IMIDAZOLINONE-RESISTANT SUNFLOWER (*Helianthus annuus*): INHERITANCE OF RESISTANCE AND RESPONSE TOWARDS SELECTED SULFONYLUREA HERBICIDES

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SUMMARY

The objective of this investigation was to determine the reaction of wild sunflower to a number of acetolactate synthase (ALS) inhibitors. We used a wild sunflower population from Kansas known to be resistant to imazethapyr and tested it with other herbicides from the group of imidazolinones (imazamox, imazapyr) and sulfonylurea herbicides (primisulfuron, rimsulfuron, thifensulfuron, prosulfuron, oxasulfuron, iodosulfuron, chlorsulfuron and triflusulfuron). Other objectives were to assess the possibility of controlling volunteer imidazolinone-resistant sunflower by means of sulfonylurea herbicides. The obtained results showed that the previously detected resistance includes not only imazethapyr but also imazamox and imazapyr Imidazolinone-resistant wild sunflower is susceptible to the normal use rates of chlorsulfuron, iodosulfuron, oxasulfuron, prosulfuron and rimsulfuron. This means that it will be possible to use these herbicides in the future to control imazethapyr-resistant sunflowers in areas where no resistance to sulfonylurea herbicides has been registered. F_1 progeny of wild sunflower crossed to the cultivated sunflower exhibited partial resistance to imazethapyr, imazamox and imazapyr in field conditions. Another aim of this piece of research was to determine the genes responsible for this resistance as well as the mode of inheritance. In order to achieve this aim, we crossed wild *Helianthus annuus* resistant to imazethapyr with susceptible inbred line Ha-26. In the greenhouse, we made the F_2 generation of this interspecific cross as well as the BC_1 and BC_2. The results showed that the resistance is semi-dominant in F_1 the generation. The segregation ratio in the F_2 generation is 1:2:1 for semi-dominance. The incorporation of this characteristic into the cultivated sunflower would significantly improve the control of weeds in sunflower, since the currently used methods do not provide an efficient weed control in this crop species.

INTRODUCTION

Imidazolinones inhibit the acetolactate synthase (ALS) enzyme, which is responsible for the synthesis of the amino acids valine, leucine and isoleucine. Sulfonylurea herbicides, triazolopyrimidines and pyrimidyl oxybenzoates possess the same target site. Thus far, 58 weed species that developed resistance to inhibitors of ALS have been found in 14 countries (Heap, 1999). Imazetaphyr-resistant sunflower was found for the first time in northeastern Kansas in soybean fields where imazethapyr had been previously applied for 7 consecutive years (Al-Khatib et al, 1998). In a phytotoxicity test performed by Al-Khatib et al. (1998), the imazethapyr-resistant sunflower biotype displayed a 170-fold greater resistance than the susceptible one and 210-fold greater resistance of the target site. Also, imazethapyr-resistant sunflower was highly resistant to imazamox, slightly resistant to thifensulfuron and chlorimuron and susceptible to cloransulam (Baumgartner et al., 1999). Chlorimuron- and halosulfuron-resistant sunflower has been registered in Missouri as well (Johnson et al., 1997). Cross-resistance of weeds to herbicides from different groups is a common occurrence, and there are also instances where a weed species that is resistant to a particular herbicide responds differently to the other herbicides from the same group. An example of this is the existence of *Xanthium strumarium* biotypes that are resistant to imazaquin, imazethapyr and chlorimuron and the parallel existence of another biotype of this species that is resistant only to imazethapyr (Sprague et al., 1997).

The incautiousness that led to the use of imazethapyr%for a number of years in the same field created a problem, but it also aroused the interest of sunflower breeders and herbicide manufacturers in the utilization of this trait to improve weed control in this crop. The use of weed resistance to some herbicides to develop resistant cultivated plants by traditional breeding methods was an earlier practice. Thus, Canola (*Brassica napus*) resistant to triazines and *Lactuca sativa* resistant to sulfonylurea herbicides were developed using sources of resistance from *Brassica campestris* (Beversdorf et al., 1988) and the weed species *Lactuca seriola* (Mallory - Smith et al., 1991), respectively. Today, imidazolinone-resistant corn and oilseed rape are grown on a significant acreage. In early 1999, furthermore, the Cyanamid company launched the so-called CLEARFIELD Production System, which involves use of specially designed plants of various species that are resistant to imidazolinones and other herbicides from this group. For the future, there are plans for the system to incorporate more key cultivated crops, including sunflower (Anon., 1999). In a study imazethapyr-resistant wild sunflowers, it has been confirmed that an efficient and selective control of the floriferous parasite *Orobanche cernua* is possible using this herbicide (Alonso et al., 1998).

The objective of this investigation was to determine the reaction of wild sunflower to a number of acetolactate synthase inhibitors and to assess the possibilities of controlling volunteer imidazolinone-resistant sunflower by means of sulfonylurea herbicides. Also, we wanted to see if the mode of inheritance of resistance to imazethapyr could be confirmed, since that would increase the chances for a transfer of this trait to commercial sunflower inbreds.

MATERIALS AND METHODS

During 1999, the reaction of imazethapyr-resistant sunflower to a number of selected ALS-inhibiting herbicides was studied in the greenhouse and under field conditions. In 1998, in the field, plant resistance was tested and homozygosity confirmed using 140 g a.i./ha imazethapyr. Achenes of imazethapyr-resistant sunflower from Kansas were first germinated, then the seedlings were transplanted into containers with a nutrient substrate. After that, at the four-leaf stage, the young plants were transplanted into a field in four 3-m long rows with

three replications. At the 4-6 leaf stage, herbicides were applied using the knapsack sprayer, 300 l/ha of water, and a pressure of two bars. The plants used in the greenhouse experiment were obtained the same way - with prior germination and transplantation of two seedlings into 0.5 l containers 10-cm in diameter with artificial lighting for 16 hours a day. Watering were performed as needed. At the four-leaf stage, herbicides, shown in table 1, were applied using the laboratory sprayer. There were four replications of each herbicide treatment.

Tab. 1. Herbicides used in the study

		Rate g a.i./ha	
Herbicide	Product	Greenhouse trial	Field trial
Imazethapyr	Pivot 100E	70 and 140	70 and 140
Imazamox	Bolero	40 and 80	40 and 80
Imazapyr	Arsenal	-	240 and 480
Primisulfuron	Tell 75WG	30 and 60	30
Rimsulfuron	Tarot 25DF	12.5 and 25	12.5
Prosulfuron	CGA-152 005 75WG	15 and 30	15
Thifensulfuron	Harmony 75DF	7.5 and 15	7.5
Oxasulfuron	Dynam 75WG	75 and 150	75
Chlorsulfuron	Glean 75DF	15 and 30	-
Iodosulfuron	Hussar	5 and 10	5
Triflusulfuron	Safari 50DF	15 and 30	15 and 30

The following herbicides and rates were used to test the F_1 generations (common sunflower from Kansas x Ha-26): imazethapyr (70, 140 and 280 g a.i./ha), imazamox (20, 40 and 80 g a.i./ha) and imazapyr (120, 240 and 480 g a.i./ha). Twenty days after the herbicide application, phytotoxicity was estimated on a scale of 0-100% (0% - without injury, 100% - utter wilting), fresh mass of the above-ground plant parts was measured (two plants in the greenhouse, five in the field), and the plants were dried down to a constant mass at 105^{o}C.

RESULTS AND DISCUSSION

As an open-pollinated species, the sunflower was not considered a prime candidate for the incorporation of resistance to certain herbicides. Reasons for the hesitation included the possibility of a transfer of genes to the wild relatives and the problem of volunteer sunflowers in other cultivated crops. Information about registered sunflower resistance to imazetaphyr attracted the attention of herbologists as well as sunflower breeders and herbicide manufacturers. The former have described the mechanism of resistance to imazethapyr (Kassim et al., 1998), cross-resistance (to imazamox, chlorimuron and thifensulfuron), and distribution in the area where it was first registered (Baumgartner et al., 1999 a,b), while the latter have been working to improve weed control by introducing this trait into the cultivated sunflower.

Our trials have not only confirmed the previous sunflower responses to imazethapyr and imazamox but have also shown the studied sunflower population to be completely resistant to imazapyr rates of up to 480 g a.i./ha (Figure 2). This suggests the potential problem of using this herbicide on non-agricultural land where resistant populations of this weed and sunflower's wild relatives are present. However, ready-made preparations that contain this herbicide, such as Lightning (imazethapyr+imazapyr), can be used in the cultivated sunflower into which genes for resistance to imidazolinones have been incorporated. Because of a lack of seed of the imazethapyr-susceptible wild sunflowers from Kansas, we were not able to determine if there is a cross-resistance to sulfonylurea herbicides used in corn (primisulfuron, prosulfuron, rimsulfuron), small-grains (chlorsulfuron, iodosulfuron), soybean (oxasulfuron), sugar beet (triflusulfuron) and sorghum (prosulfuron). Using normal use and doubled herbicide rates, we were able to determine the response of the wild sunflowers to the above herbicides but not to make comparisons with he genotype of the same population that is susceptible to

imidazolinones. Although the existence of cross-resistance to the aforementioned sulfonylurea herbicides and the resistance index are yet to be determined with greater precision, the results of our study indicate that sunflower resistance to imidazolinones is not of the same type as that of IR corn, which possesses a several hundred times greater resistance to some sulfonylureas and imidazolinones (Siehl et al., 1996). Imidazolinone-resistant wild sunflower is susceptible to the normal use rates of chlorsulfuron, iodosulfuron, oxasulfuron, prosulfuron and rimsulfuron (Figures 1 and 2). This means that it will be possible to use these herbicides in the future to control imazethapyr-resistant sunflowers in areas where no resistance to sulfonylurea herbicides has been registered. Although thifensulfuron displayed a rather similar effect to that of the above-mentioned herbicides, it had been previously established that sunflower has slightly cross-resistance to this herbicide (Baumgartner et al., 1999). Triflusulfuron caused a smaller growth suppression than the other sulfonylurea herbicides. Following the growth suppression and the necrosis of the young leaves, the formation of lateral branches and regeneration were observed in the field trial. When the rate of this herbicide was doubled (a total dose of 30 g a.i./ha in split application is recommended), the reduction of plant growth exceeded 80%. The effect of primisulfuron at 30 g/ha was the closest to that of thifensulfuron, but for more reliable conclusions the existence of cross-resistance will have to be investigated. Without doubt the most precise answers will be obtained when the first isogenic inbred lines resistant to imidazolinones are developed.

The wild sunflower population from Kansas resistant to herbicides from the imidazolinone group was selfed. The resulting S_1 generation was treated with 140 g a.i./ha of imazethapyr to test its homozygosity. The results have shown that the population in question is indeed homozygous for this trait. The standard inbred line Ha-26 was also treated and has proven to be completely non-resistant to this group of herbicides. During the summer of 1998, the S_1 plants of wild sunflower were crossed with the Ha-26 inbred line. In the winter of 1998/99, The F_2 generation was produced under greenhouse conditions and two backcrosses (BC_1 and BC_2) were made. To produce the F_2 generation and the backcrosses, plants resistant to imazethapyr were used. During the 1999 growing season, the resistance was studied in a comparative trial involving the original wild sunflower population, inbred line Ha-26, F_1 and F_2 generations, and BC_2 progeny (BC_1 was studied under greenhouse conditions). The results were as follows:

- The progeny of the original population exhibited complete resistance, meaning it is totally homozygous with regard to the trait in question;
- Inbred line Ha-26 was completely susceptible to imazethapyr;
- The F_1 generation proved resistant, although slight chlorosis of the vegetative cone and apical leaves and a growth slowdown relative to the control were observed, indicating the presence of partial dominance in the inheritance of resistance to imazethapyr, imazamox, and imazapyr;
- In the F_2 generation, after the treatment with imazethapyr there were eight susceptible (S), 24 partially resistant (PR), and 12 resistant (R) plants (Table 2). The results of the chi-square test have shown that the segregation ratio is 1:2:1, i.e. that the mode of inheritance is partial dominance and that a single dominant gene is responsible for resistance to imazethapyr

Tab. 2. Segregation ratio for resistance to imazethapyr in the F_2 generation

Experimental segregation in the F_2 generation	Ratio	$\chi 2$	P
8 S : 24 PR : 12 R	1 : 2 : 1	1,09	0,50

- In the progeny of the backcrosses with the susceptible line Ha-26, we obtained a segregation ratio of 1:1 between the tolerant and susceptible genotypes and in the BC_1 and BC_2 generations (Table 3), which confirmed this was a case of a single gene.

Fig. 1. Reaction of imazethapyr-resistant sunflower to imazamox and selected sulfonylurea herbicides in greenhouse conditions

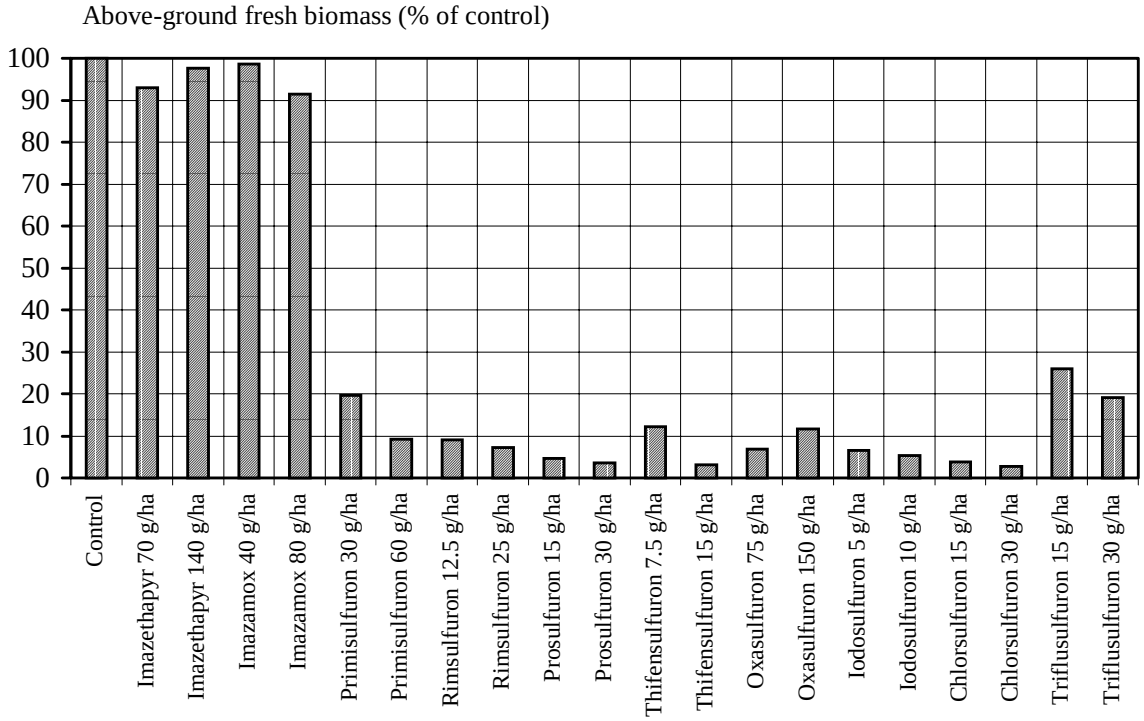
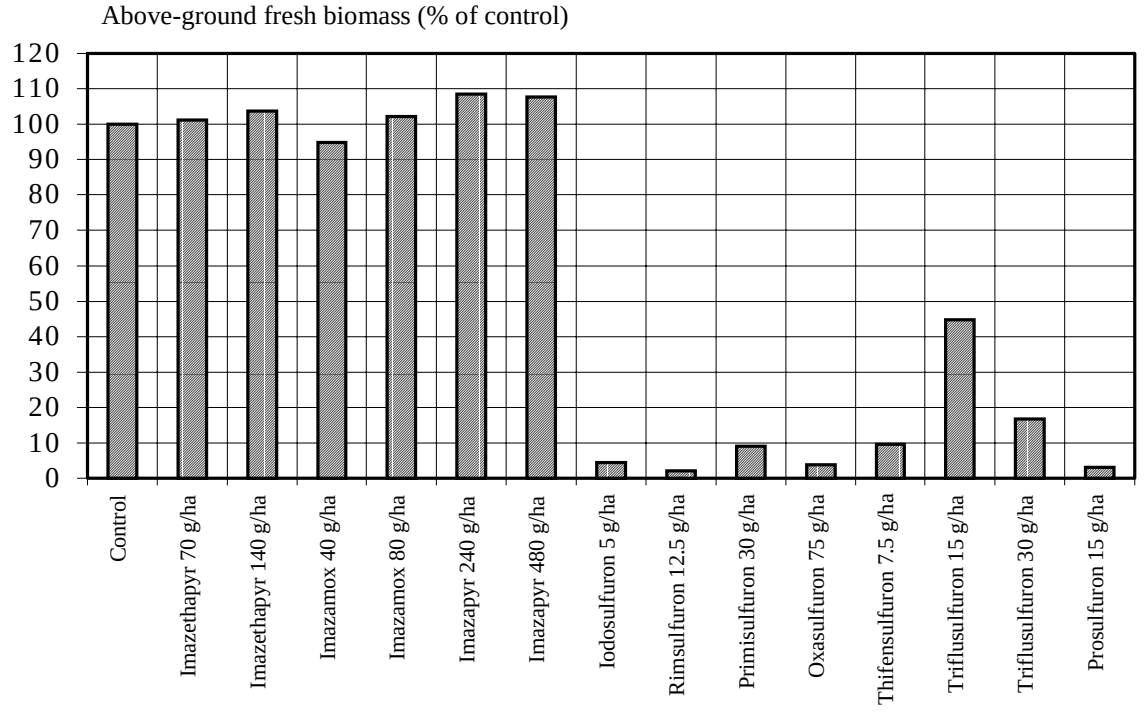


Fig. 2. Reaction of imazethapyr-resistant sunflower to imazamox, imazapyr and selected sulfonylurea herbicides in field conditions



Tab. 3. Segregation ratio for resistance to imazethapyr in the BC_1 and BC_2 generations

Experimental segregation in the BC_1 and BC_2 generations	Ratio	$\chi 2$	P
12 S : 23 PR	1 : 1	3.45	0.05
17 S : 23 PR	1 : 1	0.90	0.50
30 S : 27 PR	1 : 1	0.15	0.75
35 S : 24 PR	1 : 1	2.05	0.25
32 S : 30 PR	1 : 1	0.06	0.75
42 S : 33 PR	1 : 1	1.08	0.25
Total		7.69	0.25

Since the backcross results had been obtained from two generations (BC_1 and BC_2), we tested sample consistency and determined it indeed was there (Table 4).

Tab. 4. Sample consistency test

	Degrees of freedom	$\chi 2$	P
Sum of six $\chi 2$	6	7,69	0,25
Joint $\chi 2$	1	0,19	0,75
Difference	5	7,50	0,25

CONCLUSION

The study's results have lead to the following conclusions:

- In addition to the already known resistance to imazethapyr and imazamox, the studied sunflower is resistant to imazapyr;
- Imidazolinone-tolerant sunflower is susceptible to a number of sulfonylurea herbicides, especially to chlorsulfuron, prosulfuron, rimsulfuron and oksasulfuron;
- The response to triflusulfuron should be studied between the cotyledon- two-leaf stages and split application should be used, as recommended in the case of sugar beet;
- Resistance to imidazolinones is inherited by partial dominance and is controlled by a single gene.

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