doi: 10.17221/2/2017-PPS

Optimising Clearfield and ExpressSun Sunflower Technologies for Central European Conditions

Miroslav JURSÍK, Veronika FENDRYCHOVÁ, Michaela KOLÁŘOVÁ, Jiří ANDR and Josef SOUKUP*

Department of Agroecology and Biometeorology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, Prague, Czech Republic

**Corresponding author: jursik@af.czu.cz*

Abstract

Jursík M., Fendrychová V., Kolářová M., Andr J., Soukup J. (2017): Optimising Clearfield and ExpressSun sunflower technologies for Central European conditions. Plant Protect. Sci., 53: 265–272.

The efficacy and selectivity of herbicides and tank-mix combinations at different application terms in Clearfield and ExpressSun sunflowers were evaluated. Five-plot field trials (2011–2015) were carried out in Prague. The efficacy of tribenuron (TBM) was excellent and quite rapid on *Chenopodium album*. Its efficacy on other tested dicot weeds ranged around 90%, depending on weather conditions and growth stages of weeds. *Echinochloa crus-galli* was not controlled by TBM. The tank-mix combination of TBM + propaquizafop (PQF) caused sunflower injury when applied in a very cold growing period. Under dry conditions, PQF efficacy on *E. crus-galli* was strongly reduced. The split application of TBM, when PQF was used at the second application, was less negatively affected by herbicide antagonisms. Very good results were obtained on plots with pre-emergence treatment with dimethenamid and post-emergence with TBM. The efficacy of imazamox (IZM) on dicot weeds was strongly affected by growth stages of weed and weather conditions during application. Efficacy of IZM on *E. crus-galli* was increased by oil adjuvant, but this adjuvant reduced IZM selectivity. The best efficacy, selectivity, and Clearfield sunflower yield were on plots with the split application of IZM.

Keywords: *Helianthus annuus* L.; herbicide tolerant crop; application date; adjuvants; herbicide antagonism

Herbicide-tolerant (HT) crops (varieties) constitute a new promising trend in weed management, especially among crops for which conventional weed management is problematic due to the low level of herbicide efficacy or selectivity. Conventional sunflower varieties are very sensitive to many herbicides (Pannacci *et al.* 2007; Jursík *et al.* 2013), and the efficacy of pre-emergent sunflower herbicides is strongly affected by soil moisture conditions (Jursík *et al.* 2015). In addition, perennial and parasitic weeds are not sufficiently controlled by any sunflower herbicide.

The Clearfield and ExpressSun technologies produced without the use of biotechnology have been accepted in countries (including Europe) with restrictions of growing genetically modified crops (Tan *et al.* 2005). Both of these HT technologies have been adopted very quickly and are today used worldwide (Pfenning *et al.* 2008; Jocic *et al.* 2011; Franciscini *et al.* 2012).

Clearfield sunflower varieties are tolerant to imidazolinone herbicides (imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz, and imazaquin). An imidazolinone-tolerant wild sunflower population that was discovered in a soybean field in Kansas, USA, in 1996 (Al-Khatib *et al.* 1998) was used as the source for inserting the imidazolinone-tolerance gene into the first imidazolinone-tolerant lines (Al-Khatib & Miller 2000). The first Clearfield hybrids were commercially used in the United States, Argentina, and Turkey in 2003 (Tan *et al.* 2005). Imidazolinone herbicides control well most annual monocot weeds

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QJ1510186.

doi: 10.17221/2/2017-PPS

(FranciscHini *et al.* 2012). They also control many dicot weeds (Santos *et al.* 2012), including parasitic weeds like *Orobanche* spp. (Pfenning *et al.* 2008). Imazamox and imazapyr are used for weed control in Clearfield sunflowers in Europe.

ExpressSun sunflower varieties are tolerant to sulfonylurea tribenuron and were developed later than was the Clearfield system. ExpressSun varieties resulted from two different gene sources: (1) DuPont Chemical Company and (2) collaboration of Dr Kasim Al-Khatib and Dr Jerry Miller (Zollinger 2004). Tribenuron controls many dicot weeds, including *Cirsium arvense* (Zollinger 2004), but it does not control any grass weeds. The efficacy of tribenuron on some dicot weeds (for example *Ambrosia artemisiifolia, Galium aparine*) is lower compared to imidazolinones and tribenuron needs to be used in early growth stages or in a split application (Tuemmler & Schroeder 2013).

Both technologies need to be optimised for specific growing conditions. Determination of the suitable application date and the importance of adjuvants are the main problems for the effective use of the Clearfield technology (Elezovic *et al.* 2012). An effective and selective control of grass weeds must be defined for the ExpressSun technology. Different application rates and dates for tribenuron in ExpressSun sunflowers were tested by Godar *et al.* (2011), but they did not test any herbicide partner for grass weed control.

The objective of the present work was to compare the efficacy and selectivity of herbicides and tank-mix combinations used on different application dates in Clearfield and ExpressSun sunflowers under the conditions of Central Europe.

MATERIAL AND METHODS

Five-plot field trials were carried out in Clearfield (NK Neoma CL variety) and ExpressSun (P63LE10 variety) sunflowers at Prague, Czech Republic, Central Europe (300 m a.s.l., GPS: 50°7'N, 14°22'E) from 2011 to 2015. The study region is characterised by temperate climate (mean annual air temperature around 9°C, mean annual precipitation nearly 500 mm). The soil of the experimental fields was classified as a Haplic Chernozem and with clay content of 19%, sand content of 25%, and silt content of 56% (silt loam soil). Soil pH_{KCl} was 7.2 and sorption capacity 212 $mmol^{(+)}$/kg. Winter wheat had been the previous crop in all experimental years. Sunflower was sown on 6^{th}, 3^{rd}, 20^{th}, 14^{th}, and 15^{th} of April (in 2011, 2012, 2013, 2014, and 2015, respectively). Plots were established as randomised blocks with three replications. The area of plots was 21 m^2 (3 × 7 m). A precise small-plot sowing machine was used for drilling. The row spacing was 0.75 m, and the in-row plant spacing was 0.18 m. The experimental field was naturally infested by *Chenopodium album* L., *Echinochloa crus-galli* L., *Amaranthus retroflexus* L., *Mercurialis annua* L., and *Solanum physalifolium* Rusby. Weed densities were recorded on the area of 1 m^2 on untreated plots. Mean weed densities for individual species are given in Table 1.

A description of the experimental treatments is shown in Table 2. Herbicides were applied using a small-plot sprayer with Lurmark 015F110 nozzles at a spray volume of 250 l/ha (water) and a pressure of 0.25 MPa. The herbicides used are described in Table 3. Three application terms were used. Pre-emergent (PRE) application followed sunflower sowing (on the same day). The first post-emergent (POST 1) application was performed when sunflowers exhibited the first pair of true leaves, and the second post-emergent (POST 2) application was done when sunflowers showed six true leaves. Application dates in experimental years are displayed in Table 4.

Herbicide efficacy and selectivity were assessed by an estimation method according to guideline 1/93 (3) of the European and Mediterranean Plant Protection Organisation (EPPO). A percentage scale from 0% to 100% was used in assessing herbicide efficacy and phytotoxicity. Assessments of sunflower phytotoxicity

Table 1. Weed infestation (mean ± standard deviation) on untreated check plots in experimental years

Weed species	Weed density (plants/m^2)				
	2011	2012	2013	2014	2015
Amaranthus retroflexus	7 ± 4	32 ± 10	7 ± 5	14 ± 7	7 ± 4
Chenopodium album	49 ± 25	52 ± 15	16 ± 8	7 ± 4	65 ± 25
Echinochloa crus-galli	27 ± 15	45 ± 25	53 ± 16	62 ± 29	13 ± 7
Mercurialis annua	15 ± 7	8 ± 5	6 ± 4	7 ± 3	6 ± 4
Solanum physalifolium	14 ± 7	13 ± 9	30 ± 10	8 ± 5	7 ± 5

doi: 10.17221/2/2017-PPS

Table 2. Tested herbicide treatments

Herbicide (+ adjuvant)	Application rate (g a.i./ha)	Application term
Untreated check	–	–
FRC + DMA	500 + 720	PRE
TBM (+NIS)	22 (+225)	POST 2
DMA → TBM (+NIS)	1000 → 22 (+225)	PRE → POST 2
TBM + PQF	22 + 100	POST 2
TBM (+NIS) → TBM + PQF	11 (+225) → 11 + 100	POST 1 → POST 2
IZM	50	POST 2
IZM (+EFPA-A)	50 (+1000)	POST 2
IZM → IZM	25 → 25	POST 1 → POST 2

→ split application; FRC – flurochloridone; DMA – dimethenamid; TBM – tribenuron methyl; PQF – propaquizafop; IZM – imazamox; NIS – non-ionic surfactant (isodecyl alcohol ethoxylate); EFPA-A – esterified fatty and phosphoric acids adjuvant; PRE – pre-emergent; POST 1 – early post-emergent (sunflower BBCH 12); POST 2 – post-emergent (sunflower BBCH 16)

Table 3. Description of herbicides and adjuvants used

Active ingredient of herbicide (adjuvant)	Trade name	Formulation	Content of g a.i./l (kg)	Supplier
Flurochloridone	Racer	EC	250	Makteshim Agan
Dimethenamid	Outlook	EC	720	BASF
Tribenuron	Express	SX	500	DuPont
Imazamox	Pulsar	SL	40	BASF
Propaquizafop	Agil	EC	100	Makteshim Agan
Isodecyl alcohol ethoxylate	Trend 90	EC	900	DuPont
Fatty acids methyl esters and phosphoric acid alkyl ester	Dash	HC	900	BASF

were done 1 week after the final herbicide application and shortly before the canopy closure. Efficacy was assessed shortly before the canopy closure. Flower heads from two middle rows were hand harvested shortly before full maturity (during September). Flower heads were protected against birds with special wire netting. Achene yields were adjusted to 8% moisture content. In 2015, the yield was not measured because a hailstorm had damaged the sunflowers.

The meteorological characteristics of the study region from the day of sunflower sowing to the end of growing season are shown in Table 5.

Table 4. Application dates in experimental years

	2011	2012	2013	2014	2015
PRE (sowing)	06.04.	03.04.	20.04.	14.04.	15.04.
POST 1	28.04.	03.05.	13.05.	05.05.	11.05.
POST 2	17.05.	15.05.	29.05.	03.06.	29.05.

PRE – pre-emergent; POST 1 – early post-emergent (sunflower BBCH 12); POST 2 – post-emergent (sunflower BBCH 16)

The experimental data were evaluated using the Statgraphics Plus 4.0 software package. A one-way ANOVA was used, and the contrasts between treatments were verified by LSD test ($\alpha = 0.05$). Bartlett's test was used to determine whether efficacy data did not violate the assumption of homogeneity of variance. If necessary, log transformation of $X + 1$ or arcsine square root percent transformations were done for some data columns. In such cases, the multiple comparisons tests were applied to the transformed data.

RESULTS

PRE treatment with flurochloridone (FRC) + dimethenamid (DMA) fully controlled *Chenopodium album* in most experimental years. In 2015 the efficacy of this tank-mix (TM) combination was 95%, and in 2011 the efficacy was the lowest among all tested herbicide treatments (77%). Tribenuron methyl (TBM) with non-ionic surfactant (NIS) fully controlled *C. album* in most experimental years, the

doi: 10.17221/2/2017-PPS

Table 5. Meteorological characteristics during the time after the application of herbicides

Meteorological characteristics		2011	2012	2013	2014	2015	Climatic normal
Total precipitation (mm)	April	16	44	27	23	26	34
	May	36	17	112	131	29	71
	June	60	47	173	20	39	72
	July	120	80	54	92	32	70
	August	70	56	85	43	60	65
Mean temperature (°C)	April	12.3	9.6	9.6	11.5	9.1	8.0
	May	15.0	16.0	12.7	13.0	13.7	13.0
	June	18.2	18.0	16.8	17.2	16.8	16.1
	July	17.8	19.4	20.1	20.7	21.6	17.8
	August	19.1	20.0	18.5	17.2	22.9	17.4

exception being 2014, when the efficacy of this herbicide was 96%. The split application of TBM fully controlled *C. album* in all experimental years. Imazamox (IZM) efficacy was significantly lower (by 8–20%) compared to TBM + NIS in all experimental years. Esterified fatty and phosphoric acid adjuvant (EFPA-A) significantly increased the efficacy of IZM in 2013–2015 (by 4–6%), but the efficacy of this combination (IZM + EFPA-A) was always significantly lower as compared to TBM + NIS. IZM showed the highest efficacy (96–100%) while used in the split application (Figure 1).

The efficacy of PRE treatment on *Echinochloa crus-galli* ranged between 75 and 100% in experimental years. The efficacy of TBM was zero. Large differences in the efficacy of the TM combination of TBM + propaquizafop (PQF) were recorded between experimental years (53–97%), and only in 2011 and 2013 was the efficacy of the TM combination of TBM + PQF sufficient (more than 90%). The split application of TBM where PQF was used with TBM (at a half application rate) at the second application term was more effective (Figure 1). PRE treatment with DMA controlled *E. crus-galli* well (efficacy more than 95%). Only in 2011 (dry April) was the efficacy of DMA insufficient (54%). The efficacy of IZM ranged between 88 and 99% in experimental years. EFPA-A significantly increased the efficacy of IZM in 2012, 2013, and 2015, and the split application of IZM significantly increased efficacy only in 2013 and 2015.

The efficacy of PRE treatment on *Mercurialis annua* was greatly affected by experimental years and ranged between 0 and 100% (Figure 1). The efficacy of TBM (+NIS) was relatively stable (85–92%) in

Table 6. Sunflower injury one week after POST 2 application in experimental years

Herbicide-tolerant technologies	Herbicide treatments	Phytotoxicity (%)				
		2011	2012	2013	2014	2015
ExpressSun	FRC + DMA	1[cd]	0[c]	0[a]	0[b]	0[c]
	TBM (+NIS)	0[d]	0[c]	0[a]	0[b]	0[c]
	TBM + PQF	0[d]	18[a]	0[a]	0[b]	0[c]
	DMA → TBM (+NIS)	0[d]	0[c]	0[a]	0[b]	0[c]
	TBM (+NIS) → TBM + PQF	0[d]	0[c]	0[a]	0[b]	0[c]
Clearfield	IZM	8[ab]	0[c]	0[a]	8[a]	1[b]
	IZM (+EFPA-A)	12[a]	2[b]	0[a]	10[a]	8[a]
	IZM → IZM	3[bc]	1[bc]	0[a]	1[b]	0[bc]
P-value (0.05)		0.0001	0.0001	–	0.0001	0.0002
Data transformation		AT	LT	–	LT	AT

→ split application; FRC – flurochloridone; DMA – dimethenamid; TBM – tribenuron methyl; PQF – propaquizafop; IZM – imazamox; NIS – non-ionic surfactant (isodecyl alcohol ethoxylate); EFPA-A – esterified fatty and phosphoric acids adjuvant; values within a column with the same letter are not significantly different at the 5% *LSD* level (*P* = 0.05); AT – arcsine square root % transformation; LT – log transformation of X+1, – no data transformation applied

doi: 10.17221/2/2017-PPS

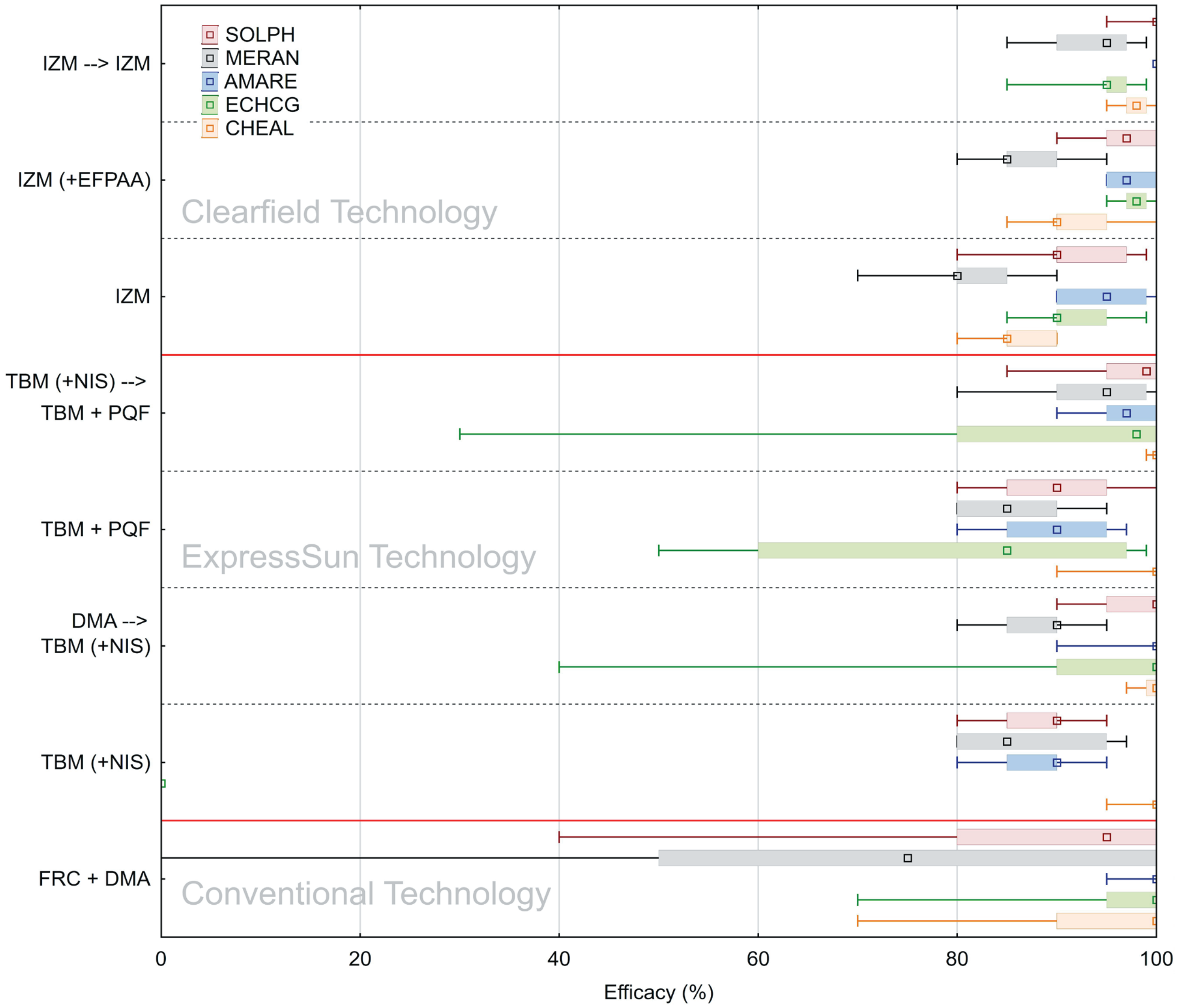

Figure 1: Box-plots displaying differences in efficacy between different treatments on selected weeds during the five-year period (box displays 25th, median, and 75th percentile, whiskers extend to minimum and maximum value)

→ split application; FRC – flurochloridone; DMA – dimethenamid; TBM – tribenuron methyl; PQF – propaquizafop; IZM – imazamox; NIS – non-ionic surfactant (isodecyl alcohol ethoxylate); EFPA-A – esterified fatty and phosphoric acids adjuvant; SOLPH – *Solanum physalifolium*; MERAN – *Mercurialis annua*; AMARE – *Amaranthus retroflexus*; ECHCG – *Echinochloa crus-galli*; CHEAL – *Chenopodium album*

experimental years. The split application of TBM significantly increased the efficacy (by 11–13%) in most years. The efficacy of IZM ranged between 75% and 87%, and split application or EFPA-A addition significantly increased the efficacy (by 6–24%).

The efficacy of PRE treatment on *Solanum physalifolium* ranged between 53 and 100% in experimental years (Figure 1). The efficacy of TBM (+NIS) was sufficient (83–92%). The best efficacy (93–100%) was shown by TBM when DMA was applied PRE and TBM (+NIS) was applied at the POST 2 application term. The split application of TBM also showed very good efficacy (96 to 100%), but in 2013 the efficacy of split application of TBM was only 87%. The efficacy of IZM ranged between 87 and 98%, and split application or EFPA-A addition significantly increased the efficacy (on 95–100% or 93–100%, respectively).

Amaranthus retroflexus was the most sensitive of the tested weeds (Figure 1). DMA applied PRE fully controlled *A. retroflexus.* The efficacy of TBM was around 90% and that of IZM was about 95%. The split application of both herbicides significantly increased their efficacies in most test years. The split application of IZM fully controlled *A. retroflexus.*

doi: 10.17221/2/2017-PPS

Figure 2. ExpressSun sunflower injury caused by the tank-mix combination of tribenuron methyl + propaquizafop

Figure 3. Leaf chloroses on Clearfield sunflower after the application of imazamox (50 g/ha) with oil adjuvant

No symptoms of phytotoxicity were recorded after the application of TBM (+NIS) to ExpressSun hybrid P63LE10 in any experimental year. The TM combination of TBM + PQF damaged ExpressSun sunflower only in 2012 (Table 6). The main symptoms of TBM phytotoxicity were bud necrosis and growth retardation (Figure 2). Selectivity of IZM to Clearfield hybrid Neoma was sufficient (phytotoxicity was 0% to 8% one week after application), but the addition of EFPA-A increased phytotoxicity (to 0–12%). The split application of IZM, on the other hand, reduced phytotoxicity (0 to 3%). The main symptom of IZM phytotoxicity was leaf chlorosis (Figure 3). With the exception of 2014 (phytotoxicity 5% shortly before the canopy closure), the regeneration of sunflowers after IZM injury was quite rapid (phytotoxicity 1–3% shortly before the canopy closure).

The lowest achene yields (1.03–2.68 t/ha) were recorded on untreated plots in each experimental year. The highest differences in yields between experimental years were on plots treated only PRE (1.38–4.70 t/ha). The ExpressSun hybrid showed the highest yield (2.72–4.93 and 2.79–4.64 t/ha, respectively) on plots treated with the split application of TBM (when PQF was added at the second application) and on plots treated PRE with DMA and subsequently treated POST with TBM (+NIS). The Clearfield hybrid showed the highest yield on plots where the split application of IZM was used (3.19–5.33 t/ha). Yield differences between the split

Table 7. Yield of sunflower seeds in tested treatments in experimental years

Herbicide-tolerant technologies	Herbicide treatments	Yield of achenes (t/ha)			
		2011	2012	2013	2014
ExpressSun	untreated check	1.09^{e}	1.30^{c}	2.26^{a}	2.68^{ef}
	FRC + DMA	1.38^{e}	4.70^{a}	2.89^{a}	3.94^{bc}
	TBM (+NIS)	3.85^{bcd}	3.68^{b}	2.70^{a}	3.21^{de}
	DMA → TBM (+NIS)	3.99^{bc}	4.64^{a}	2.79^{a}	4.00^{b}
	TBM + PQF	3.27^{d}	4.22^{ab}	2.56^{a}	3.76^{bcd}
	TBM (+NIS)→ TBM + PQF	4.93^{a}	3.60^{b}	2.72^{a}	3.98^{bc}
Clearfield	untreated check	1.03^{e}	1.34^{c}	2.13^{a}	2.33^{f}
	IZM	3.31^{d}	1.79^{c}	2.67^{a}	3.63^{bcd}
	IZM (+EFPA-A)	3.45^{cd}	1.89^{c}	2.45^{a}	3.36^{cd}
	IZM → IZM	4.29^{ab}	4.04^{ab}	3.19^{a}	5.33^{a}
P-value		0.0001	0.0001	0.0783	0.0001
Data transformation		–	–	–	–

Values within a column with the same letter are not significantly different at the 5% LSD (P = 0.05) level; – no data transformation applied; → split application; FRC – flurochloridone; DMA – dimethenamid; TBM – tribenuron methyl; PQF – propaquizafop; IZM – imazamox; NIS – non-ionic surfactant (isodecyl alcohol ethoxylate); EFPA-A – esterified fatty and phosphoric acids adjuvant

doi: 10.17221/2/2017-PPS

and single application of IZM were significant in most experimental years (Table 7).

DISCUSSION

The efficacy of PRE treatment was strongly affected by soil moisture. In years with low precipitation during April and in the first half of May, the efficacy of FRC + DMA was lower compared to POST treatments, especially for *M. annua*, which is able to emerge from a deeper soil layer (Magyar & Lukács 2002). The lower efficacy of PRE treatment resulted in a reduction of sunflower yield, which was most visible in 2011, when the lowest total precipitation and highest mean temperature were recorded in April. Similar results were recorded by McCullough *et al.* (2013) and Jursík *et al.* (2015), who tested the efficacy of PRE herbicides under different weather conditions.

The efficacy of TBM (+NIS) was excellent and quite rapid on *C. album*, but the efficacy on other tested dicot weeds ranged around 90% depending on weather conditions and growth stages of weeds. *E. crus-galli* was not controlled by TBM. TM combinations of sulfonylureas with inhibitors of ACCase can cause a decrease of ACCase inhibitor efficacy and crop selectivity (Isaacs *et al*. 2003; Kammler *et al*. 2010; Matzenbacher *et al*. 2015). Sunflower phytotoxicity of the TM combination of TBM + PQF was recorded in 2012, when a very cold period occurred after a very hot start to the growing season (minimum ground temperature ranged between 5°C and –5°C five days before and after application). The efficacy of PQF decreased strongly in 2012 and 2014 and partially in 2015. This reduced efficacy was probably influenced by an absence of precipitation prior to herbicide applications (less than 10 mm two weeks before application). The split application of TBM, where PQF was used at the second application term, was less negatively affected by herbicide antagonisms (no problem with phytotoxicity and higher efficacy of PQF on *E. crus-galli* and TBM on dicot weeds). Very good results (higher efficacy, no phytotoxicity, and the highest yield) were obtained on plots treated PRE with DMA and subsequently treated POST with TBM (+NIS) in most test years. Only in 2011 (with very dry April) the efficacy was insufficient and the yield was reduced. Similar results were recorded by Sarpe *et al.* (2002) and Jursík *et al.* (2015) in maize and sunflower, respectively, wherein those authors observed the good efficacy of DMA in moist and moderate soil conditions that was in contrast with low efficacy in extremely dry conditions. The results of Elezovic *et al*. (2012) demonstrated the positive effect of PRE treatment on Clearfield sunflowers. According to their recommendation, the POST weed control following the PRE herbicide treatment can be delayed by approximately two weeks as compared to a crop where no PRE herbicide control was used.

The efficacy of IZM on dicot weeds (mainly *C. album*) was strongly affected by the growth stages of weeds and weather conditions shortly before and on the application date. The efficacy of IZM on *E. crus-galli* usually increased with the EFPA-A addition, but this adjuvant also reduced the selectivity of IZM. Similar results were reported by Jursík *et al*. (2011) and Idziak and Woznica (2013) based on field experiments in sunflower and maize, respectively, where the efficacy and selectivity of flumioxazin and nicosulfuron were strongly affected by tested adjuvants. In particular, methylated seed oil significantly increased efficacy and decreased selectivity. In our study, the best efficacy, selectivity, and Clearfield sunflower yield were achieved on plots where the split application of IZM was used. Elezovic *et al*. (2012) and Knezevic *et al.* (2013) concluded that weeds in Clearfield sunflower plots with as many as three or four true leaves must be controlled by imidazolinone herbicides. Later the weed control can lead to unacceptable sunflower yield losses (greater than 5%).

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Received: 2017–01–02
Accepted after corrections: 2017–04–07
Published online: 2017–05– 03