of three sulfonylureas on the seedling growth of corn plants, the dose-response and the possible reasons of the differential inhibition caused by sulfonylureas.

MATERIALS AND METHODS

Seeds of corn (*Zea mays* L.) cultivar, hybrid Tainung No.1 (TN1), were planted in 12-cm diameter pot filled with sand in the greenhouse at the National Chung-Hsing University during January to April in 1996. Each pot was supplied with 25 ml of Hogland's nutrient solution every other day, and with 25 ml of water on the day without application of nutrient solution during the experimental period (Hogland and Arnon, 1950). Plants were applied with various rates of three sulfonylureas, i.e. bensulfuron-methyl (Londax, 0.083%, DuPont Co.), pyrazosulfuron-ethyl (Sirius, 10%) and imazosulfuron (10%), respectively, at the fourth leaf stage of corn plants. A given rate of each herbicides were diluted to 25 ml and applied directly into each pot. The experimental design was complete random design (CRD) with four replications, but the dry matter production was determined with ten replications.

Corn seedlings were collected at the third week after treatment and the sandy soil surrounding roots was carefully washed out. The plant height of each seedling was measured and both the shoot- and root-part were separated, oven-dried at 80°C for 48 hours and weighed. All of the data related to dose-response were transformed and fitted by log-logistic analysis model with equation f(x)=d+[(a-d)/1+Exp[b(log(x)-log(c))]] (Seefeldt *et al.*,1995). The four parameters, a, b, c and d, indicate the upper

limit, slope, GR_{50} and lower limit of the log-logistic curve, respectively. In this study, GR_{50} was defined as the dosage inhibiting 50 % of plant height growth.

Based on the results of dose-response curve, 20g a.i/ha of three sulfonylureas were applied and the seedlings were sampled 0, 1, 2 and 3 weeks after treatment to measure the dry matter production and analyze the branched-chain amino acids. Total free amino acids were determined by ninhydrin reagent (Rosen, 1957) and read the absorbance at 570 nm with spectrophotometer (Model L-4200, Hitachi Co.). Three branched-chain amino acids were determined by OPD (ortho-phthal-di-aldehyde) reagent (Joseph and Marsden, 1986). The fresh sample was ground in liquid nitrogen and homogenized with methanol at 4°C for 10 min. The mixture was centrifuged at 7,500 g for 10 min. Fifty μL of supernatant were mixed with 250μL of OPD reagent (adding 10 μL mercaptoethanol into 2 mL OPD stock, freshly prepared before use). Twenty μL of the mixture were injected into HPLC system consisting of 5 μm, RPC-18 column (4.6x250 mm), and a fluorescence detector with 360 nm of excitation wavelength and 455 nm of emission wavelength. The mobile phase consisted of buffer A (0.05M phosphate buffer: methanol=4:1) and buffer B (0.05M phosphate buffer: methanol=1:4), and run by using a gradient of buffer B as: 0- 10%(10 min.)- 85%(40 min.)- 0%(45 min.)- 0%(55 min.) with 1.0 mL/min. of flow rate. The data were specified as means and standard errors (S.E.) of the sample mean with four replicates.

RESULTS AND DISCUSSION

To estimate the dose-response of seedling growth of corn, cv. Tainung No.1 (TN1), in response to three sulfonylureas, i.e. bensulfuron-methyl (Londax), pyrazosulfuron-ethyl (Sirius) and imazosulfuron, plant height and dry matter production of corn plants were measured. Experimental results indicated that the dosage response of plant height to three given sulfonylureas could satisfied the log-logistic model mentioned by Seefeldt *et al.*(1995). Plant height growth was extremely inhibited by pyrazosulfuron-ethyl than other two sulfonylureas (Fig. 1). In four continuous months, the GR_{50} value of pyrazosulfuron-ethyl was 4.8-7.27 g a.i/ha while two others had larger values. The finding suggests that the plant height growth of corn seedlings is extremely sensitive to pyrazosulfuron-ethyl, less or slightly sensitive to imazosulfuron and bensulfuron-methyl.

In this experiment, dry matter production of corn seedlings was also investigated to estimate its dose-response to three sulfonylureas. Experimental results showed that the upper limits (parameter a) of dose-response curve in response to three herbicides were similar (Table 1). Although the GR_{50} values of both bensulfuron-methyl and

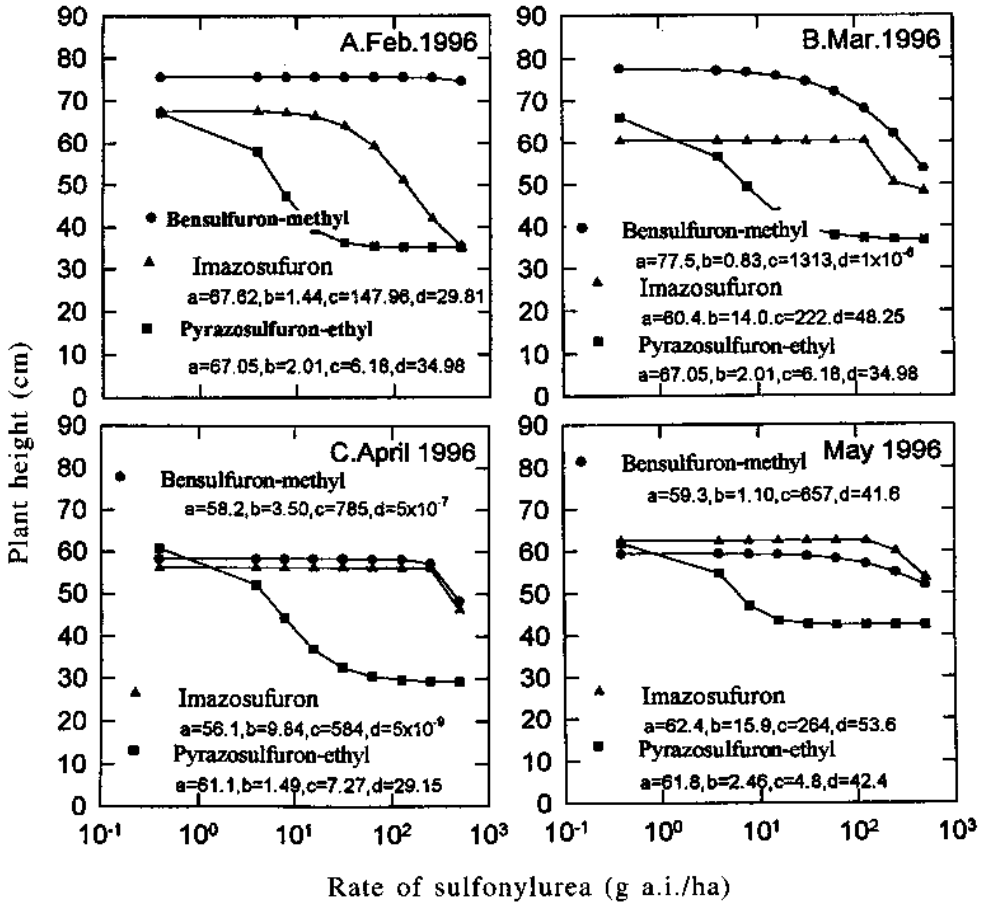

Fig. 1. Dose response of corn seedlings in continuous months to sulfonylureas, i.e. bensulfuron-methyl, imazosulfuron and pyrazosulfuron-ethyl. Data are analyzed by log-logistic analysis model. The response curves are fitted by the equation y=f(x)=d+[(a-d)/1+exp[b(log(x)-log(c))]] and the four parameters, a,b,c and d, represent the upper limit, slope, GR_{50} and lower limit, respectively. Each point shown is a transformed value by log-logistic function with 4 replicates.

pyrazosulfuron-ethyl were approximate (9.7 and 14.9 g a.i/ha), the lower limits (parameter d) under the application of bensulfuron-methyl, imazosulfuron and pyrazosulfuron-ethyl were 0.86, 1.07 and 0.60 g a.i/ha, respectively. In calculating the decrease percentage of lower limit as compared with upper limit, there were 49, 30 and 84% of reduction for lower limits in response to bensulfuron-methyl, imazosulfuron and pyrazosulfuron-ethyl (Table 1). It is suggested that the dry matter production of corn seedlings is extremely sensitive to pyrazosulfuron-ethyl than bensulfuron-methyl and imazosulfuron.

Table 1. Effects of sulfonylurea herbicides, i.e. bensulfuron-methyl, imazosulfuron and pyrazosulfuron-ethyl, on the dry matter production of corn (*Zea mays* L.) seedlings. The dose-response relationships were denoted by the log-logistic curve: y=f(x)=d+[(a-d)/1+exp[b(log(x)-log(c))]] and the four parameters, a, b, c and d, represent the upper limit, slope, GR_{50} and lower limit, respectively. The data was obtained from 4 months, Feb., Mar., April and May, in 1996 with 10 replicates.

Sulfonylurea	a	b	c	d	[(a-d)/a]*100
	-g/plant-		-g/plant-	-g/plant-	-%-
Bensulfuron-methyl	1.70±0.23	71.2±61.0	9.7±1.7	0.86±0.23	49
Imazosulfuron	1.54±0.21	0.61±0.47	44.3±25.2	1.07±0.13	30
Pyrazosulfuron-ethyl	1.49±0.17	2.56±1.72	14.9±3.9	0.60±0.06	84

To explore the herbicidal action of three adopted sulfonylureas, the average value of three GR_{50} values of dry matter production in response to three herbicides, ca. 20g a.i/ha of dosage, was adopted in the subsequent experiment. After applying 20 g a.i/ha of each herbicide, total dry matter production of corn seedlings was not inhibited until one week after treatment and was significantly reduced to 87, 83 and 67% of

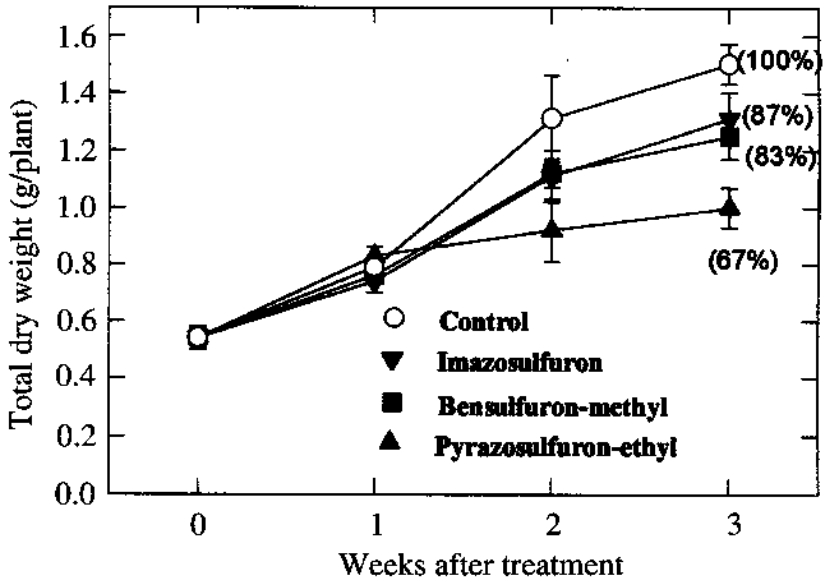

Fig. 2 Effects of sulfonylureas (20 g a.i/ha) on the dry matter production of corn seedlings. Vertical bars indicate the standard errors of the sample mean with 10 replicates.

the control at the third week by imazosulfuron, bensulfuron-methyl and pyrazosulfuron-ethyl, respectively (Fig. 2).

Since 1985, the ALS enzyme has been referred as the target enzyme of sulfonylurea herbicides (Stidham, 1991). The ALS is a critical enzyme in the first reaction of branched-chain amino acids synthesis; the inhibition of this enzyme may cause starvation of branched-chain amino acids and subsequent death of plants. In addition, the inhibition of ALS activity may also result in the accumulation of some toxic metabolites, i.e. 2-ketobutyrate and 2-aminobutyrate (Shaner and Singh, 1993). However, Shaner and Singh (1993) demonstrated that growth inhibition following imazaquin treatment is not due to the accumulation of 2-ketobutyrate and/or 2- aminobutyrate in corn plants, and suggested that starvation of the branched-chain amino acids may be the primary cause of growth retardation.

In this experiment, total free amino acids of leaves were decreased to 77, 41 and 30% of control by the imazosulfuron, bensulfuron-methyl and pyrazosulfuron-ethyl treatments within one week (Fig. 3). However, the reductions were diminished immediately. The changes of free amino acids apparently can not accurately reflect the inhibitory effect of growth. As well known, the action of sulfonylureas blocks the biosynthesis of branched-chain amino acids, i.e. valine, isoleucine and leucine;

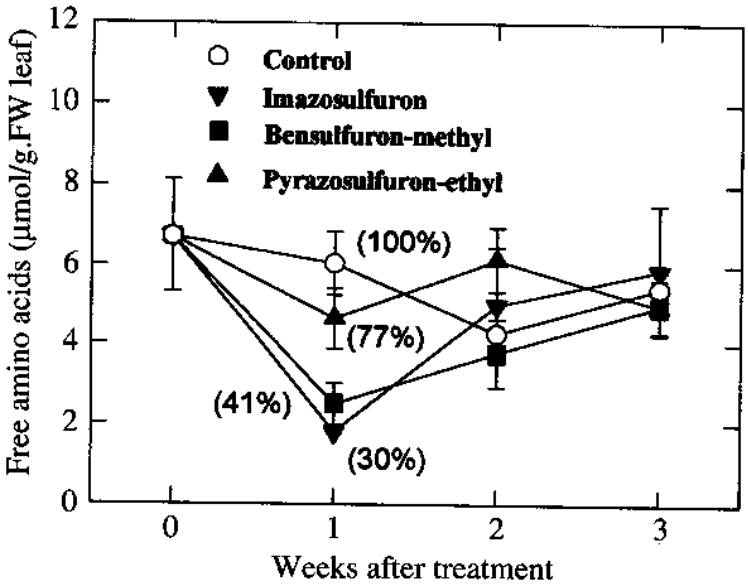

Fig. 3. Changes in total free amino acids in leaves of corn seedlings as affected by sulfonylurea herbicides, i.e. bensulfuron-methyl, imazosulfuron and pyrazosulfuron-ethyl, with application rate of 20 g a.i/ha.

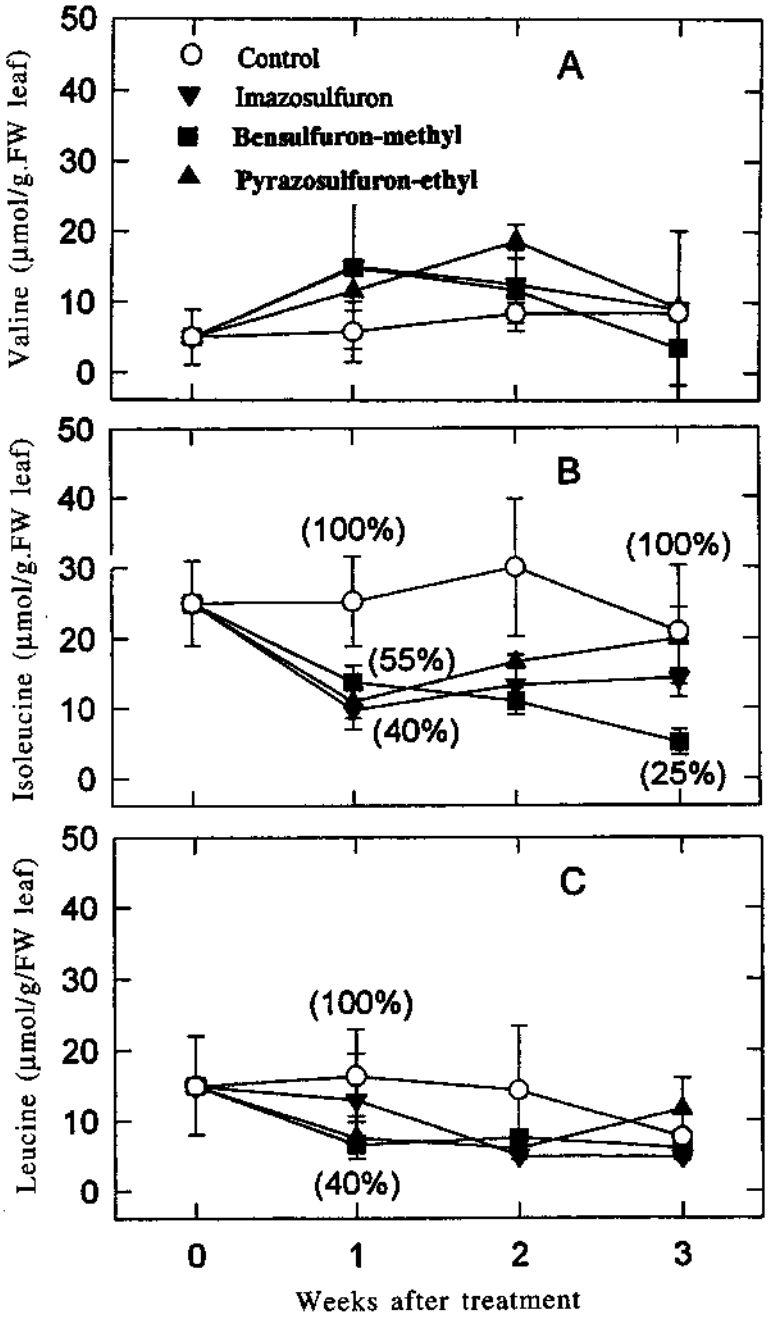

Fig. 4. Changes in the branched-chain amino acids, A) valine B) isoleucine and C) leucine contents in leaves of corn seedlings as affected by three sulfonylurea herbicides.

thus, the changes of these three amino acids were expected to assay. Experimental results indicated that the isoleucine was decreased to 40-45% of control within one week after three sulfonylurea treatments and decreased more to 25% by bensulfuron-methyl three weeks later (Fig. 4B), though little changes in valine after treatments (Fig. 4A). Also, the leucine slightly declined, ca. 20%, by imazosulfuron and decreased more, ca. 40-46%, by bensulfuron-methyl and pyrazosulfuron-ethyl one week after treatment (Fig. 4C). This finding suggests that the reduction of both leucine and isoleucine by sulfonylureas within one week might be involved in the growth inhibition of corn seedling. Due to that the ALS enzyme is a target enzyme of sulfonylureas, all branched-chain amino acids were expected to be decreased by the herbicides. Experimental results indicated that the valine was not decreased but increased slightly by three sulfonylureas. Therefore, it might be occurred that the common precursor of both valine and leucine, i.e. alpha-ketoisovalerate, was competitively metabolized by two pathways after the sulfonylureas application. Above results contradict a previous report in *Salmonella typhimurium* (Epelbaum *et al.*, 1996), which showed that the levels of the keto acid precursor of valine, but not of the precursor of isoleucine, were drastically decreased by sulfometuron-methyl. Therefore, whether certain inhibitory effects exist other than ALS activity directly by sulfonylureas requires further investigation.

In Taiwan, sulfonylureas are used in weed control of rice field. Their influences on the growth of land-crops are seldom studied. Due to the advantages of low rate and low toxicity as compared with other kinds of herbicides, the sulfonylureas have been recommended for the near decade. To reduce the herbicidal injury of corn seedling caused by sulfonylureas, it is suggested to use imazosulfuron, other than bensulfuron-methyl and pyrazosulfuron-ethyl, in corn filed.

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