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TOLERANCE OF SUNFLOWER (*Helianthus annuus* L.) TO IMAZETHAPYR

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SUMMARY

The response of RIMI [imazethapyr-tolerant (T)] sunflower (*Helianthus annuus* L.) hybrid and the local imazethapyr-sensitive hybrid "Zoltan" (S) was investigated under controlled conditions. Hybrids grown in pots were treated post-emergence with imazethapyr at the two pairs of leaves stage. Visual injury evaluation and vegetative parameters were recorded. ALS (acetolactate synthase) enzyme activity was estimated *in vivo* 24 h after imazethapyr application. Tolerance level was determined based on the resistance ratio index (ED_{50} - herbicide dose causing 50% growth inhibition of T hybrid/ ED_{50} of S hybrid). Significant differences were noted between the hybrids in the *in vivo* ALS activity and vegetative parameters. Indexes of resistance ranged between 5 and 452 for vegetative parameters, whereas the index for ALS activity was 210. The data confirmed the high level of tolerance of the hybrid Rimi as compared with the hybrid Zoltan.

Key words: **ALS activity, *Helianthus annuus* L., imazethapyr, sensitive, tolerance**

INTRODUCTION

Growing herbicide-tolerant (HT) crops is a relatively new technological achievement in weed control, allowing the improvement of weed management, particularly for some troublesome weed species (Heap and LeBaron, 2001). There are risks associated with the adoption of HT crops such as the transfer of resistance genes to other plants within the same species, between species or other organisms (Hall *et*

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al., 2000) and the potential occurrence of cultivated HT crops as volunteers (Kwon and Kim, 2001).

Until the present in Serbia, a limited number of studies has dealt with the response of the new HT hybrids to herbicides (Malidža *et al*., 2000, Jocić *et al*., 2001), and their use as a component of the weed management practice is still at an early stage (Malidža *et al*., 2002, 2003; Malidža and Orbović, 2004).

Sunflower (*Helianthus annuus* L.) is a cultivated annual crop considered to be a staple product, however as a volunteer, it tends to become a troublesome weed in the following year. Several cases of resistance of wild sunflower to ALS-inhibiting herbicides have been reported so far (Heap, 2006; White *et al*., 2002; Al-Khatib *et al*., 1998), attracting considerable attention among sunflower breeders interested in transferring the resistance trait to cultivated plants. Success in acquiring plants tolerant to imidazolinones and sulfonyl ureas using conventional methods of selection, mutagenesis and direct gene transfer has been reported (Dyer, 1996).

This work was aimed at determining the level of tolerance of the imidazolinone-resistant (RIMI) sunflower hybrid to imazethapyr. The hybrid had been previously selected for tolerance to imidazolinones using a wild sunflower population that evolved resistance to imazethapyr.

MATERIAL AND METHODS

Seeds of the susceptible (S) hybrid (*cv*. "Zoltan") and the tolerant (T) hybrid (RIMI) of sunflower were obtained from Institute of Field and Vegetable Crops in Novi Sad. In all experiments, plants were grown in pots containing a commercial growth medium (Flora Gard TKS1, Germany), under controlled conditions (16 h/8 h light/dark photoperiod, light intensity 300 $\mu Em^{-2}s^{-1}$, 23°C and 60-70% RH). A water-soluble fertilizer (Polyfeed 20 : 20 : 20, Haifa Chemicals, Israel) was added to irrigation water once a week. The plants were irrigated when needed. Irrigation was applied when needed. Imazethapyr (Pivot 100-E, 100 g a.i. l^{-1}, SL, BASF, Germany) was applied post-emergence, at the two pairs of leaves stage, in the amounts of 0, 80, 100, 200 and 400 g a.i. ha^{-1} using a laboratory sprayer equipped with an 8001-E nozzle delivering 200 l ha^{-1} at 276 kPa. The experiment was conducted twice in a completely randomized design with three replications per treatment.

Visual rating of plant injury (0-100 scale; 0=no injury; 100=total destruction), and several vegetative parameters: plant height, leaf length, leaves area, fresh leaf weight, and shoot fresh and dry weight, were recorded at 0, 7, 14 and 21 DAT. Leaf silhouette area on paper was employed as a method of leaf area determination (Džamić *et al*., 1999).

In vivo ALS inhibition study was conducted according to a procedure described by Lovell *et al.* (1996). Foliar application of 0.26 g ml^{-1} of CPCA (1,1-cyclopropane dicarboxylic acid 97%, Sigma-Aldrich, Germany) solution containing 0.25% v/v non-ionic surfactant (Trend 90, Du Pont de Nemours, France), was done 21 h after her-

bicide application. Three hours after CPCA application, 0.2 g of the youngest plant tissue was sampled from each plant, frozen at -20°C to improve cell destruction, finely ground and stored frozen. Twenty-four hours later, the samples were gradually thawed, 3 ml of distilled water was added and the tube incubated at 60°C for 5 min followed by incubation at 25°C for 45 min. Aliquots (3 ml each) were then transferred from each sample into new tubes, 75 µl 6N H_2SO_4 were added and incubated at 60°C for 30 min to decarboxylate the acetolactate to acetoin. Acetoin was determined as described in Ray (1984) at 525nm (Biochrom Novospec II spectrophotometer, LKB, Austria). The OD values obtained were converted into µg of acetoin using a standard curve. Dose-response curves were plotted and the ED_{50} (herbicide concentration causing 50% inhibition of the ALS activity) values were determined. Student's T-test (SPSS 8.0 program package) was used to analyze and interpret the obtained data. Tolerance level was determined based on the ratio between the ED_{50} of T hybrid and the ED_{50} of S hybrid (Zoltan). The level of resistance of the investigated populations was determined based on fresh weight reduction (Moss *et al.*, 1999).

RESULTS AND DISCUSSION

Highly significant differences ($P<0.01$) were found between the S and T hybrids in various vegetative parameters in the three evaluations, but no difference was detected between the untreated S and T hybrids indicating a lack of fitness (Figure 1). Visual injury of the S hybrid was clearly recorded 7 DAT with imazetaphyr while negligible injury was observed in the T hybrid even when treated with the highest application rate of herbicide (Figure 2a). This agrees with the differences found in biomass production between susceptible and resistant wild populations of *H. annuus* exposed to imazethapyr (White *et al.*, 2002). However, the shoot dry weight and plant height of the S and T hybrids showed no statistically significant difference 7 days after treatment, but differences emerged 14 and 21 DAT (Figures. 2b, 2c).

Based on the vegetative parameters tested, the relationship between the T and S hybrids of *H. annuus* (resistance ratio) ranged between 5 and 452 depends on the parameter checked (Table1). Similarly, index of resistance (IR) of 65 was determined based on biomass reduction, for *Amaranthus quitensis* H.B.K. population that evolved resistance to imazethapyr (Tuesca & Nisensohn, 2001). Furthermore, based on dry weight reduction, an IR of 170 was reported in imazethapyr-resistant wild population of *H. annuus* (Al-Khatib *et al.*, 1998) which is similar to the level of tolerance to imazethapyr found in the present study. Total shoot dry weight was found to be the most sensitive parameter for distinguishing between T and S hybrids. The T hybrid was 452 times less susceptible to imazethapyr than the S hybrid regarding this parameter. Studying sulfonylurea effects to *Lindernia micrantha* D., Itoh *et al.* (1999) have found that although this group of herbicides

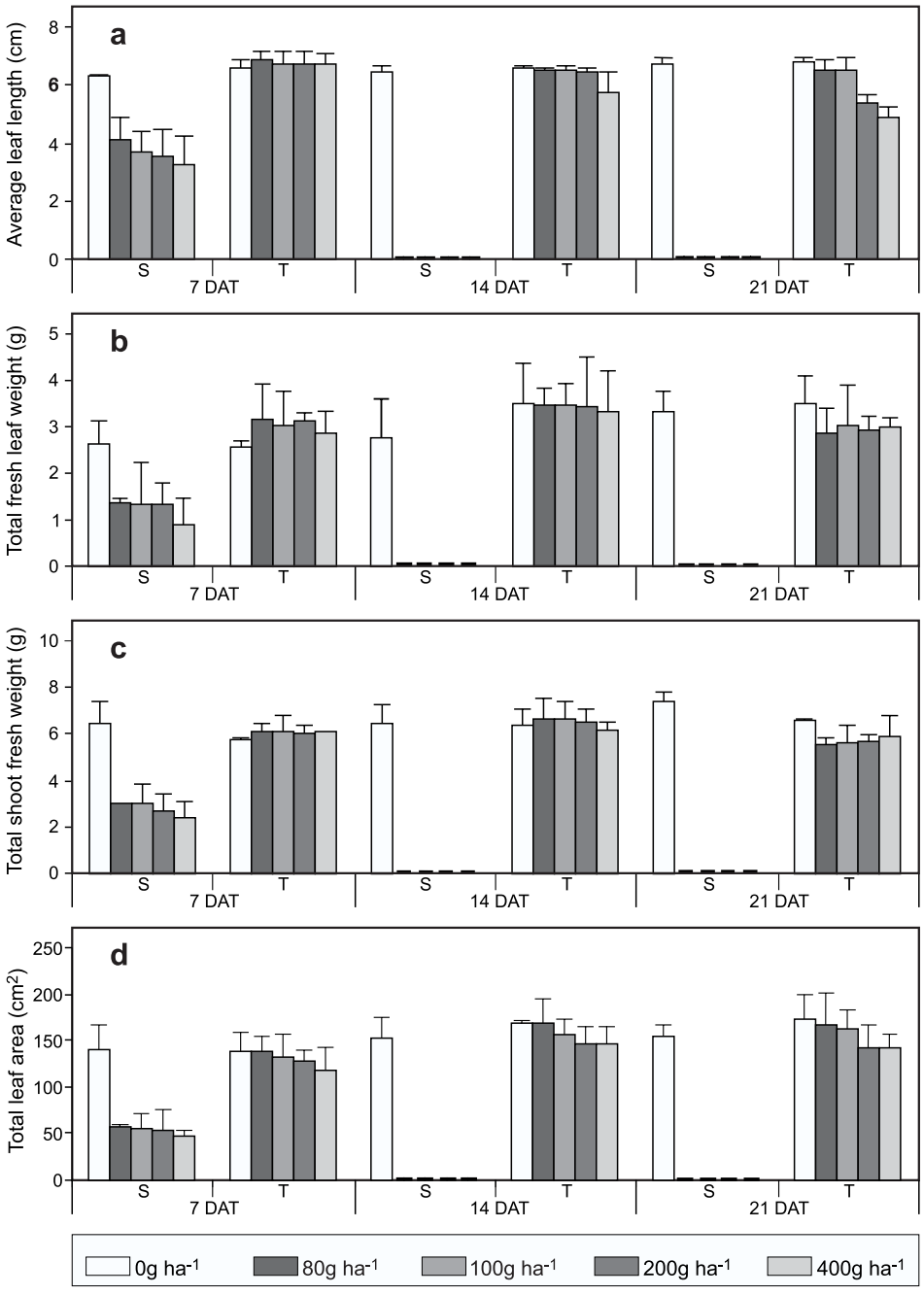

Figure 1: Average leaf length (a), total fresh leaf weight (b), total shoot fresh weight (c) and leaf area (d) of S and T hybrids of Helianthus annuus treated with imazethapyr.

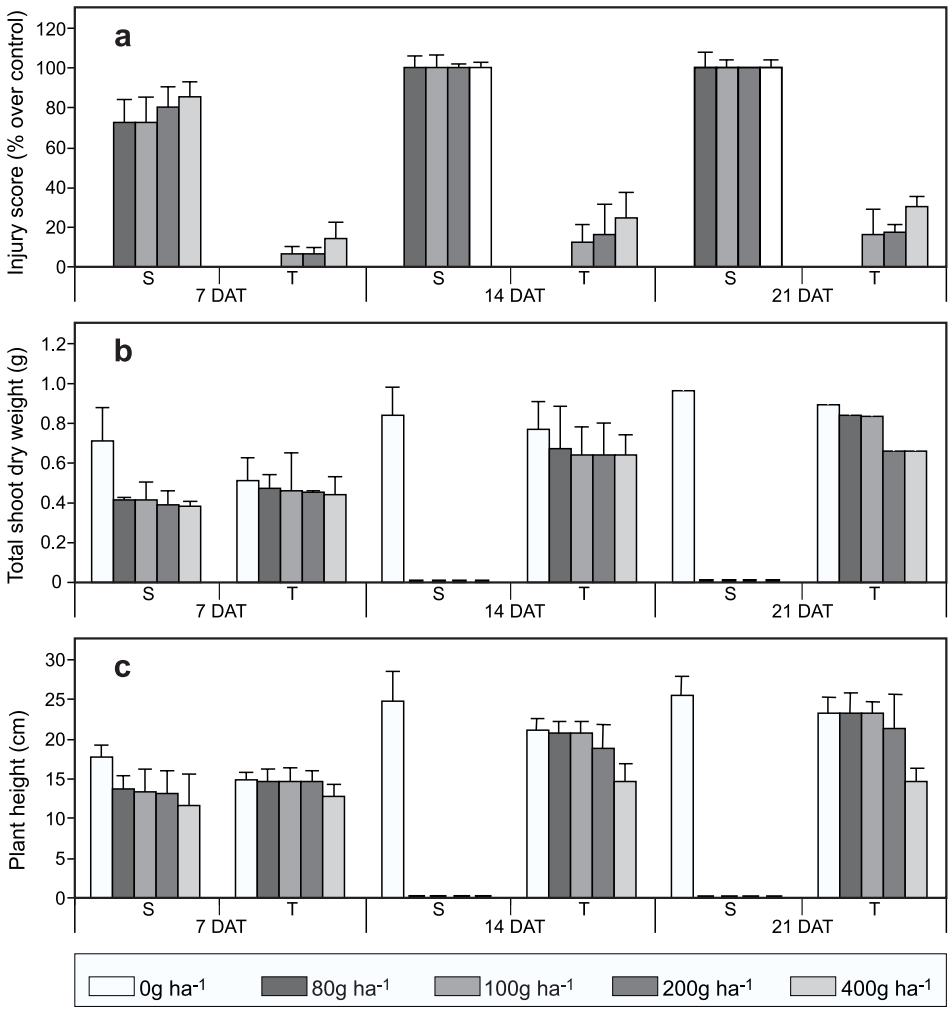

Figure 2: Injury score (a), the shoot dry weight (b) and plant height (c) of S and T hybrids of H. annuus treated with imazethaphyr

Table 1: Levels of tolerance to imazethapyr of *H. annuus* T hybrid according to vegetative parameters as determined 7 DAT

Parameters measured	RatioT : S
Plant height (cm)	5.00
Average leaf length (cm)	291.90
Total fresh leaf weight (g)	177.20
Total fresh shoot weight (g)	70.53
Total dry shoot weight (g)	452.40
Total leaf area (cm^2)	94.73

causes decrease in these parameters in both biotypes (R and S), the R biotype produced more dry weight than the S one under all rates of herbicide applied, which makes dry weight a reliable parameter for monitoring sulfonylurea-resistance of the said species. The level of tolerance of the T hybrid was also determined based on a decrease in total fresh shoot weight of the susceptible hybrid using a scale proposed by Moss *et al.* (1999). A high level of tolerance (5*) of the T hybrid was hereby confirmed.

Using t-test to compare between the hybrids we found that the ALS enzyme activity highly differed ($p<0.01$) in all treatments, except control (Figure 3). The value indicates that the degrees of ALS inhibition by imazethapyr in the T and S hybrids (resistance ratio) is 210 folds higher as compared to the S hybrid. A identical value of resistance index (IR=210) had been reported by Al-Khatib *et al.* (1998) for *in vitro* ALS activity in a wild population of *H. annuus*. As the imazethapyr tolerance trait is inherited as a partially dominant feature (Malidža *et al.*, 2000), the plant has to be homozygous if full tolerance is to be achieved. Currie *et al.* (1995) had shown that in vitro ALS extracts from a heterozygous maize hybrid (Pioneer IR) was 6 times more tolerant to imazethapyr, while homozygous plants were >62 times more tolerant. Al-Khatib *et al.* (1998) had investigated ALS activity *in vitro*, while our experiment was conducted *in vivo*, so that our results are incomparable. Simpton and Stoller (1996), who studied thifensulfuron and imazethapyr effects on ALS in SU-tolerant soybean (*Glycine max*), concluded that direct correlation between *in vivo* and *in vitro* ALS reactions was almost impossible because herbicide concentration reaching the site of primary activity could not be determined, so that the herbicide activity *in vivo* may differ from its activity *in vitro*. The data acquired showed that the T hybrid was by far more tolerant to imazethapyr than the wild population of the same species (IR=39) detected by White *et al.* (2002).

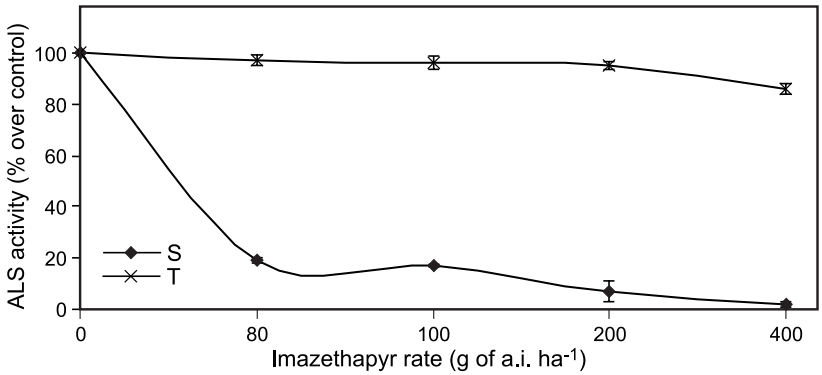

Figure 3: ALS in vivo activity in S and T hybrids after treatment with imazethapyr

Although the mechanism of tolerance itself was not the subject of the current investigation, the data presented on ALS activity indicate that the observed different in response between the hybrids resulted from different sensitivity of the enzyme.

Our results agree with a number of other reports confirming that the enzyme's reduced sensitivity was the cause of resistance of various weed species to ALS inhibitors (Sprague *et al.,* 1997; Sibony *et al.*, 2001; Jander *et al.*, 2003; Osuna and de Prado, 2003; Duran-Prado *et al.*, 2004; Hanson *et al.*, 2004; Maertens *et al.*, 2004; Park and Mallory-Smith, 2004; McNaughton *et al.*, 2005; Trucco *et al.*, 2006). A high level of tolerance of the T hybrid as compared to an S hybrid, was confirmed based on several vegetative measurements and examination of the *in vivo* ALS activity, and further proved by a decrease in total shoot fresh weight of the susceptible hybrid. Total shoot dry weight was found the most reliable of all vegetative parameters tested for distinguishing between hybrids response to imazethapyr. *In vivo* ALS activity also confirmed an earlier observation of tolerance existing in the T hybrid, and proved that the mechanism of tolerance of the hybrid investigated is an altered target site.

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TOLERANCIA A IMAZETAPYR EN GIRASOL (*Helianthus annuus* L.)

RESUMEN

Se investigaron las respuestas del híbrido de girasol (*Helianthus annuus* L.) tolerante a imazethapyr (T) "RIMI" y la del híbrido local "Zoltan", susceptible (S) a dicho herbicida bajo condiciones controladas. Los híbridos fueron cultivados en maceta y tratados con imazethapyr en post-emergencia al estado de dos pares de hojas. Se registraron evaluaciones de daño y parámetros vegetativos. Se estimó la acción de la enzima acetolactato sintetasa (ALS) *in vivo* a las 24 h de la aplicación de imazethapyr. Se determinó el nivel de tolerancia

sobre la base del índice de relación de resistencia (ED_{50}-dosis de herbicida que causa un 50% de inhibición del crecimiento del híbrido T/ED_{50} del híbrido S). Se observaron diferencias significativas entre los dos híbridos para actividad de ALS *in vivo* y para parámetros vegetativos. LOs índices de resistencia mostraron un rango entre 5 y 452 para parámetros vegetativos, mientras que el índice de actividad de la enzima ALS fue 210. Los datos confirman el alto nivel de tolerancia del híbrido RIMI comparado con Zoltan.

TOLÉRANCE DU TOURNESOL (*Helianthus annuus* L.) À L'IMAZETAPYR

RÉSUMÉ

La réponse de l'hybride de tournesol "RIMI" et l'hybride locale sensible à l'imazethapyr "Zoltan" a été examiné dans des conditions contrôlées. Les hybrides qui ont poussé dans des pots étaient traités après levée avec imazethapyr au niveau des deux paires de feuilles. Une évaluation visuelle de blessure et des paramètres végétatifs ont été enregistrés. L'activité de l'enzyme ALS in vivo a été estimée à 24h après l'application d'imazethapyr. Le niveau de tolérance a été déterminé, en se basant sur l'indice du rapport de résistance (ED_{50}-dose d'herbicide causant 50% d'inhibition à la pousse de l'hybride T/ED_{50} de l'hybride S). Des différences significatives ont été notées entre les hybrides de l'activité ALS in vivo et les paramètres végétatifs. Les indices de résistance se classant entre 5 et 452 pour les paramètres végétatifs, alors que l'indice pour l'activité ALS était de 210. Les données ont confirmé la haute tolérance de l'hybride RIMI ainsi comparé à l'hybride Zoltan.