Differences in sensitivity of F1 and F2 generations of herbicide tolerant sunflower volunteers to selected acetolactate synthase inhibiting herbicides

M. Jursík[1], K. Hamouzová[1], J. Soukup[1], J. Andr[2], J. Holec[1]

[1]*Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*

ABSTRACT

The aim of this work is to describe the differences in sensitivity of Clearfield, ExpressSun and non-herbicide tolerant (HT) sunflower cultivars to several acetolactate synthase inhibitor herbicides and to find out the differences in sensitivity in F1 and F2 generations of these cultivars. Non-HT sunflower was very sensitive to most of the tested herbicides, except for nicosulfuron (efficacy 43–75%). F2 generation of non-HT sunflower showed a low level of sensitivity to imazamox (efficacy 10–43%) and to nicosulfuron, tribenuron, thifensulfuron and rimsulfuron under dry conditions (efficacy 43–83%). Sensitivity of Clearfield sunflower (cultivar with tolerance to imidazolinone herbicides) to tested sulfonylurea varied according to used herbicide and weather conditions. The highest efficacy on Clearfield sunflower showed iodosulfuron (97–100%) > florasulam (88–100%) > foramsulfuron (40–98%) > thifensulfuron (12–99%) > rimsulfuron (37–99%) > tribenuron (0–87%) > nicosulfuron (0–78%). ExpressSun sunflower (cultivar with tolerance to tribenuron) was the most tolerant to all tested herbicides. The lowest efficacy on F1 generation showed tribenuron (0%) > imazamox (0–17%) > nicosulfuron (0–43%) > thifensulfuron (0–57%) > rimsulfuron (0–70%) > foramsulfuron (8–75%) > florasulam (75–96%) > iodosulfuron (87–98%). Sensitivity of F2 generation of ExpressSun to tested herbicide was usually higher as compared to F1 generation.

Keywords: *Helianthus annus*; herbicide efficacy; acetolactate synthase inhibiting herbicides; Clearfield; ExpressSun

Herbicide tolerant (HT) crops (cultivars) are a new and perspective trend in weed management, especially in crops where conventional weed management was problematic due to low level of herbicide efficacy or selectivity. Conventional cultivars of sunflower are very sensitive to many herbicides, especially to those used for a post-emergent application (Pannacci et al. 2007). Efficacy of pre-emergent sunflower herbicides decreased under dry conditions (Jursík et al. 2013). In addition, perennial and parasitic weeds are not sufficiently controlled by any sunflower herbicide.

An imidazolinone-tolerant wild sunflower population that was discovered in soybean field in Kansas, USA in 1996 (Al-Khatib et al. 1998) was used as a source for insertion of imidazolinone-tolerance gene into the first imidazolinone-tolerant lines (Al-Khatib and Miller 2000). Ala205Val mutation in acetolactate synthase (ALS) gene was shown to confer resistance to imidazolinone-tolerant sunflower (Bruniard 2001). Two lines (IMISUN-1 and IMISUN-2) were developed with resistance to imidazolinone herbicides (Al-Khatib and Miller 2000). Pro197Leu mutation in ALS gene was shown to confer resistance to sulfonylurea herbicides in the wild biotype of sunflower (Kolkman et al. 2004). Two lines were developed with resistance to sulfonylurea herbicides by introgression of mutations from wild isolates ANN-KAN and ANN-PUR, respectively, into elite breeding lines SURES-1 and SURES-2 (Miller and Al-Khatib 2004). SURES-1 and SURES-2 are homozygous for resistance to tribenuron. F1 generations produced from the crossings are completely resistant to tribenuron-methyl, pointing out to dominant way of inheritance of this trait. The exact number of genes controlling resistance is still unknown (Jocic et al. 2011). White et al. (2003) suggest that at least two copies of ALS gene are common in sunflower, while Bruniard (2001) thinks of three putative ALS genes.

Imidazolinone-tolerant sunflower cultivars (Clearfield technology) were introduced for farmers in 2003 (Turkey, Argentina and USA), sulfonylurea tolerant sunflower cultivars (ExpressSun technology) were introduced for farmers in 2007 (USA). Clearfield and ExpressSun technologies produced without the use of biotechnology were accepted in countries with restriction of growing of genetically modified crops (Tan et al. 2005), like European countries. Both these HT technologies were very quickly adopted and are used worldwide nowadays. However, these technologies bring also some new problems for growers. It is expected that: (1) volunteers of HT sunflower cultivars have lower sensitivity to other ALS inhibiting herbicides compared to conventional cultivars and (2) control of these volunteer plants is more difficult than control of volunteers of conventional cultivars of sunflower. Many studies dealt with the response of the new sunflower lines resistant to ALS inhibiting herbicides (Sala et al. 2008, 2012). Also a limited number of studies focused on resistant hybrids and their response to ALS inhibiting herbicides (Vrbnicanin et al. 2008, Bozic et al. 2012). Sensitivity of volunteer sunflower (F2 generation) to ALS inhibiting herbicides used in subsequent crops has not been well recognized, yet.

The aim of this work is to describe the differences in sensitivity of Clearfield, ExpressSun and non-HT sunflower cultivars to different ALS inhibitor herbicides to find the differences in sensitivity in F1 and F2 generations of these cultivars.

MATERIAL AND METHODS

Small plot field trials were carried out in Central Bohemia (Prague), Central Europe (300 m a.s.l.; GPS: 50°7'N, 14°22'E), in 2011–2013. F1 and F2 generations of Clearfield (Primis CL – breeder: Euralis), ExpressSun (PR64A31 – breeder: Pioneer) and non-HT (Biba – breeder: Euralis) sunflower cultivars were treated with eight ALS inhibitor herbicides (imazamox, tribenuron, nicosulfuron, florasulam, foramsulfuron, thifensulfuron, rimsulfuron and iodosulfuron) at recommended doses (Table 1). The experiment included untreated control plots. Seeds of F2 generations of the tested hybrids were collected from centre of fields where tested hybrids were grown (size of fields was 20–50 ha).

Sunflower was sown on the 12th April 2011, 4th April 2012 and 24th April 2013. The experiment was designed in split plots with three replications using herbicide as the main plot and cultivars and generations after hybridization of sunflower as the subplots. The plot size for the main plots and subplots was 30 × 3.5 m and 5 × 3.5 m, respectively. The row width was 70 cm, with an in-row plant spacing of 16 cm. Whole experimental area was pre-emergently treated by aclonifen (Bandur) + dimethenamid (Outlook) at application rare 1800 + 720 g a.i./ha) to weed control.

Table 1. Description of tested herbicide treatments

Herbicide			Adjuvant		
Active ingredient	trade name and formulation	application rate (g/ha)	active ingredient	trade name	application rate (g/ha)
Tribenuron	Express 50 SX	22.5	isodecyl alcohol ethoxylate	Trend 90	90
Imazamox	Pulsar 40 SL	50	–	–	–
Nicosulfuron	Milagro Extra 6 OD	45	–	–	–
Florasulam	Kantor SC	50	–	–	–
Foramsulfuron	Equip OD	45	–	–	–
Thifensulfuron	Refine 50 SX	11	isodecyl alcohol ethoxylate	Trend 90	90
Rimsulfuron	Titus 25 WG	15	isodecyl alcohol ethoxylate	Trend 90	90
Iodosulfuron	Husar WG	10	laurylsulphate Na	Biopower	280

Table 2. Weather conditions one month before and one month after application of herbicides

Meteorological characteristic		2011	2012	2013
Total precipitation (mm)	30 DBT	37.3	15.2	157.1
	10 DBT	19.4	0.0	98.2
	10 DAT	2.6	15.7	22.5
	30 DAT	62.6	45.8	127.9
Average of mean day temperature (°C)	30 DBT	14.4	16.4	12.9
	10 DBT	18.0	16.4	12.4
	10 DAT	18.6	16.1	18.3
	30 DAT	17.9	17.4	18.3

DBT – days before treatment; DAT – days after treatment

Tested herbicides were applied at 4–6 true leaves growth stage of sunflower on the 27th May 2011, 25th May 2012 and 7th June 2013. A small-plot sprayer was used to apply the herbicides. The water volume applied was 250 L/ha. Lurmark 015 F 80 nozzles were used, with an application pressure of 0.3 MPa. Description of the meteorological characteristics one month before and after application of tested herbicides is shown in Table 2.

The herbicide efficacy (sunflower sensitivity) was assessed by the estimation method using a percentage scale from 0% to 100% (0% – untreated; 100% – full control) according to the European and Mediterranean Plant Protection Organisation (EPPO) 1/135 (3) guidelines. The first assessment was performed 2 weeks after treatments (WAT), while the second was performed 4 WAT.

The experimental data were evaluated using the software package Statgraphics Plus 4.0 (Statpoint, Inc., Herndon, USA). A one-way and multifactorial analysis of variance was used. The contrasts between treatments were verified by the *LSD* test ($\alpha = 0.05$).

RESULT AND DISCUSSION

Multifactorial analysis. The best control of sunflower showed iodosulfuron (efficacy 97%) > florasulam (91%) > foramsulfuron (77%) > rimsulfuron (69%) > thifensulfuron (62%) > nicosulfuron (44%) > tribenuron (40%) > imazamox (27%) at 4 WAT assessment term. Differences in efficacy among all tested herbicides were significant (Table 3). The highest tolerance to tested herbicides showed ExpressSun hybrid (efficacy 45%), on the contrary, the highest sensitivity to tested herbicide showed non-HT cultivar (efficacy 82%). Differences in sensitivity to tested herbicides among tested sunflower cultivars were significant in both assessment terms (Table 3). According to the study of Bozic et al. (2012), imidazolinone tolerant hybrid was 9–64-fold more resistant than non-HT hybrid for

Table 3. Control of sunflower in dependence on used herbicides, cultivars, generations and experimental years

Factor	Efficacy (%)	
	2 WAT	4 WAT
Herbicide		
Tribenuron	46^{c}	40^{b}
Imazamox	30^{a}	27^{a}
Nicosulfuron	41^{b}	44^{c}
Florasulam	88^{g}	91^{g}
Foramsulfuron	71^{f}	77^{f}
Thifensulfuron	61^{d}	62^{d}
Rimsulfuron	66^{e}	69^{e}
Iodosulfuron	93^{h}	97^{h}
$LSD_{0.05}$	1.9	1.5
F-ratio	1032.72	2162.09
P-value	0.0000	0.0000
Cultivar		
Non-herbicide tolerant	80^{c}	82^{c}
Clearfield	63^{b}	63^{b}
ExpressSun	42^{a}	45^{a}
$LSD_{0.05}$	1.1	0.9
F-ratio	2028.31	3115.09
P-value	0.0000	0.0000
Generation after hybridization		
F1	61^{a}	64^{a}
F2	63^{b}	63^{a}
$LSD_{0.05}$	1.0	0.8
F-ratio	18.49	2.00
P-value	0.0000	0.1583
Experimental year		
2011	47^{a}	49^{a}
2012	60^{b}	65^{b}
2013	78^{c}	76^{c}
$LSD_{0.05}$	1.2	0.9
F-ratio	1396.05	1717.07
P-value	0.0000	0.0000

Means followed by the same letter within the column are not significantly different at $P < 0.05$. WAT – week after treatment vegetative parameters and about 3000-fold for ALS activity, while sulfonylurea tolerant hybrid was 14–56-fold more resistant than non-HT hybrid for vegetative parameters, and about 2600-fold for ALS activity. 2 WAT, F2 generation of sunflower cultivars showed significantly higher sensitivity to herbicide than F1 generation. 4 WAT, significant differences in sensitivity to herbicide between F1 and F2 generations were not found (Table 3). Efficacy of tested herbicides on tested cultivars of sunflower was significantly affected by weather conditions in experimental years (Table 3). The highest level of sunflower control was achieved in 2013, when precipitation was more than 3 times higher compared to 2011 and 2012 (Table 2).

One way analysis for individual years. All tested cultivars of sunflower were very well controlled by iodosulfuron (efficacy higher than 95%), but under dry conditions (2011), the ExpressSun was not satisfactorily controlled (efficacy on F1 generation 87% and on F2 generation 82%). Also florasulam controlled all tested sunflower cultivars (efficacy above 85%), except for 2011 when efficacy on ExpressSun was 75% (Tables 4 and 5). Lower herbicide efficacy under dry conditions is usually caused by strongly developed protection structure (such as wax and trichomes) on leaves surface (Stewart et al. 2010, Jursík et al. 2011).

Non-HT sunflower was very sensitive to most of the tested herbicides, except for nicosulfuron which caused only low plant injury (efficacy 47–75% on F1 generation and 43–53% on F2 generation). F2 generation of non-HT sunflower had low level of sensitivity to imazamox (efficacy 10–43%) and to some sulfonylureas (nicosulfuron > tribenuron > thifensulfuron > rimsulfuron) under dry conditions (2011 and 2012), when efficacy of these herbicides ranged from 43% to 83% (Tables 4 and 5). Single plants of F2 generation of non-HT sunflower showed different sensitivity to the tested herbicides (some plants were fully controlled while some of them were semitolerant), especially in 2012. Bruniard (2001) pointed out that some breeders observed distorted ratios in tolerance of populations deriving from progenies with different genetic backgrounds.

F1 generation of Clearfield sunflower was not controlled by imazamox, but plants of F2 generation were partly injured in 2013 (efficacy 40%, 2 WAT). Sensitivity of Clearfield sunflower to tested sulfonylurea differed significantly and was affected by used herbicide and weather conditions in experimental years (Tables 4 and 5). The highest efficacy on Clearfield sunflower showed iodosulfuron (97–100%) > florasulam (88–100%) > foramsulfuron (40–98%) > thifensulfuron (12–99%) > rimsulfuron (37–99%) > tribenuron (0–87%) > nicosulfuron (0–78%). It is known that the levels of injury in single mutants of imidazolinone tolerant breeding lines to different ALS inhibiting herbicides varied (Bruniard 2001). Inheritance of imidazolinone tolerance is not as clear as expected

Table 4. Control of sunflower cultivars 2 weeks after application

Herbicide	Efficacy (%)																	
	non-herbicide tolerant cultivar						cv. Clearfield						cv. ExpressSun					
	F1 generation			F2 generation			F1 generation			F2 generation			F1 generation			F2 generation		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
Tribenuron	80^{c}	80^{b}	93^{c}	77^{bc}	70^{b}	87^{c}	40^{c}	27^{b}	92^{c}	43^{c}	30^{b}	88^{c}	0^{a}	0^{a}	0^{a}	2^{a}	8^{a}	13^{a}
Imazamox	88^{d}	82^{bc}	85^{b}	50^{a}	33^{a}	27^{a}	0^{a}	0^{a}	10^{a}	0^{a}	0^{a}	40^{a}	0^{a}	8^{b}	27^{b}	2^{a}	25^{b}	60^{bc}
Nicosulfuron	43^{a}	50^{a}	72^{a}	47^{a}	33^{a}	77^{b}	0^{a}	20^{b}	83^{b}	33^{b}	23^{b}	73^{b}	0^{a}	37^{c}	33^{b}	8^{a}	50^{c}	50^{b}
Florasulam	85^{cd}	93^{d}	97^{c}	83^{cd}	87^{bcd}	97^{d}	87^{d}	83^{cd}	99^{cd}	85^{e}	83^{c}	99^{d}	68^{c}	85^{e}	96^{e}	70^{c}	85^{d}	96^{f}
Foramsulfuron	90de	82^{bc}	93^{c}	88de	80^{bc}	98^{d}	33^{c}	85^{d}	98^{cd}	57^{d}	82^{c}	97^{d}	17^{b}	50^{d}	68^{d}	23^{b}	50^{c}	85^{e}
Thifensulfuron	83^{cd}	93^{d}	93^{c}	82^{cd}	83^{bcd}	98^{d}	17^{b}	75^{c}	99^{cd}	40^{bc}	80^{c}	98^{d}	0^{a}	0^{a}	57^{c}	5^{a}	22^{b}	68^{cd}
Rimsulfuron	57^{b}	85^{c}	92^{c}	68^{b}	88^{cd}	99^{d}	33^{c}	83^{cd}	98^{cd}	47^{c}	95^{d}	99^{d}	0^{a}	50^{d}	70^{d}	3^{a}	50^{c}	75de
Iodosulfuron	96^{e}	99^{e}	96^{c}	97^{e}	100^{d}	99^{d}	96^{e}	96^{e}	99^{cd}	96^{f}	100^{d}	99^{d}	65^{c}	92^{e}	97^{e}	63^{c}	90^{d}	96^{f}
$LSD_{0.05}$	7	5	5	10	17	5	9	9	7	10	11	7	5	7	8	7	10	10

Means followed by the same letter within the column are not significantly different at $P < 0.05$

Table 5. Control of sunflower cultivars 4 weeks after application

Herbicide	Efficacy (%)																	
	non-herbicide tolerant cultivar						cv. Clearfield						cv. ExpressSun					
	F1 generation			F2 generation			F1 generation			F2 generation			F1 generation			F2 generation		
	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
Tribenuron	90^{c}	87^{bc}	95^{bc}	70^{b}	70^{c}	60^{b}	23^{c}	0^{a}	87^{c}	33^{b}	10^{b}	77^{c}	0^{a}	0^{a}	0^{a}	0^{a}	8^{a}	18^{a}
Imazamox	90^{c}	93^{cd}	93^{b}	43^{a}	30^{a}	10^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	3^{a}	0^{a}	7^{b}	17^{b}	3^{ab}	20^{b}	60^{c}
Nicosulfuron	47^{a}	71^{a}	75^{a}	43^{a}	43^{b}	53^{b}	0^{a}	37^{b}	78^{b}	37^{b}	43^{c}	60^{b}	0^{a}	43^{c}	33^{c}	10^{b}	60^{c}	50^{b}
Florasulam	93^{c}	96^{d}	98^{cd}	90^{d}	85^{de}	98^{c}	88^{e}	90^{c}	100^{d}	88^{e}	90^{de}	97^{d}	75^{c}	85^{f}	96^{f}	75^{d}	90^{e}	97^{f}
Foramsulfuron	92^{c}	96^{d}	96^{bc}	90^{d}	90^{e}	99^{c}	40^{d}	90^{c}	98^{d}	60^{d}	93^{e}	98^{d}	8^{b}	75^{e}	68^{e}	33^{c}	75^{d}	85^{e}
Thifensulfuron	90^{c}	95^{d}	96^{bc}	82^{c}	80^{d}	98^{c}	12^{b}	85^{c}	99^{d}	47^{c}	87^{d}	95^{d}	0^{a}	0^{a}	57^{d}	3^{ab}	12^{a}	71^{d}
Rimsulfuron	73^{b}	85^{b}	93^{b}	72^{b}	83^{de}	99^{c}	37^{d}	85^{c}	98^{d}	50^{c}	92^{de}	99^{d}	0^{a}	57^{d}	70^{e}	2^{a}	65^{c}	75^{d}
Iodosulfuron	100^{d}	100^{d}	100^{d}	100^{e}	100^{f}	100^{c}	97^{f}	100^{d}	100^{d}	97^{f}	100^{f}	100^{d}	87^{d}	97^{g}	98^{f}	82^{d}	95^{e}	97^{f}
$LSD_{0.05}$	6	7	4	8	9	7	8	6	4	6	6	7	6	6	8	8	6	8

Means followed by the same letter within the column are not significantly different at $P < 0.05$

from the DNA sequencing results. Miller and Al-Khatib (2000) studied the inheritance pattern of the trait and concluded that the tolerance appears to be controlled additively by at least two genes. Wolff et al. (1992) reported that the variations in response to herbicide among expected homogeneous tolerant and susceptible populations are either due to modifying genes or environmentally induced cultivars. Single plants of F2 generation of Clearfield sunflower showed different sensitivity to tribenuron, nicosulfuron, foramsulfuron and thifensulfuron in 2012 and 2013.

ExpressSun sunflower was the most tolerant to all tested herbicides. The lowest efficacy on F1 generation showed tribenuron (0%) > imazamox (0–17%) > nicosulfuron (0–43%) > thifensulfuron (0–57%) > rimsulfuron (0–70%) > foramsulfuron (8–75%) > florasulam (75–96%) > iodosulfuron (87–98%). Nevertheless, high differences were observed among experimental years (Tables 4 and 5). Low sensitivity of ExpressSun to imazamox was very surprising because mutation of ALS gene in position 197 results usually in no or low tolerance to imidazolinone herbicides (Kolkman et al. 2004). However, Park et al. (2012) tested the level of resistance of thifensulfuron resistant *Sonchus asper* population to some other ALS inhibiting herbicides and the lowest level of resistance was recorded for imazamox.

Sensitivity of F2 generation to tested herbicides was usually higher compared to F1 generation, especially in 2013, when there was very intensive precipitation before and after application (Table 2). Single plants of F2 generation of ExpressSun showed different sensitivity to imazamox, nicosulfuron and foramsulfuron in 2012 and 2013.

In conclusion, the control of volunteers of HT cultivars of sunflower by ALS inhibitors is more difficult than the control of volunteers of conventional hybrids. Especially volunteers of ExpressSun cultivars are very tolerant to the most of ALS inhibitor herbicides used. Tolerance of sunflower volunteers to herbicides is higher under dry conditions, when the protection barriers on leaf surface are better developed.

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Corresponding author:

Doc. Ing. Miroslav Jursík, Ph.D., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra agroekologie a biometeorologie, Kamýcká 129, 165 21 Praha, Česká republika
phone: + 420 224 382 787, fax: + 420 224 382 780, e-mail: jursik@af.czu.cz