

## Herbicidal Action Mechanism of Flucetosulfuron

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### 플루세토설푸론의 제초활성 작용기작

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**ABSTRACT** This study was conducted to investigate the herbicidal action mechanism of flucetosulfuron. At the whole plant level, the GR<sub>50</sub> values (the dose rate required for 50% growth inhibition) of flucetosulfuron for *Echinochloa crus-galli* were 0.6 and 4.6 g ai ha<sup>-1</sup> by soil and foliar application, respectively, while those for rice were 183 and 223 g ai ha<sup>-1</sup>, respectively, demonstrating high activity against *E. crus-galli* with good safety to rice. In the assay by dipping leaf and root of *E. crus-galli* in flucetosulfuron solution at 0.42 ppm, the LT<sub>50</sub> values (dipping period required for 50% growth inhibition) of flucetosulfuron were 48.7 and 5.5 hrs for leaf and root, respectively, demonstrating that flucetosulfuron applied to the flooded soil is more readily absorbed via root than leaf. In the leaf excision assay, the LT<sub>50</sub> values (excision time after spot-treatment required for 50% growth inhibition) of flucetosulfuron were 5.2 and 0.7 hrs for *E. crus-galli* and *Brassica napus*, respectively, while those of glyphosate and pyribenzoxim were much greater, indicating that flucetosulfuron treated on a leaf is more quickly translocated than glyphosate and pyribenzoxim. In *in vitro* acetolactate synthase (ALS) enzyme assay, I<sub>50</sub> values (concentration required for 50% activity inhibition of ALS) of flucetosulfuron were  $9.38 \times 10^{-5}$  and  $8.38 \times 10^{-5}$  M for rice and *E. crus-galli*, respectively, confirming that flucetosulfuron inhibited ALS similarly to other sulfonylurea herbicides. Overall results show that flucetosulfuron is readily absorbed to both leaf and root, translocated in the plants relatively quickly and inhibits ALS.

**Key words:** acetolactate synthase (ALS); *Echinochloa crus-galli*; flucetosulfuron; sulfonylurea; translocation.

### INTRODUCTION

Flucetosulfuron is a new sulfonylurea herbicide and known to control broadleaf and sedge weeds such as *Monochoria vaginalis*, *Rotala indica*, *Scirpus juncooides*, *Eleocharis kuroguwai*, etc. by post-

emergence application in rice cultivation (Kim et al., 2003; Koo et al., 2003). In this terms, flucetosulfuron is similar to existing sulfonylurea herbicides, but its unique advancement is its high efficacy against *Echinochloa crus-galli* as compared with other sulfonylurea herbicides. Due to its broad spectrum

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covering not only broadleaf and sedge weeds but also *E. crus-galli*, its straight product is now registered as an one-shot herbicide with a brand name of Fluxo<sup>®</sup> in Korea (KCPA 2004). Although flucetosulfuron was firstly developed for rice use, it also shows good activities against cereal weeds including *Galium aparine*, *Matricaria perforata*, *Papaver rhoeas*, and *Stellaria media* with a good safety to cereal crops (Kim et al., 2003). Lee et al. (2006) reported that air temperature, overflow of the flooded water after soil application, and soil nitrogen fertility significantly affected the herbicidal activity of flucetosulfuron.

Although many studies were conducted to evaluate the herbicidal performance of flucetosulfuron under various conditions (e.g., Lee et al., 2006), little effort has been made to investigate its herbicidal action mechanisms. It is expected that flucetosulfuron is a systemic herbicide like other sulfonylurea herbicides, so its absorption and translocation may be important processes for its herbicidal action. Like other sulfonylurea herbicides, the target site of flucetosulfuron is assumed to be acetolactate synthase (ALS), a responsible enzyme for the biosynthesis of branched amino acids such as leucine, isoleucine, and valine. No study has been made for its absorption and translocation. Therefore, studies on absorption and translocation together with *in vitro* activity on the target site are essential and thus will provide more clear understanding of its mechanism.

In this study, we investigated the absorption and translocation of flucetosulfuron using bioassay methods, and conducted an *in vitro* ALS assays to investigate its mechanism of herbicidal action.

## MATERIALS AND METHODS

### Whole plant assay

Pot experiments were conducted in the glasshouse

of LG Life Sciences R&D Park in Daejeon, Korea, to evaluate the herbicidal activities of flucetosulfuron on rice (*Oryza sativa* L.) and *E. crus-galli* by foliar and soil application.

For foliar application, each 10 plants of rice (cv. Chucheong) and *E. crus-galli* were grown in a plastic pot (150 cm<sup>2</sup>) in the glasshouse maintained at 30/23 (day/night)±3°C. At 4-leaf stage, the herbicide was sprayed using a CO<sub>2</sub>-pressurized belt-driven sprayer (R&D Sprayer, USA) equipped with an 8001E flat fan nozzle (Spraying System Co., USA) adjusted to deliver 300 L ha<sup>-1</sup>. Application rates of flucetosulfuron (50% WG) were 10-320 g ai ha<sup>-1</sup> for rice and 2.5-80 g ai ha<sup>-1</sup> for *E. crus-galli*. Treated plants were then returned to the glasshouse and watered by sub-irrigation as needed. Fresh weight was measured at 20 days after application.

For soil application, 6 plants of 14 days-old rice seedlings were transplanted in a plastic pot (200 cm<sup>2</sup>) containing paddy soil, and seeds of *E. crus-galli* were sown in the same pot as rice. The plants were grown in a submerged paddy condition at 3 cm water depth in the glasshouse. At 3-leaf stage of *E. crus-galli*, flucetosulfuron (0.07% GR) and pyrazosulfuron-ethyl (0.07% GR) were applied onto the flooded soil in the pots at 1.25, 2.5, 5, 10, 20, 40, and 80 g ai ha<sup>-1</sup> for *E. crus-galli*, and 10.5, 21, 42, 63, 84, 168, and 336 g ai ha<sup>-1</sup> for rice. Fresh weight was measured at 34 days after application.

The experiments were conducted in a completely randomized design with three replicates.

### Measurement of absorption and translocation

Bioassay methods were used to measure absorption and translocation of flucetosulfuron. For absorption and translocation of flucetosulfuron applied to the flooded soil, two separate experiments were conducted. In root absorption experiment, *E. crus-galli* grown up to 3.5-leaf stage was sampled by carefully washing

soil off from the roots. The roots of *E. crus-galli* were then dipped in the 0.42 ppm flucetosulfuron solution, prepared by dissolving flucetosulfuron 0.07% GR in distilled water, and distilled water as an untreated control, for 0, 4, 12, 24, 48, 72, 96, and 120 hours, washed the roots with enough water, and then transplanted in new pots containing puddled paddy soil until assessment at 14 days after the 120 hours dipping treatment. In foliar absorption experiment, the third leaf of *E. crus-galli* at 3.5-leaf stage was dipped in 0.42 ppm of flucetosulfuron and distilled water as an untreated control for the same periods of time as the above. After the periods of dipping, the dipped leaves were rinsed with enough water. The plants were then maintained in the glasshouse at the flooded condition by regular irrigation until assessment at 14 days after the 120 hours dipping treatment.

Leaf excision method (Koo et al., 2000) was used to measure absorption and translocation of flucetosulfuron applied to foliage. Dose rates for spot treatment were determined to be 1.0 and 10  $\mu\text{g plant}^{-1}$  of flucetosulfuron, 2.0 and 20  $\mu\text{g plant}^{-1}$  of pyribenzoxim, 0.5 and 20  $\mu\text{g plant}^{-1}$  of glyphosate for *E. crus-galli* and *Brassica napus*, respectively, from a preliminary experiment. Spot-treatment was made on the third and the second leaf of *E. crus-galli* at 3.5-leaf stage and *B. napus* at 2.5-leaf stage, respectively, and the treated leaves were excised sequentially at different timings; 1, 3, 6, 12, 24, 48, 72, and 96 hours after the spot-treatment. Fresh weights of treated *E. crus-galli* and *B. napus* were measured at 14 days after the last leaf excision at 96 hours after the spot-treatment.

#### *In vitro* ALS assay

ALS was extracted from green seedlings of rice and *E. crus-galli* at 3-leaf stage. The enzyme extraction and assay were conducted using a modified protocol

of Ray (1984). The concentrations of flucetosulfuron and pyrazosulfuron-ethyl as a reference herbicide were ranged  $1 \times 10^{-10} \sim 1 \times 10^{-3}$  M.

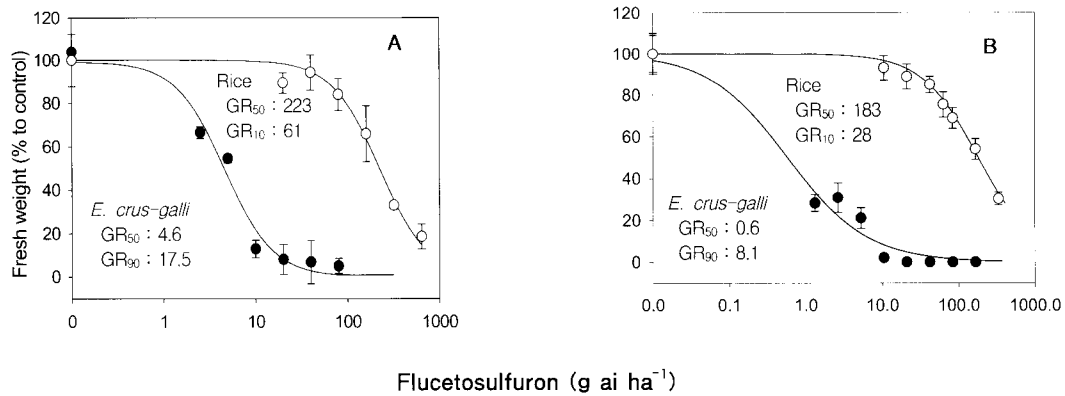
#### Statistical analysis

All measurements were initially subjected to analysis of variance (ANOVA). The standard dose-response model (Streibig, 1980) was fitted to the observed data to estimate GR<sub>50</sub>, a dose rate required for 50% growth inhibition, I<sub>50</sub>, a concentration required for 50% inhibition of ALS activity, and LT<sub>50</sub>, a time required for 50% growth inhibition. All statistical analyses were conducted using Genstat 5 (Genstat Committee, 1997).

## RESULTS AND DISCUSSION

#### Activity against whole plants

When flucetosulfuron was applied foliarly, the GR<sub>50</sub> values were 4.6 and 223 g ai ha<sup>-1</sup> for *E. crus-galli* and rice, respectively (Fig. 1A). In the case of soil application, the GR<sub>50</sub> values were 0.6 and 183 g ai ha<sup>-1</sup> for *E. crus-galli* and rice, respectively (Fig. 1B). Although these results indicate good selectivity of flucetosulfuron between rice and *E. crus-galli*, a simple comparison of GR<sub>50</sub> values is not sufficient enough to determine a practical selectivity. For more biologically practical determination of the selectivity, it is necessary to compare between the GR<sub>90</sub> value for *E. crus-galli* and the GR<sub>10</sub> value for rice (Hwang et al., 2006). The GR<sub>90</sub> value for weed and the GR<sub>10</sub> value for crop are practically acceptable levels of weed control and crop injury, respectively. The selectivity index (GR<sub>90</sub>/GR<sub>10</sub>) of flucetosulfuron, therefore, was approximately 3.5 for both foliar and soil applications. The selectivity index greater than 1 indicates that the herbicide tested can be practically used to manage the weed in the crop cultivation.

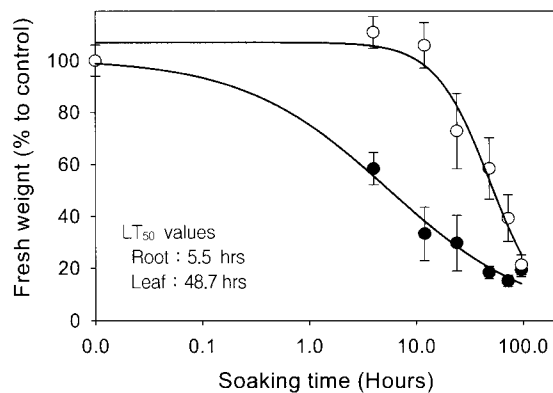


**Figure 1.** Fresh weights of rice (○) and *E. crus-galli* (■) treated with flucetosulfuron to foliage (A) and the flooded soil (B). The vertical bars represent the standard deviations of three replicates and the solid lines represent fitted values to the standard dose-response model.

Therefore, the selectivity index, 3.5, of flucetosulfuron clearly demonstrates that flucetosulfuron has a good safety to rice with a good herbicidal activity in controlling *E. crus-galli*.

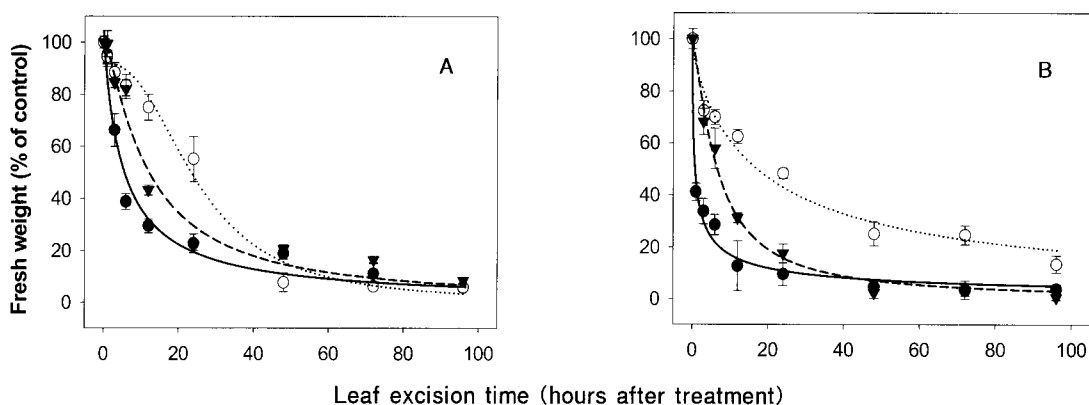
### Absorption and translocation

To measure the absorption of flucetosulfuron to root and leaf of *E. crus-galli*, bioassay test was conducted by dipping their roots and leaves in the 0.42 ppm flucetosulfuron solution for different periods of time, respectively. The fresh weight of *E. crus-galli* decreased logarithmically with increasing the periods of dipping time (Fig. 2). By fitting the observed data to the standard dose-response model, LT<sub>50</sub> values, the periods of dipping time for 50% growth inhibition, were estimated for absorptions to root and leaf. The LT<sub>50</sub> value for root absorption was 5.5 hours of dipping period, while leaf absorption was 48.7 hours, required much longer period than root absorption. One of the reasons of this difference may be physicochemical difference in leaf and root epidermis. In general, the plant leaf epidermis, particularly its upper epidermis, contains high quantity of epicuticular wax covering leaf surface, so the quantity of epicuticular wax on leaves could affect



**Figure 2.** Fresh weight of *Echinochloa crus-galli* dipped its leaf (○) and root (■) in 0.42 ppm flucetosulfuron solution for different periods of time. The vertical bars represent the standard deviations of three replicates and the solid lines represent fitted values to the standard dose-response model.

herbicide uptake indirectly by their effects on leaf wettability (Holloway et al., 1980). The leaf cuticle is a detrimental barrier to the retention and penetration of herbicide solution, so it may take much longer for herbicides dissolved in water at a very low concentration without adjuvant to be absorbed via leaf. Water without adjuvant has surface tension of 72 mN m<sup>-1</sup> and is repulsed from the leaf surface of *E. crus-galli*, while water with adjuvant may have



**Figure 3.** Fresh weights of *Echinochloa crus-galli* (A) and *Brassica napus* (B) spot-treated with flucetosulfuron (■), pyribenzoxim (○), and glyphosate (▼) on their leaves and excised treated leaves at different timings after treatment. The vertical bars represent the standard deviations of three replicates and the solid lines represent fitted values to the standard dose-response model.

much lower surface tension and retain on the leaf (Harr et al., 1991). Conversely, root epidermis has low content of epicuticular wax with many root hairs, which may allow herbicides dissolved in water to be absorbed easily.

Using the leaf excision method (Koo et al., 2000), the translocation of flucetosulfuron via leaf was investigated in detail. The minimal times of flucetosulfuron, glyphosate, and pyribenzoxim required to get 50% reduction in the fresh weight of *E. crus-galli* were estimated to be 5.2, 11.7 and 25.1 hours after spot treatment, respectively (Fig. 3A and Table 1). In the case of *B. napus*, those were 0.71, 6.7, and 18.3 hours after spot treatment, respectively (Fig. 3B and Table 1). These results indicated that flucetosulfuron translocated most rapidly in both *E. crus-galli* and

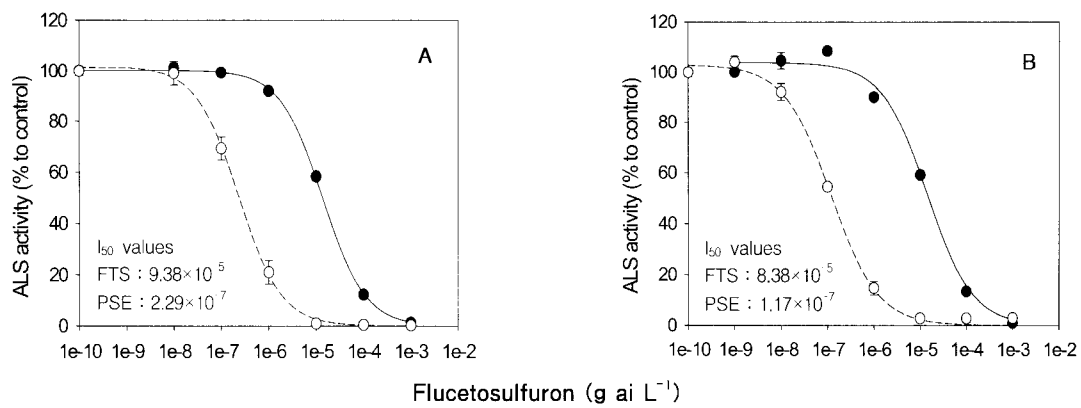
*B. napus*, followed by glyphosate and pyribenzoxim.

#### Activity against ALS

To confirm if ALS is a target site of flucetosulfuron and to quantify its activity on the target site in comparison with pyrazosulfuron-ethyl, *in vitro* assay using ALS extracted from rice and *E. crus-galli* was conducted. The ALS  $I_{50}$  values of flucetosulfuron for rice and *E. crus-galli* were  $8.38 \times 10^{-5}$  and  $9.38 \times 10^{-5}$  M, respectively. However, the ALS  $I_{50}$  values of pyrazosulfuron-ethyl were  $2.29 \times 10^{-7}$  and  $1.17 \times 10^{-7}$  M for rice and *E. crus-galli*, respectively (Fig. 4). Thus, the  $I_{50}$  values of flucetosulfuron were about 100-1000 times greater than those of pyrazosulfuron-ethyl. Other sulfonylurea herbicides such as chlorsulfuron, sulfometuron-methyl, and chlorimuron-ethyl had similar  $I_{50}$  values to that of pyrazosulfuron-ethyl (Beyer et al., 1988; Gerwick et al., 1990). However, in whole plant assay, flucetosulfuron showed about 3 times greater activity against *E. crus-galli* than pyrazosulfuron-ethyl (Koo et al., 2003). It is unique that, despite this marked weakness in *in vitro* ALS inhibition activity, flucetosulfuron had stronger whole plant activity than pyrazosulfuron-ethyl in *E. crus-galli*. Therefore, in the herbicidal action at the whole

**Table 1.**  $LT_{50}$  and  $LT_{90}$  values in inhibiting *Echinochloa crus-galli* and *Brassica napus*, whose leaves were spot-treated with herbicides.

Herbicide	<i>E. crus-galli</i>		<i>B. napus</i>	
	GR <sub>50</sub>	GR <sub>90</sub>	GR <sub>50</sub>	GR <sub>90</sub>
Flucetosulfuron	5.2	51.3	0.7	27.0
Pyribenzoxim	25.1	60.5	18.3	236.4
Glyphosate	11.7	66.8	6.7	36.2



**Figure 4.** Activities of ALS extracted from rice (A) and *E. crus-galli* (B) and treated with flucetosulfuron (FTS, ■) and pyrazosulfuron-ethyl (PSE, ○) at a range of concentrations. The vertical bars represent the standard deviations of three replicates and the solid lines represent fitted values to the standard dose-response model.

plant level, flucetosulfuron is likely to involve some unknown physiology in the potent herbicidal action. In terms of selectivity, the difference in *in vitro* ALS I<sub>50</sub> values between rice and *E. crus-galli* was not great; therefore, differential ALS sensitivity does not seem to contribute significantly to the selectivity between rice and weeds.

### Conclusion and further works

Our studies confirmed that flucetosulfuron is highly active against *E. crus-galli* and safe to rice, demonstrating good selectivity between *E. crus-galli* and rice. Absorption and translocation studies showed that flucetosulfuron could be absorbed via both root and shoot, implying that flucetosulfuron may use both xylem and phloem for its translocation. All aerial parts of plants are covered by a cuticle, which represents a heterogeneous interface between a plant and the atmosphere. The cuticle is composed of an insoluble cutin framework and soluble waxes (soluble in nonpolar solvents). Soluble waxes are dispersed throughout the cuticle, but are more predominant towards the outer surface of the cuticle. Therefore, the outer surface is highly lipophilic and the environment becomes increasingly hydrophilic as the inner surface

of the cuticle is approached. A pot experiment with *Aeschynomene indica* showed that adjuvant significantly affect the herbicidal activity of flucetosulfuron against this weed (data not shown), but its mechanism has not been investigated in detail. Further work is required, particularly to quantify the amount of flucetosulfuron absorbed and translocated into plant parts by using radio-labelled flucetosulfuron, and to investigate adjuvant effects on its absorption and translocation.

*In vitro* ALS enzyme assay revealed that flucetosulfuron inhibited plant ALS as other sulfonylurea herbicides, but with no significant difference between ALS enzymes extracted from rice and *E. crus-galli*, suggesting that selectivity between the two species would not be based on sensitivity difference at the target site. The significantly greater I<sub>50</sub> value and stronger whole plant activity of flucetosulfuron than pyrazosulfuron-ethyl suggest likely involvement of some unknown physiology in the potent herbicidal action. Therefore, further investigation is needed to understand the relationship between the weak *in vitro* ALS activity and the potent whole plant activity, and the selectivity mechanism of flucetosulfuron between rice and *E. crus-galli*.

## 요 약

본 실험은 flucetosulfuron에 대한 벼와 피의 생물적 반응과 제초활성 작용기구를 연구하고자 수행되었다. 피와 벼에 대한 활성평가 결과 피에 대한 GR<sub>50</sub>값은 토양 및 경엽처리의 경우 각각 0.6과 4.6 g ai ha<sup>-1</sup>이었으나, 벼에 대한 값은 각각 183과 223 g ai ha<sup>-1</sup>로 피에 대한 높은 제초활성과 벼에 대한 안전성을 보여 주었다. 피의 잎과 뿌리를 0.42 ppm flucetosulfuron 용액에 침지하여 흡수이행을 평가한 결과 50% 생육억제에 필요한 침지기간은 잎의 경우 48.7시간, 뿌리는 5.5시간으로 뿌리를 통해 흡수이행이 빨리 이루어졌다. 엽 절취법에 의해 잎을 통한 이행을 평가한 결과 50% 생육억제를 위한 flucetosulfuron의 점적처리 후 엽 절취까지의 기간이 피의 경우 5.2시간, 유채는 0.7시간으로 대조약제인 glyphosate와 pyribenzoxim보다 빨리 흡수 이행되었다. Acetolactate synthase (ALS)에 대한 flucetosulfuron의 I<sub>50</sub>값은 벼의 경우 9.38×10<sup>-5</sup> M, 피는 8.38×10<sup>-5</sup> M로 다른 설폰닐우레아 제초제와 마찬가지로 ALS를 억제하였다. 종합적으로 flucetosulfuron은 경엽과 뿌리를 통해 흡수 이행되며, 경엽 이행은 glyphosate와 pyribenzoxim보다 비교적 빠르며, 이행된 flucetosulfuron이 식물체내에서 ALS의 활성을 억제함으로써 제초활성을 나타낸다.

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