RIMSULFURON UPTAKE, TRANSLOCATION, METABOLISM AND ALS SENSITIVITY TO RIMSULFURON IN TWO MAIZE HYBRIDS

Absorción, translocación, metabolismo y sensibilidad de la ALS al rimsulfuron en dos híbridos de maíz

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SUMMARY

Research was conducted to determine the role in selectivity of uptake, translocation, metabolism and ALS (acetolactate synthase) activity of rimsulfuron in two maize (*Zea mays* L.) hybrids ('Cargill 2127', tolerant, and 'Pioneer 3897', sensitive) grown at temperatures of 14°C and 21°C. Forty eight hours after treatment (HAT), uptake of rimsulfuron was 40% and 67% in 'Pioneer 3897', and 26% and 43% in 'Cargill 2127' at 14°C and 21°C, respectively. Neither total translocation nor allocation of rimsulfuron in various organs differed greatly between the hybrids. Translocation of ^{14}C-rimsulfuron was greater at 21°C (53%) than at 14°C (23%), 48 HAT. In 'Pioneer 3897' over 65% and 30% of the total ^{14}C-activity present in plant extracts was recovered as the parent compound within 24 HAT, at 14°C and 21°C, respectively. However, in 'Cargill 2127' detoxification of rimsulfuron was not affected by temperature, and 27% of the ^{14}C-total activity was recovered as the parent compound. Crude ALS extracts from 'Pioneer 3897' and 'Cargill 2127' maize seedlings were treated with various doses (0.001, 0.005, 0.01, 0.1 and 1.0 μM) of rimsulfuron. Based on ALS specific activity, I_{50} values differed sligthly between the two hybrids (I_{50} 'Pioneer 3897' = 0.091 μM and I_{50} 'Cargill 2127' = 0.142 μM). These results suggest that the mechanism of rimsulfuron tolerance in maize could be mainly explained by differential herbicide uptake and detoxification, with translocation and ALS sensitivity having little effect on differential tolerance of these maize hybrids to rimsulfuron. On the other hand, the greater uptake and translocation of rimsulfuron at 21°C, compared to 14°C, could explain the observed herbicide injury in maize at high temperatures under field conditions.

Key words: Herbicides, sulfonylureas, rimsulfuron, selectivity mechanism, uptake, translocation, metabolism, ALS, acetolactate synthase, maize.

RESUMEN

Esta investigación fue realizada con el objetivo de determinar el papel de la absorción, trnaslocación, metabolismo y actividad de la ALS (acetolactato sintetasa) en la selectividad del herbicida rimsulfuron en dos híbridos de maíz ('Cargill 2127', tolerante, y 'Pioneer 3897', sensible), bajo dos condiciones de temperatura, 14°C and 21°C. Cuarenta y ocho horas después del tratamiento (HDT) la absorción del herbicida fue 40% y 67% en 'Pioneer 3897', y 26% y 43% en 'Cargill 2127' a 14°C y 21°C, respectivamente. Ni la translocación total del herbicida ni su distribución en diferentes organos de la planta fueron apreciablemente diferentes entre los dos híbridos. La translocacion del ^{14}C-rimsulfuron fue mayor a 21°C (53%) que a 14°C (23%), 48 HDT. En 'Pioneer 3897' más del 65% y 30% de la actividad del ^{14}C presente en los extractos de plantas fue recuperada como molécula parental a las 24 HDT, a 14°C y 21°C, respectivamente. Sin embargo, en 'Cargill 2127' la detoxificacion del rimsulfuron no fue afectada por la temperatura, y 27% del total de actividad del ^{14}C se recuperó como molécula parental. Extractos crudos de la ALS de plántulas de 'Pioneer 3897' y 'Cargill 2127' se sometieron a dosis variables del herbicida (0.001, 0.005, 0.01, 0.1 y 1.0 μM). Con base en la actividad específica de la ALS, los valores de I_{50} fueron muy cercanos entre los dos hibridos (I_{50} 'Pioneer 3897' = 0.091 μM e I_{50} 'Cargill 2127' = 0.142 μM). Estos resultados sugieren que el mecanismo de tolerancia del rimsulfuron en el maíz podría explicarse principalmente por diferencias en la absorción y en el metabolismo; la translocación y la actividad de la ALS contribuyeron poco en este mecanismo de selectividad. Por otra parte, la mayor absorción y translocación del rimsulfuron a 21°C en comparación con 14°C, podría explicar la fitotoxicidad causada por el herbicida en el máiz bajo condiciones de campo.

Palabras claves: Herbicidas, sulfonylureas, rimsulfuron, mecanismo de selectividad, absorción, translocación, metabolismo, ALS, acetolactate synthase, maíz.

INTRODUCTION

There are at least four mechanisms in plants which affect response to herbicides. These include: (1) modification of the site of action of the herbicide, (2) differences in uptake and translocation of the herbicide so that it is not able to reach the site of action, (3) ability of the plant to degrade the herbicide, and (4) compartmentation of the

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herbicide or of its toxic metabolites (Coupland, 1991; Shaner, 1991; Shaner & Mallipudi, 1991). It is likely that the most important single factor contributing to species differences in response to herbicide action is the ability of tolerant plants to detoxify a herbicide (Hathway, 1986). On the other hand, the occurrence of differential tolerance to a specific herbicide among crop cultivars and among weed biotypes seems to be due to herbicide metabolism (Hathway, 1986). In fact, several cases of differential tolerance to a herbicide among crop cultivars were attributed to differences in the rate of herbicide metabolism (Hathway, 1986; Shaner & Mallipudi, 1991).

Two mechanisms appear to account for the tolerance of crops to sulfonylurea herbicides. Differential metabolism would play the predominant role (Sweetser *et al.,* 1982; Brown *et al*., 1991a; Brown *et al*., 1991b; Shaner, 1991), whereas changes at the site of action would have less importance (Novosel & Renner, 1995; Sterling & Jochen, 1995). Many references support the hypothesis that plant metabolic pathways and rate of metabolism are the basis for the selectivity of sulfonylurea herbicides between crops and weeds (Beyer *et al.,* 1988; Brown, 1990; Brown & Kearney, 1991; Brown *et al*., 1991a). Clear exceptions to this are the cases of genetically altered plants which have a resistant form of the ALS enzyme acquired through deliberate mutation (crop species), or unintended transformation (weed species) (Beyer *et al.,* 1988; Brown & Kearney, 1991; Shaner, 1991; Till *et al.,* 1991).

At least seven metabolic reactions leading to sulfonylurea herbicides detoxification have been identified: (1) aryl and alkyl-hydroxylation, (2) de-esterification, (3) homoglutathion conjugation, (4) aliphatic hydroxylation, (5) o-demethylation, (6) urea hydrolysis, and (7) sulfonamide cleavage (Beyer *et al.,* 1988; Brown, 1990; Brown & Kearney, 1991; Brown *et al*., 1991a; Brown *et al*., 1991b). Aryl and alkyl hydroxylation are one of the first reactions of the metabolism of sulfonylurea herbicides (Sweetzer *et al.,* 1982; Hutchinson *et al.,* 1984; Beyer *et al.,* 1988; Hatzios, 1991; Brown *et al*., 1991a; Brown *et al*., 1991b). Also, homoglutathion conjugation is an important metabolic pathway for these herbicides (Brown *et al*., 1991a). Direct involvement of P_{450} monooxygenases in aryl and alkyl hydroxylation and o-dealkylation of sulfonylureas have been successfully measured *in vitro* using plant microsomal preparations (Werck-Reichhart, 1995).

Rimsulfuron is a selective postemergence herbicide for the control of annual and perennial grasses and some broad-leaved weeds in maize (Palm *et al*., 1989; Everaerer, 1991; Anonymous, 1996). Maize tolerance to rimsulfuron is based on a higher rate of metabolism of the active compound to inactive metabolites, as compared to sensitive species such as blackgrass (*Alopecurus myosuroides*), johnsongrass (*Sorghum halepense*), and sorghum (*Sorghum bicolor*) (Palm *et al.,* 1989; Everaerer, 1991).

Nicosulfuron, a herbicide closely related to rimsulfuron, is rapidly hydroxylated in maize plants to an inactive compound, which is subsequently conjugated to glucose mediated via UDP-glucosyl transferase (Brown *et al*., 1991a). Obrigawitch *et al.* (1990) reported that maize metabolized over 90% of absorbed nicosulfuron within 20 HAT, and that metabolites are inactive against ALS. In contrast, there was no significant degradation of this herbicide in johnsongrass (*Sorghum halepense*) leaves at 24 HAT (Brown *et al*., 1991a). Kimura *et al*. (1989) found that nicosulfuron is metabolized in maize within six hours, while sensitive species cannot detoxify it after 48 HAT. Similarly, metabolism is the mechanism responsible for primisulfuron tolerance in maize (Harms *et al.,* 1990). Hinz and Owen (1996) also reported a rapid detoxification of nicosulfuron and primisulfuron in maize, with a half-life of less than 4h.

The selectivity of rimsulfuron in maize could be variable regarding the cultivar (Green & Ulrich, 1994; Fuentes, 1997). Maize is a variable species and has exhibited various responses to primisulfuron and nicosulfuron, two herbicides registered for selective weed control in maize (Monks *et al*., 1992; Morton & Harvey, 1992; O'Sullivan *et* al., 1995). Sweet maize cultivars are, generally, susceptible to rimsulfuron (Everaerer, 1991) while others are tolerant (Green & Ulrich, 1994).

Previous field experiments indicated that tolerance of maize to rimsulfuron is related to heat unit requirements and genotypes. Hybrids with more than 2 700 MHU (maize heat unit) were the most tolerant requirements (Fuentes, 1997). Moreover, previous growth-chamber and greenhouse experiments showed that rimsulfuron caused more injury to maize at high temperatures (Fuentes, 1997).

The objective of this study was to compare rimsulfuron uptake, translocation, and metabolism at two different temperatures, as well as ALS sensitivity to this herbicide, in a tolerant and a sensitive maize hybrid, in order to identify the mechanism of rimsulfuron tolerance in maize at the intra-specific level.

MATERIALS AND METHODS

General growing conditions

Research was conducted to determine the role in selectivity of uptake, translocation and metabolism of ^{14}C-rimsulfuron in maize, as well as ALS sensitivity to this herbicide. Two maize hybrids were compared: 'Cargill 2127' (tolerant) and 'Pioneer 3897' (sensitive). Uptake, translocation and metabolism experiments were carried out at temperatures of 14°C and 21°C. In all experiments, two seeds were planted in each plastic pot (8-cm diameter and 6-cm deep) in horticultural grade vermiculite, and grown under glasshouse conditions. After emergence, plants were thinned to one plant per pot. Plants were fertilized twice daily with 50-ml of a Hoagland nutrient solution.

In absorption, translocation and metabolism experiments, plants were placed in growth chamber, set for 14°C or 21°C

constant temperature, one day before ^{14}C-rimsulfuron treatment. Lighting from fluorescent and incandescent lamps provided a photosynthetic photon flux density (PPFD) of *ca.* 380 μmol m^{-2} sec^{-1} during a 14-h photoperiod. Plants stayed under these conditions until assessment.

Uptake and translocation studies

'Cargill 2127' and 'Pioneer 3897' plants were treated at the 4-leaf stage with ^{14}C-rimsulfuron (^{14}C-2-pyridine) with a specific activity (sp. act.) of 51.7 μCi mg^{-1} and 99% purity. Radiolabeled rimsulfuron was dissolved in 1 ml acetonitrile, divided in 10 vials, and kept at -4°C until use. A ^{14}C-rimsulfuron mixture was prepared adding fifteen ml 50 mM K-phosphate buffer (pH 7.0), 10% (v/v) ethanol, and 0.5% (v/v) Tween-20™ (Sigma Chemical, St. Louis, Missouri, U.S.A.). A 2-cm marked zone in the middle of the third fully developed leaf was treated with 0.110 μCi ^{14}C-rimsulfuron solution spotted in 10 droplets of 1μl, which corresponds to a field rate of 47.93 g a.i. ha^{-1} in 200 L water of a broadcast application. Five droplets were applied with a microsyringe on each side of the mid-vein on the adaxial surface of the leaf.

Plants were assessed at 6, 24 , and 48 hours after treatment (HAT). Recovery of the non-absorbed radioactivity was assessed as follows. The treated zone was cut and shaken for 30 sec in 5 ml of the following three mixtures: (1) water (50 mM K-phosphate buffer, pH 7.0) + 0.5% (v/v) Tween-20™; (2) water:ethanol (90:10 v/v) + 0.5% (v/v) Tween-20™; (3) water:acetone (90:10 v/v) + 0.5% (v/v) Tween-20™. A 1-ml aliquot from each leaf wash was added to a 10 ml CytoSynt™ (ICN, St. Laurent, Québec, Canada, H4T 9Z9) scintillation cocktail. Radioactivity of each leaf wash was assayed separately by liquid scintillation spectroscopy counting (LSSC) in a 1217 Rackbeta™ Liquid Scintillation Counter (LKB Wallac Oy, Turku 10, Finland). Counts min^{-1} (cpm) were corrected to desintegration min^{-1} (dpm) by an automatic external standard quenching curve.

Radioactivity in maize seedling tissues was recovered by using a tissue solubilizer procedure. The roots of harvested plants were washed with distilled water to remove vermiculite and each plant was sectioned into seven parts: (1) Treated zone (Tzn), (2) apex of the treated leaf (TL), (3) TL-base, (4) TL- sheath, (5) TL-above tissues, (6) TL-below tissues, and (7) roots. Thereafter, fresh weight of each tissue section was recorded. Tissues of each part were finely cut with a scissors and a 200 mg sample was solubilized with 0.5 ml of Scintigest SO-X-10™ (Fisher Scientific, Fair Lawn, New Jersey 07410, U.S.A.). Twenty four hours later, tissues were homogenized with a Polytron™ (Brinkman Instruments Canada, Rexdale, Ontario, Canada M9W 4Y5) and bleached with 400 μl of a 4% sodium hypochlorite solution and 400 μl of a 6% hydrogen peroxide solution. Thereafter, samples were neutralized with 100 μl concentrated acetic acid and kept in the dark for 24 hours. Fifteen-ml of CytoSynt™ scintillation coktail was added at each vial and radioactivity was measured by LSSC, as above.

The absorbed ^{14}C was expressed as percent of the total ^{14}C recovered, and calculated for each treatment as equal to: [∑dpm in plant sections/(∑dpm in plant sections+∑dpm in leaf washes)]∗100. Total ^{14}C-rimsulfuron translocation was expressed in percent and calculated as : [∑dpm in plant sections other than the treated zone/∑dpm in plant sections]∗100. ^{14}C-distribution in plant sections (percent) was calculated as : [dpm in each plant section/∑dpm in plant sections]∗100.

Two-10 μl aliquots of the ^{14}C-rimsulfuron treatment mixture were collected on each day of treatment to verify the amount of ^{14}C-herbicide applied. Recovery of the applied ^{14}C exceeded 90% for all treatments (data not shown).

The experiment was repeated twice with two replicates of each treatment. Data were subjected to standard analysis of variance (ANOVA) at the 0.05 level under a factorial structure of treatments (Factor A=hybrid, Factor B=temperature and Factor C=time) using a randomized complete block design. Significant interaction of hybrid by temperature by time of absorption and total translocation variables were examined by means of contrast.

Metabolism study

In metabolism study, maize seedlings were treated at the 3-leaf stage. A 1.5 cm zone in the middle of the second youngest leaf was treated with 0.0963 μCi of ^{14}C-rimsulfuron mixture (^{14}C-2-pyridine, sp. act. of 51.7 μCi mg^{-1} and 99% purity). An 8 μl aliquot of the ^{14}C-rimsulfuron mixture was applied to each plant, which corresponds to a dose of 41.97 g ai ha^{-1} in 200 L water of sprayed. Four 1 μl droplets were applied onto each side of the mid-vein. Preparation of the ^{14}C-rimsulfuron mixture and leaf wash procedure to recover the non-absorbed ^{14}C-rimsulfuron were as in the uptake and translocation experiment. Non absorbed radioactivity was determined by LSSC as in previous experiment. Plants were harvested at 6, 12, and 24 HAT, and fresh weight of whole maize seedlings was recorded.

The method for extracting radioactivity from tissues and the HPLC (high performance liquid chromatography) system for the separation of the parent compound and metabolites was described by Mekki (1994). Treated seedlings (shoot plus roots) were frozen in liquid N_2 and stored at -80°C until ^{14}C-material extraction. Plants were homogenized for 60 sec using a Polytron™. Thereafter, the extracts were filtered through a solid phase extraction (SPE) filter column and the pellets was re-extracted twice. Homogenization of samples was carried out on ice in 5 ml of an acidified aqueous acetonitrile solution (acetonitrile:water 70:30 (v/v) plus 0.1 % (v/v) acetic acid).

The filtrates were vacuumed to near dryness on a rotary evaporator (BUCHÏ®, model 112696, Switzerland) at 45°C. The chlorophyll pigments were separated by a solvent partitioning procedure. The parent compound and its metabolites were separated in K phosphate-buffer (pH 7.0), and chlorophyll in ethyl acetate. After partitioning three times, and bleaching with a 4% sodium hypochlorite solution, residual radioactivity in the ethyl acetate

fraction was measured by LSSC. The aqueous fractions were freeze-dried with a SpeedVac® (AS 160 model, Savant) for three hours. The concentrated extracts were re-dissolved in 300 µl K phosphate-buffer (pH 7.0) and filtered through a 4 mm-nylon® mesh (Micro Separation, Honeoye Falls, New York, 14472, U.S.A) with a 0.2 µm pore size diameter to remove plant debris. The filtrate volume of each sample was quantified and kept at -10°C for one day until HPLC analysis.

Two sub-samples of 50 µl of radioactive extract of each treatment were analized using a LKB Bromma®-HPLC system (LKB 2158 Uvicord SD, BROMMA, Sweden). The samples were injected into a reverse-phase C_{18} column (Partisil-10 ODS-3, Whatman®, Clifton, New Jersey 07014, U.S.A.). Samples were eluted with a mobile phase, operated at a constant flow rate of 1 ml min^{-1} and composed of (A) acetonitrile, and (B) HPLC grade water acidified with 0.1 % (v/v) of phosphoric acid. The elution method comprised three steps: (1) from 0 to 8 min, A was 20% and B was 80% of the mobil phase; (2) from 8 to 9 min, A increased linearly to 90%, and B decreased linearly to 10%; and (3) from 9 to 16 min, A increased linearly to 100%, and B decreased linearly to 0%. Two fractions were collected, the first one, from 0 to 11 min, corresponding to metabolites, and the second one, from 11 to 16 min, containing the parent compound. The radioactivity of the fractions was assayed using LSSC. Three samples of ^{14}C-rimsulfuron standard were run. Rimsulfuron was identified by comparing retention time with the standard. No attempt was made to identify metabolites. Recovery of ^{14}C exceeded 87% in all samples.

The experiment was repeated twice with two replicates of each treatment. Data were subjected to standard analysis of variance (ANOVA) at the 0.05 level under a factorial structure of treatments (Factor A=hybrid, Factor B=temperature and Factor C=time) in a randomized complete block design. Significant interaction of hybrid by temperature by time was examined using single degree contrast.

Acetolactate Synthase (ALS) Inhibition Study

The procedure used to extract and assay ALS activity was that standardized by Singh *et al.* (1988). Using a pestle and mortar, 10 g of maize seedling leaves at the 4 leaf-stage were powdered in liquid N_2 with polyvinyl-polypyrolidone (PVPP; 0.2 g g^{-1} fresh weight). Powder was transferred to a test tube and extracted in 25 ml of 100 mM K-phosphate buffer (pH 7.5) containing 10 mM sodium pyruvate, 5 mM $MgCl_2$, 100 µM flavin adenine dinucleotide (FAD), 5 mM ethylene diamine tretra acetic acid (EDTA), 1mM valine, 1mM leucine, 10 mM cysteine and 10% glycerol (v/v). Prior to the homogenization with a Polytron™, 0.00383 g of dithiothreitol (DDT) and 9 µl of antifoam-A™ (Sigma, St. Louis, MO 63178, U.S.A.) per 25 ml buffer were added.

The homogenate was filtered through four layers of cheesechloth and centrifugated at 25000 *g* for 20 min at 4°C. The supernatant was kept on ice and brought to 50% saturation with $(NH_4)_2SO_4$ in 20 min. The mixture was then recentrifuged under the same conditions as above and the supernatant discarded. The $(NH_4)_2SO_4$ precipitated pellet was dissolved in 3 ml of equilibration K-phosphate buffer [50 mM K-phosphate buffer (pH 7.5) containing 5 mM $MgCl_2$ and 100 mM NaCl]. The 3-ml sample was fractioned and passed through two desalting columns (PD-10 Sephadex™ G-25 column, Pharmacia, Piscataway, New Jersey 68854, U.S.A.), which had been equilibrated with the K-phosphate buffer. The elutes were collected and combined for use in ALS activity assays. All operations were carried out in a cold room at 4°C.

ALS activity was estimated by measuring acetolactate, after its conversion to acetoin. Each reaction mixture consisted of 100 µl enzyme crude extract, 350 µl assay buffer [(50 mM K-phosphate buffer (pH 7.0) that contained 10 mM sodium pyruvate, 10 mM $MgCl_2$, 1mM thiamine pyrophosphate (TPP), and 10µM FAD], and 50 µl of each herbicide solution. Inhibition of ALS activity was measured over rimsulfuron concentration rates of 0, 0.001, 0.005, 0.01, 0.1 and 1 µM. The reaction mixture was incubated at 37°C for 1 h and the reaction was stopped with 50 µl H_2SO_4 (6N). The reaction product, acetolactate, was allowed to decarboxylate at 60°C for 15 min and the acetoin formed was incubated with creatine (0.17%) and α-naphtol (1.7%) to carried out a colorimetric assay (Westerfield, 1945). Sample-tubes were centrifuged at 5 000 *g* at room temperature for 25 min prior to measurement of acetoin. Optical densities were read at 520 nm against a water blank, and measured two times in each sample.

Acetoin concentration was calculated using a standard curve. Quantification of proteins in the enzyme extract was done with a protein assay kit from Sigma (Sigma™, St. Louis, Missouri 63178, U.S.A.). ALS specific activity was calculated for each treatment and data were expressed as a percent of the untreated control. Data expressed as percent were transformed to probits values. A probit value of 5, is equal to 50% inhibition of ALS specific activity (I_{50}). The I_{50} value for each hybrid was determined from a linear regression equation. The experiment was repeated six times.

RESULTS AND DISCUSSION

Uptake and translocation studies

The temperature by hybrid by time interaction was significant (Table 1). Single degree contrast showed that rimsulfuron uptake follows a linear trend (Table 1; Figure 1). Fourty eight hours after treatment (HAT), uptake of rimsulfuron was 40% and 57% in 'Pioneer 3897', and 26% and 43% in 'Cargill 2127', at 14°C and 21°C, respectively (Figure 1). The greater rimsulfuron uptake by 'Pioneer 3897' could be related to its cuticular properties

Table 1. F-statistics and error mean squares for analyses of rimsulfuron uptake, translocation out of the treated zone, and metabolism, in two maize hybrids.

Source of variation	df	Herbicide uptake (% of total ^{14}C-recovered)	Herbicide translocated out of the treated zone (% of ^{14}C-absorbed)	Metabolism (% of ^{14}C-rimsulfuron in sample extracts)‡
		F-statistics	F -statistics	F -statistics
Block	1	391.16 *	0.10 ns	6.13 ns
Temperature (T)	1	9046.00 **	248.12 *	236.77 **
Error mean square (a)	1	*0.0675*	*11.25*	*10.27*
Hybrid (Hyb)	1	0.84 ns	3.72 ns	99.66 **
T x Hyb	1	2.19 ns	0.019 ns	54.60 **
Time	2	20.74 **	30.19 **	13.34 **
Time x Hyb	2	219.61 **	77.27 **	1.49 **
T x Time	2	5.21 *	36.50 **	1.71 ns
T x Hyb x Time	2	3.26 *	55.80 **	3.41 *
T x Hyb x Time linear	1	3.98 *	11.24 **	5.04 *
T x Hyb x Time quadratic	1	2.54 ns	100.36 **	0.19 ns
Error mean square (b)	34	*17.20*	*11.25*	*35.07*
Total	47			

‡ ^{14}C parent form in sample extracts, expressed as percent of the total ^{14}C recovery

* ** F-statistic significant at 0.05 or 0.01 level, respectively

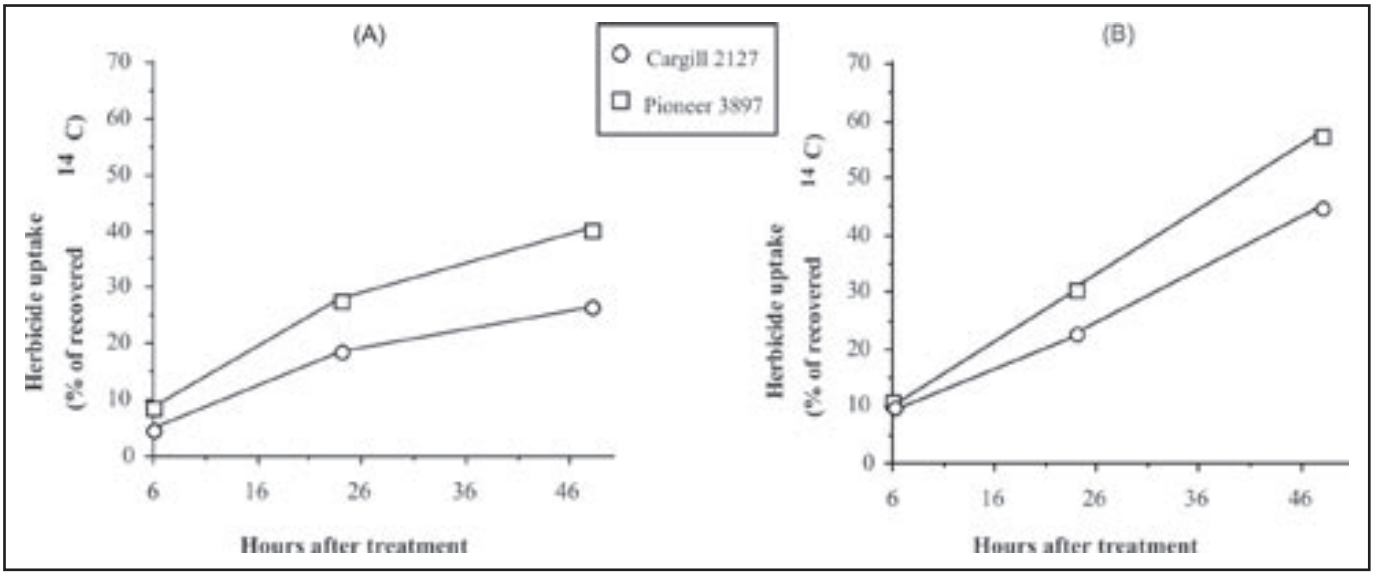

Figure 1. ^{14}C-rimsulfuron uptake (expressed as % of total ^{14}C recovered) by 'Cargill 2127' and 'Pioneer 3897' at (A) 14°C and (B) 21°C; 6, 24 and 48 hours after foliar treatment. S y = 2.07.

(Avato *et al,.* 1987). In both hybrids, rimsulfuron uptake increased 25% as the temperature increased from 14°C to 21°C (Figure 1). Mekki & Leroux (1995) reported same rimsulfuron uptake values between 23% to 46%, in selected annual weed species. In other studies, differences in sulfonylurea uptake were poorly correlated with plant sensitivity (Brown, 1990; Brown *et al.,* 1991b). Nevertheless, there are at least two documented cases with sulfonylurea herbicides which the absorption would contribute, at least partially, to differential inter-specific tolerance. The moderate tolerance of pitted morningglory (*Ipomoea lacunosa* L.) and entireleaf morningglory (*Ipomoea hederacea* var. *integriuscula* Gray) was reported to be due to reduced uptake, with only 1% of the total applied radioactivity absorbed after 72 hours of exposure (Moseley *et al.,* 1993). In another study, it was shown

that compared to wheat, *Lolium perenne* takes up *via* roots relatively more triasulfuron, which is translocated in higher quantity to the shoot and metabolises the active compound more slowly than wheat. Thus, the uptake, translocation and the rate of metabolism seem to be the reasons for the selective action of triasulfuron in wheat (Meyer & Müller, 1989).

The temperature by hybrid by time interaction was significant for total translocation of ^{14}C-rimsulfuron (Table 1). Herbicide translocation followed both a linear and quadratic trend (Table 1; Figure 2). Herbicide translocation at 14°C did not differ between hybrids. At 21°C and 24 HAT, 'Cargill 2127' translocated 6% more herbicide than 'Pioneer 3897', but this difference was negligible 48 HAT. Twenty-four and 48 HAT, translocation of the herbicide was greater at 21°C than at 14°C, but not at 6 HAT. Averaged over hybrids, herbicide translocation 48 HAT was 53% *vs.* 23% at 21°C and 14°C, respectively (Figure 2).

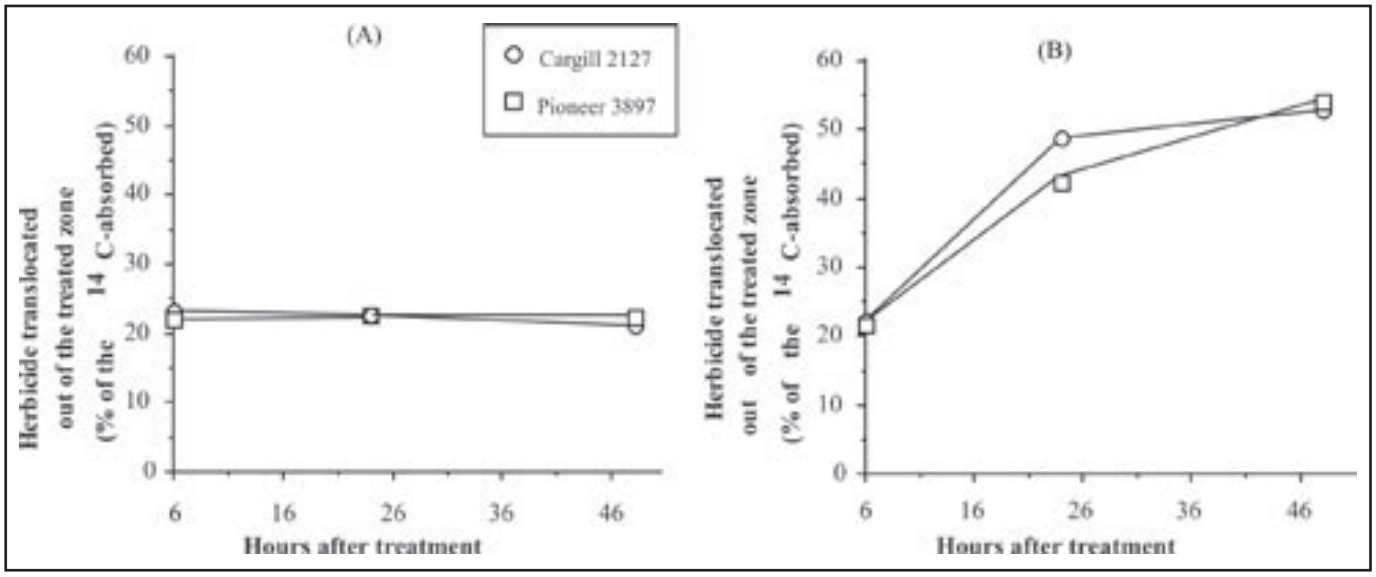

Figure 2. ^{14}C-rimsulfuron translocated out of the treated area, expressed as % of herbicide absorbed in 'Cargill 2127' and 'Pioneer 3897' at (A) 14°C and (B) 21°C, 6, 24 and 48 hours after foliar treatment. S y = 1.68.

Distribution of ^{14}C-rimsulfuron in various plant organs did not differ between hybrids (Figure 3). Both hybrids retained more than 50% of the absorbed ^{14}C-rimsulfuron in the treated zone. By 48 HAT, rimsulfuron had translocated mainly to the apex of the treated leaf (TL). The ^{14}C-rimsulfuron recovered in this plant-section averaged 10% and 41% at 14°C and 21°C, respectively. The ^{14}C-recovered in other tissues (TL-base, TL-sheath, TL-above tissues, TL-below tissues and roots) ranged from 1% to 6%, and did not differ between the two temperatures tested (Figure 3). These results agree with data reported by Eberlein *et al*., (1989). Maize inbreds retained in the treated leaf more than 85% of the ^{14}C-DPX-M6316 absorbed. Mekki & Leroux (1995) also reported that most of the absorbed ^{14}C-nicosulfuron and ^{14}C-rimsulfuron remained in the treated leaf of five annual weed species tested. The ^{14}C-rimsulfuron quantified in the treated leaf of these weed species ranged from 65% to 85% at 48 HAT.

Membrane permeability to the sulfonylurea herbicides is not carrier-mediated, but instead depends on their relative lipophilicity and pKa (Brown, 1990). The uptake and translocation of sulfonylureas proceed through an acid-trapping mechanism that is influenced by their physico-chemical properties and is driven by the energy-dependent pH gradient between apoplastic (pH 5.0) and symplastic (pH 7.0) compartments (Brown, 1990). Rimsulfuron is a weak-acid and lipophilic compound with a K_{ow} (octanol/water partition coefficient) of 1.94 at pH 5.0 and 0.034 at pH 7.0 (Palm *et al*., 1989; Worthing & Hance, 1991). This suggest that the low rimsulfuron translocation in plants could be attributed to its stronger association with lipoidal components during foliar uptake, rather than to its low phloem-mobility (Mekki & Leroux, 1995).

Metabolism study

The temperature by hybrid by time interaction was significant (Table 1). Rimsulfuron metabolism followed a linear pattern (Table 1; Figure 4). Detoxification of herbicide in 'Cargill 2127' was not affected by temperature. At both 14°C and 21°C, 27% of the total ^{14}C-rimsulfuron absorbed, was recovered as the parent compound in extracts from 'Cargill 2127' plants assessed at 24 HAT. In contrast at 14°C, plant extracts of 'Pioneer 3897' contained around 65% of the herbicide parent form at any time of assessment (Figure 4). At 21°C, differences in the pattern of rimsulfuron metabolism from the two hybrids were less noticeable as compared to 14°C. However, at 6 HAT 'Cargill 2127' had detoxified 8% more herbicide than 'Pioneer 3897'.

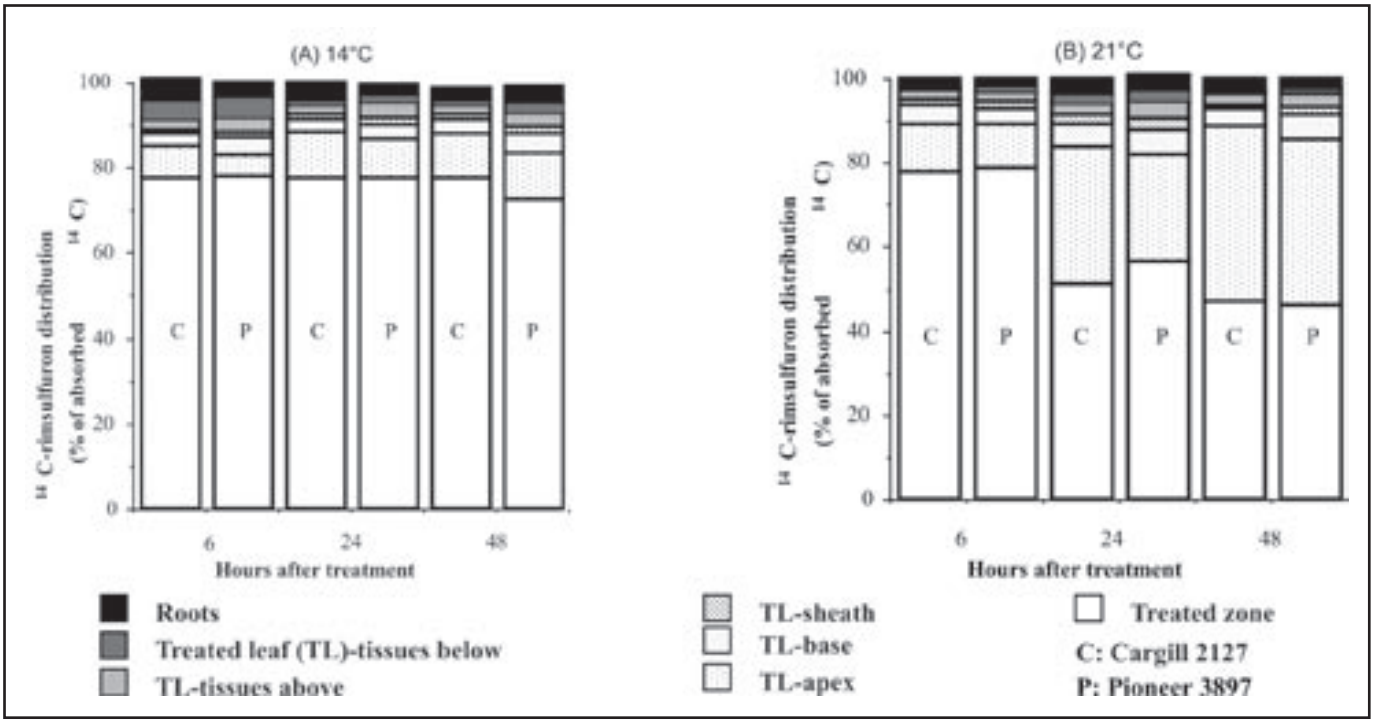

Figure 3. Distribution of ^{14}C-rimsulfuron in various plant sections of 'Cargill 2127' and 'Pioneer 3897' seedlings at (A) 14°C and (B) 21°C, 6, 24 and 48 hours after treatment.

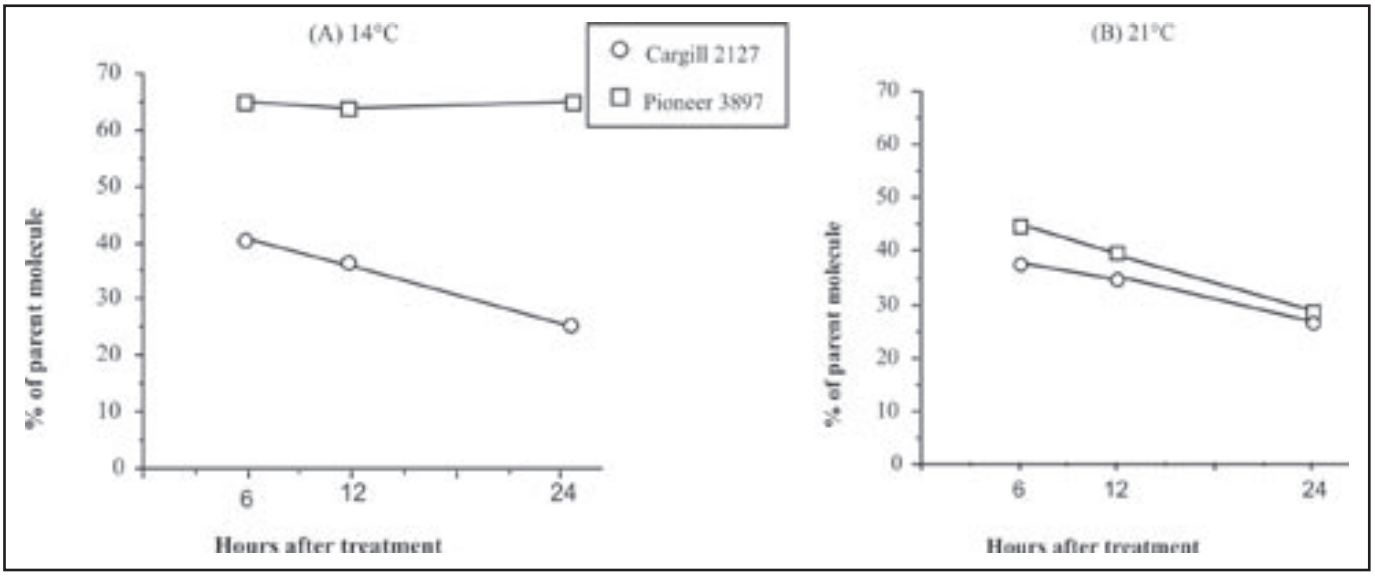

Figure 4. ^{14}C-rimsulfuron degradation (expressed as % of ^{14}C-rimsulfuron absorbed) in 'Cargill 2127' and 'Pioneer 3897' under (A) 14°C and (B) 21°C, 6, 12 and 24 hours after foliar treatment. S y = 2.96.

It has been established that maize tolerance to rimsulfuron is based on the higher rate of metabolism of the active compound to inactive metabolites, as compared to sensitive species (Palm *et al.,* 1989). Palm *et al.* (1989) reported a rapid degradation of rimsulfuron in maize leaves, with a half-life of 6 to 7 hours. N'Tchobo (1994) reported a half-life of 2 to 3 hours for nicosulfuron plus rimsulfuron (1:1 premix) in maize cells culture. In the present work, 'Cargill 2127' had metabolized around 60 % of the absorbed at 6 HAT. Compared with metabolism profiles of rimsulfuron in five annual weed species reported by Mekki (1994), which degraded less than 40% of the parent compound 48 HAT, 'Cargill 2127' can metabolize rimsulfuron at a high rate.

Rimsulfuron elution time under our separation conditions ocurred at 13 to 14 min (Figure 5). We assume that ^{14}C-activity in the fraction collected from 2 to 11 min contains herbicide metabolites, and that ^{14}C-activity in the fraction collected from 11 to 16 min contain

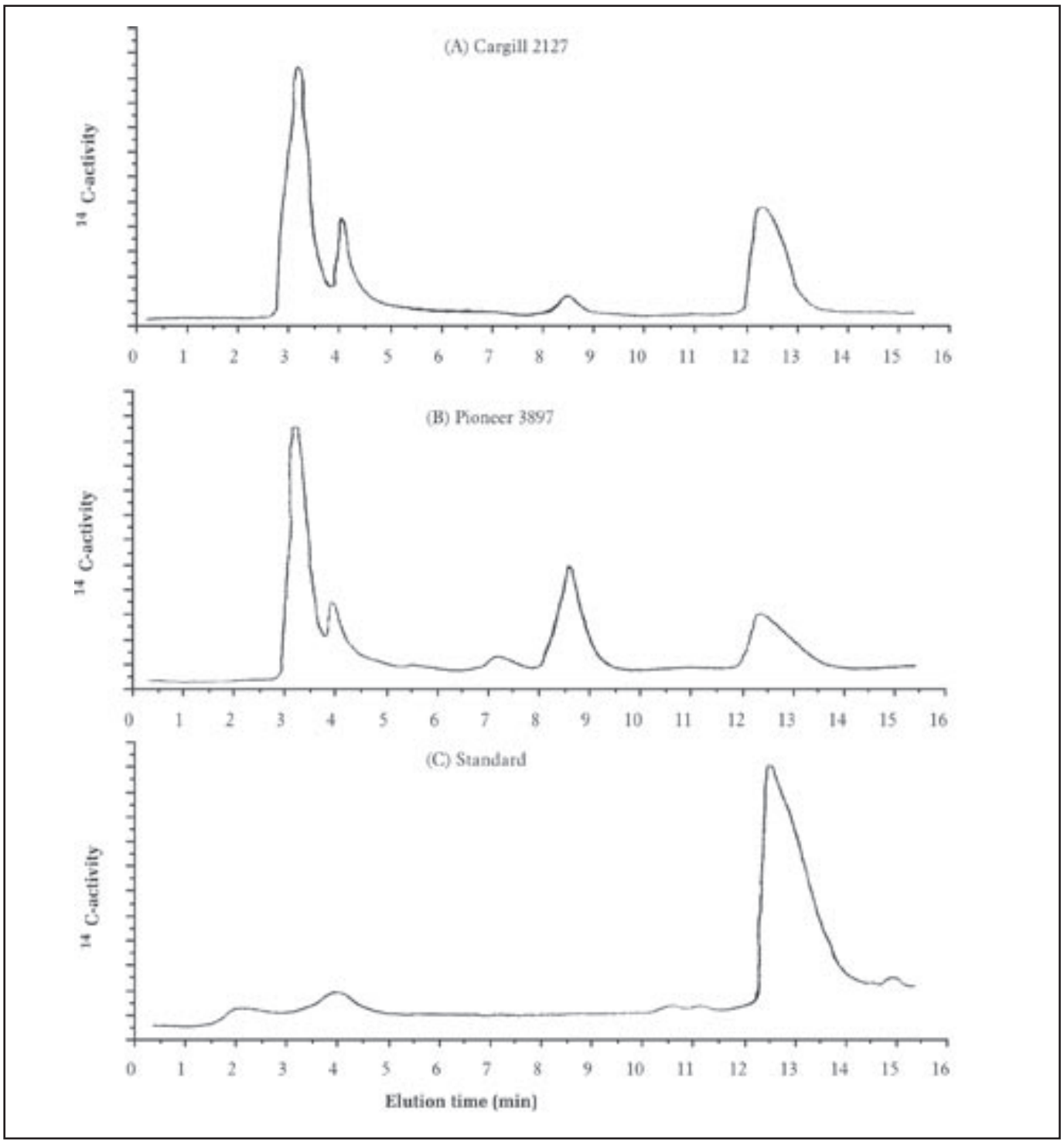

Figure 5. Typical HPLC-chromatogram from C_{18}-HPLC separation of ^{14}C-rimsulfuron from (A) 'Cargill 2127', (B) 'Pioneer 3897' plant extracts, and (C) standard.

^{14}C-rimsulfuron. Two major metabolites were found in extracts from treated seedlings of both 'Cargill 2127' and 'Pioneer 3897' (Figure 5). Retention times for those metabolites (3 and 9 min) were nearly identical, suggesting that similar pathways for rimsulfuron metabolism may operate in both hybrids. In our study, metabolites were not identified. In soil and aqueous environments the major rimsulfuron metabolite identified is [1-(3-ethylsulfonyl)-2-pyridinil]-4,6-dimetoxy-2-pyrimidineamine (Schneiders *et al.,* 1993).

Major metabolites of nicosulfuron have been identified. Nicosulfuron is rapidly metabolized to 5-hydroxypyrimidinyl inactive derivative, which is subsequently conjuga-

ted to glucose (Brown *et al*., 1991a). Many pyrimidinyl sulfonylureas are chemically-susceptible to 5-hydroxylation, yet maize tolerance is exceptionally rare among the hundreds of pyrimidinyl sulfonylurea compounds tested (Brown *et al*., 1991a). Most of the commercial pyrimidines are highly injurious to maize (Brown 1990; Brown & Kerney, 1991; Brown *et al.,* 1991a). Thus, sulfonylureas pyrimidines like rimsulfuron, primisulfuron and nicosulfuron are exceptional cases among sulfonylurea herbicides registered in maize. Fonné-Pfister *et al*., (1990) have shown aryl hydroxylation of primisulfuron mediated by cytochrome P_{450}'s. Therefore, one might speculate that cytochrome P_{450}'s also metabolize rimsulfuron in maize.

Acetolactate Synthase (ALS) Inhibition Study

ALS specific activity values for inhibition of ALS (I_{50}) by rimsulfuron were 0.091 µM for 'Pionner 3897' and 0.142 µM for 'Cargill 2127' (Figure 6). ALS-based tolerance to sulfonylurea herbicides has been reported for

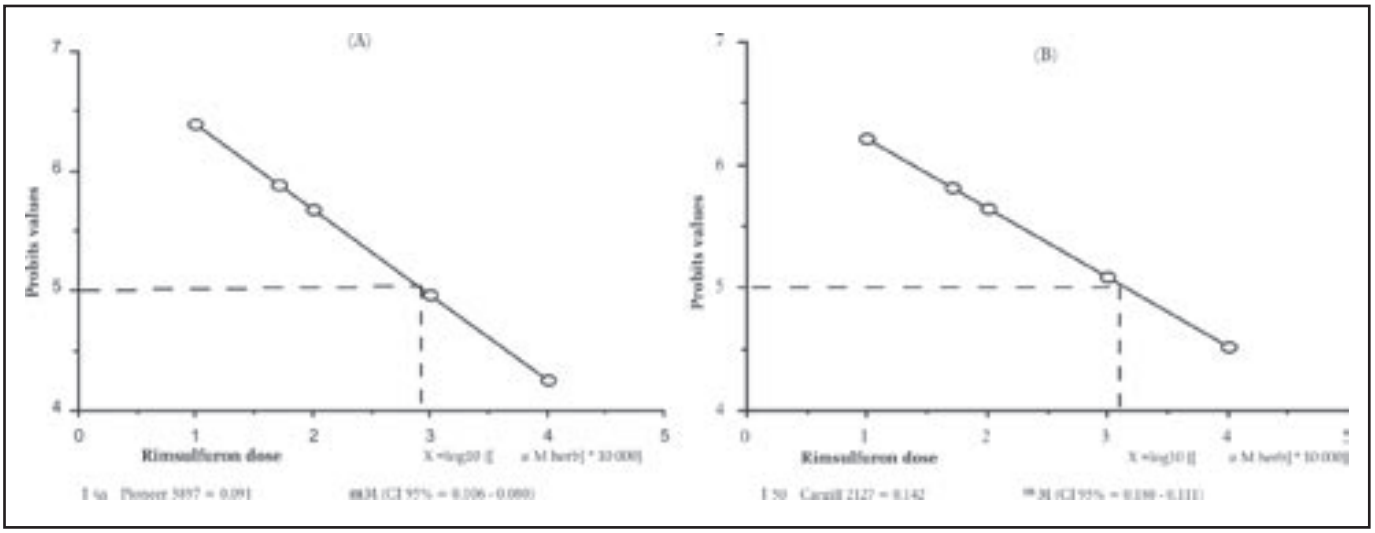

Figure 6. ALS sensitivity of 'Pioneer 3897' (A) and 'Cargill 2127' (B) maize hybrids to rimsulfuron. A probit value of 5, is equal to 50% inhibition of ALS specific activity (I_{50}). I_{50} was calculated from a linear regression equation as follow: $Y_{\text{'Pioneer 3897'}} = 7.104 - 0.713x$ (R^2=0.763*); for a probit value = 5, x = 2.9583. Then, if X =$\log_{10}$ {[*u*M herb] * 10 000}, (antilog 2.9583)/10 000 = 0.091*u*M.; $I_{50\ \text{'Pioneer 3897'}}$ = 0.091*u*M (CI (confidence interval) 95% = 0.080 - 0.106). $Y_{\text{'Cargill 2127'}} = 6.772 - 0.562x$ (R^2=0.850*); for a probit value equal to 5, x = 3.1523. Then, if X = $\log_{10}$ {[*u*M herb] * 10 000}, (antilog 3.1523)/10 000 = 0.142*u*M; $I_{50\ \text{'Cargill 2127'}}$ = 0.142 *u*M (CI 95% = 0.111 - 0.180). * = significant at $P < 0.05$.

several crop and weed species (Chaleff & Mauvais, 1984; Till *et al*., 1991; Saari *et al.,* 1994). In these cases, the I_{50} for ALS inhibition by various sulfonylurea herbicides was more than 4-fold for resistant than for susceptible species or lines (Saari *et al.,* 1994). Therefore, the differential tolerance at the whole plant level between 'Cargill 2127' and 'Pioneer 3897' could not be explained by the small difference in their ALS sensitivities. In previous field experiments, rimsulfuron at 20 g a.i. ha^{-1} caused a maximum injury of 14% and 51% (mean of four visual phytotoxicity evaluations) in 'Cargill 2127' and 'Pioneer 3897', respectively (Fuentes, 1977).

ALS specific activity does not explain differences in tolerance to rimsulfuron between 'Cargill 2127' and 'Pioneer 3897'. Specific activity [(nmol acetoin min^{-1}) (mg $protein^{1}$)] was 5.64 for 'Pioneer 3897' and 5.38 for 'Cargill 2127'. Green and Ulrich (1994) characterized the response of various maize inbred lines to rimsulfuron. Dose-response analysis at the whole plant level showed that varieties could vary more than 40 000-fold in sensitivity. But all had similar ALS sensitivity (I_{50} ranged from 0.06 to 0.03 µM). Only the ALS-modified XA-17 gene of the cultivar 'Pioneer 3180 IR' was about 30-fold less sensitive to rimsulfuron, as compared to the various maize inbreds tested (Green & Ulrich, 1994). Similarly, the degree of rimsulfuron tolerance among five annual weed species under greenhouse conditions was not correlated with ALS sensitivity. I_{50} values differed slightly among the weed species tested (Mekki & Leroux, 1994).

Based on the rimsulfuron dose used in the uptake and translocation experiment (47.93 g ai ha-1 in 200 L water), and based on the uptake and metalolism rates, calculations showed that 0.21 and 0.38 µg of rimsulfuron $plant^{-1}$ remained in 'Pioneer 3897' seedlings 24 HAT at 14°C and 21°C, respectively; compared with 0.11 and 0.13 µg $plant^{-1}$ at 14°C and 21°C, respectively, in 'Cargill 2127'. It seems that the differences in tolerance to rimsulfuron between maize cultivars could be mainly explained by differential uptake and herbicide metabolism. Both hybrids studied have the same level of ALS sensitivity to rimsulfuron and the same pattern of herbicide translocation. Consequently, ALS sensitivity and translocation does not play a major role in the tolerance of maize to rimsulfuron. Diehl *et al.* (1993) also repor-

ted that the sensitivity mechanism of a 'Pioneer' maize hybrid (ALSSI) extremely susceptible to ALS-inhibiting herbicides is due to a lack of herbicide metabolism.

On the other hand, the greater uptake and translocation of rimsulfuron at 21°C compared to 14°C could explain the phytotoxicity of this herbicide in maize at high temperature under field conditions. Rimsulfuron at a dose of 15 g a.i. ha^{-1} is able to reduce ALS activity in maize by 65% at 24 HAT (Martinetti *et al.,* 1995) despite the ability of maize to rapidly metabolize the herbicide. Hence any factor that increases the amount of rimsulfuron at the site of action will cause injury at the whole plant level. However, 'Pioneer 3897' was one of the most sensitive hybrids to rimsulfuron under field and greenhouse conditions, phytotoxicity was not severe enough to lead to plant death (Fuentes, 1977).

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