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Results of the study of cross-resistance and effect of herbicide on crops in the production of cycloxydim-tolerant maize (*Zea mays* L)

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Abstract

In Hungary, monocot weed species are present as a significant yield limiting factor. In practice there is a great demand for efficiently applying agrochemicals against monocot weeds at a later period and without causing phytotoxicity. Field trials were carried out in 2010 and 2011. The trials aimed to understand whether the cycloxydim-tolerant (CT) maize have cross-resistance to herbicides expressing different graminicide action (quizalofop-p-tefuril, haloxyfop-r-methyl ester, propaquizalofop, fluazifop-p-butyl). The obtained results confirm that CT maize has significant tolerance to cycloxydim active substance. Lower rates of other types of graminicides neither damage maize plants nor reduce the yield, while application of higher rates used to control perennial weeds do. According to the authors' conclusions, no other types of graminicides can be used to perform chemical weed control in CT maize. Post-emergent use of cycloxydim showed excellent efficacy against monocot weeds: *Echinochloa crus-galli*, *Setaria verticillata*, *Panicum miliaceum*. It is well-known that growing genetically modified maize is greatly restricted in Europe, therefore the published scientific results provide good option for the control of monocot weeds in the maize growing areas.

Keywords: ACCase inhibitor, graminicide, ACCase resistance, monocotyledon weeds, weed control, herbicide tolerant maize, cycloxydim

Introduction

In Hungary, weed species present in maize fields have greatly changed for the last 60 years. Based on the information of The Fifth National Weed Survey published in 2009, the predominant annual monocot weed species are barnyardgrass (*Echinochloa crus-galli*), yellow foxtail (*Setaria pumila*), wild-proso millet (*Panicum miliaceum*), green foxtail (*Setaria viridis*), large crabgrass (*Digitaria sanguinalis*), while the perennial species are johnsongrass (*Sorghum halepense*), quackgrass (*Elymus repens*) and bermudagrass (*Cynodon dactylon*) (Novák et al, 2009). The major issue is presented by the perennial johnsongrass causing great herbological problems also in the Mediterranean region (Andújar et al, 2011), in several Central European countries (Stefanovic et al, 2007; Tsvetanka and Marinov-Serafimov, 2007; Tyr et al, 2011) and in Hungary.

As far as maize is concerned, the chemical weed control of dicots and monocots must be separated because, in general, dicot weed species cause much less problems in maize due to the great selection of efficient weed management programs.

The spread of the perennial weed species and the mostly drought weather of recent years resulted in a shift towards the post-emergent applications of chemicals in maize. The various sulfonylurea (SU) derivatives serve as a basis for the post-emergent control of monocots, for example: nicosulfuron, rimsulfuron, foramsulfuron and their combinations. The SU preparations show great efficacy in controlling barnyardgrass, wild-proso millett, foxtail species, but only up to their one-three leaf stages (Krausz et al, 2000; Bunting et al, 2005; Hennigh and Al-Khatib, 2010; Damalas et al, 2012). Two applications may even suppress johnsongrass (Camacho et al, 1991; Eleftherohorinos and Kotoula-Syka, 1995). When applying them against monocot weeds post-emergently, the adequate timing may pose difficulties because the various species emerge at different dates due to their different biology. Furthermore, it is possible that the early or normal post-emergent treatments do not show suitable weed control efficacy, because some species which germinate later (mainly foxtail species and wild-proso millet) (Leon et al, 2004) are not contacted with the herbicide. The SU preparations may be applied only up to three-five leaf stages of maize because, if applied later, they may cause phytotoxicity (Swanton et al, 1996). Certain active substances must be used in combination with a special compound (Bunting et al, 2004). In dry weather, adjuvant shall be mixed into the spray in order to have adequate efficacy (Kapusta et al, 1994; Torma et al, 2011).

It is necessary to include a herbicide into the pest management program of maize which can be applied without any risk of phytotoxicity and which show great efficacy in controlling developed individu-

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als of all monocot species, similarly to glyphosate in Roundup Ready maize.

The aryloxyphenoxypropionate (APP), the cyclohexanedione (CHD) and the phenylpyrazolin (PPZ) (pinoxaden) derivative herbicides are authorized for post-emergent applications to control grasses (Winton-Daniels et al, 1990; Askew et al, 2000; Hofer et al, 2006). The plant destruction is primarily caused by the inhibition of fatty acid biosyntheses trough the blocking of the Acetyl-coenzyme A carboxylase (ACCase) (EC 6.4.1.2) enzyme (Rendina and Felts 1988). Resistance may develop to them on the basis of the change of the target-site, when plants develop an insensitive ACCase. They are genetically governed as a result of a single point mutation (Murray et al, 1995). The target-site resistance at the binding sites of herbicide and enzyme - carboxyl transferase domain (Xiang et al, 2009; Yu et al, 2010) – is the result of the incurring exchange of amino acid. Indeed, the different exchanges of amino acid developing at various positions are responsible for the formation of each type of ACCase resistance (Deléye et al, 2002a, 2002b, 2005; Yu et al, 2007).

Development of maize tolerant to ACCase inhibitor started at the beginning of the 90s by the selection of mutants from tissue cultures (Parker et al, 1990a, 1990b; Marschall et al, 1992). Sethoxydim-tolerant (ST) maize varieties were produced in the mid-1990s. Excellent herbicidal efficacy was achieved in ST maize (Dotray et al, 1993) however it had no particular economic importance because both the glyphosate- and the gluphosinate-tolerant genetically modified (GM) versions were effective in controlling both monocots and dicots (Tharp and Kells 1999; Loux et al, 2011). In Europe production of GM plants is legally governed and restricted and, in addition, that of GM herbicide-tolerant (HT) plant has not spread due to the public thinking favouring “GM-free” regions (James, 2010). In Hungary, a moratorium has been introduced for the production of GM plants. In Europe and in Hungary, the production of non-GM HT plants has increasing economic importance, as the imidazolinone (IMI) and SU tolerant sunflower (Kukorelli et al, 2011), the IMI tolerant rape (Ádámszki et al, 2011) or the cycloxydim-tolerant maize.

The CTM gene is responsible for the cycloxydim-tolerance (CT) of maize. The various breeding institutions have introduced it into the grown hybrids (Vancetovic et al, 2009; Széll et al, 2010). In Hungary CT-maize can be used for growing since 2008.

When producing HT plants, it is recommended to thoroughly know the phytotoxic effect of the herbicide on the plants. High tolerance makes possible the use of herbicide combinations, even the use of higher rates does not cause plant damages, and in addition, the late post-emergent application becomes possible. One objective of this study was to find out whether the multiple dose of cycloxydim has a grain yield decreasing effect. Several members of ACCase-inhibitors are used in weed management systems worldwide. Tolerance of CT-maize to different doses of ACCase inhibiting herbicides was surveyed and certain conclusions were drawn asking whether they can be used for weed control in maize. Efficacy of weed management against monocot and dicot species using cycloxydim tolerance was studied.

Materials and Methods

Field trials were carried out near the city of Győr in North-West Hungary. The areas were close to each other under the coordinates of 47.65°N, 17.69°W (2010), 47.64°N, 17.69°W (2011). Soil type: meadow alluvial soil. Soil properties in 2010: pH: 7.5, K_A: 39.2, humus content: 2.97; in 2011: pH 7.61, K_A 41.3, humus content: 4.12.

In the various years of the experiments, similar cultural techniques were used. Preceding crop on the area: winter wheat, nutrition management: 105 kg ha^{-1} Nitrogen, 35 kg ha^{-1} Potassium, and 35 kg ha^{-1} Phosphorus were applied. Sowing was made by seed driller (Monosem), using 69 000 plants ha^{-1}. The sown hybrid: ES Ultrafox ® (cycloxydim-tolerant, FAO 340, Euralis). Sowing was made on 3 May 2010 and 24 April 2011.

On the experimental areas 800 g ha^{-1} bentazone + 225 g ha^{-1} dicamba were used at the 3-leaf stage of maize in order to suppress dicot species. No other treatments with plant protection products were made.

The study was conducted in plots of four rows, 7 m each (21 m^2). Treatments were made in four replicates in randomized complete block design. Treatments (with graminicides) were post-emergently made at five-seven leaf stage of maize (BBCH: 15-17) (Table 1). Applications were made with hand sprayer and AD12004 Lechler nozzles, using 270 l ha^{-1} water volume at 3 bar pressure.

The rates of the preparations applied: (1) doses to control annual species; (2) doses to control perennial species (these two types of herbicide applications are generally accepted in herbological practice); (3) double of the rates to control perennial species (in provocative treatments double dosages for perennial monocots control were used. In practice crop injury can occur due to the overlapping during spraying).

The studied preparations in 2010: cycloxydim, quizalofop-p-tefuril, haloxyfop-r-methyl ester. In 2011: cycloxydim, quizalofop-p-tefuril, haloxyfop-r-methyl ester, propaquizafop, fluazifop-p-butyl. Adjuvant (0.185 l ha^{-1} methyloleate + 0.185 l ha^{-1} methyl palmitate) was mixed to cycloxydim, quazilafop-p-tefuril, haloxyfop-r-methyl ester, propaquizalofop, based on the recommended pest management (Table 1).

In order to evaluate the results of the phytotoxicity studies, a control area was included into each treatment, where weed control was performed using a combination of 160 g ha^{-1} dicamba + 50 g ha^{-1} topramezone. Areas without weed management were

Table 1 - Experimental treatments in the two years of research (2010, 2011).

Treatments Herbicide (ai.)	Dosage (g ha^{-1})	Maize phenology (BBCH) 2010	2011	Date of treatments 2010	2011
cycloxydim[1]	150	15-16	16-17	24. 06	31. 05
	400	15-16	16-17	24. 06	31. 05
	800	15-16	16-17	24. 06	31. 05
quizalofop-p-tefuril[1]	40	15-16	16-17	24. 06	31. 05
	120	15-16	16-17	24. 06	31. 05
	240	15-16	16-17	24. 06	31. 05
haloxyfop-r-methyl ester[1]	55	15-16	16-17	24. 06	31. 05
	215	15-16	16-17	24. 06	31. 05
	430	15-16	16-17	24. 06	31. 05
propaquizafop[1]	75	-	16-17	-	31. 05
	150	-	16-17	-	31. 05
	300	-	16-17	-	31. 05
fluazifop-p-butyl	120	-	16-17	-	31. 05
	375	-	16-17	-	31. 05
	750	-	16-17	-	31. 05
bentazone + dicamba	800 + 225	13	13	12. 06	21. 05
dicamba + topramezone	160 + 50	13	13	12. 06	21. 05

[1]Adjuvant was mixed to the herbicides.

also included to study the herbicide efficacy of cycloxydim tolerant techniques.

Phytotoxicity and herbicide efficacy were evaluated one and two weeks after treatments and in August, at the time of ripening. Visual evaluation of phytotoxicity was made using a scale of 0 to 100 (0 = no damage, 100 = plant death), in percentage, expressing with one single number. Phytotoxicity assessment followed the method of EPPO (2007a, b). In addition, grain yield were performed on the treated plots following the harvest with Wintersteiger plot-combine. Grain production was converted to 13.5% grain moisture.

The weed control efficacy for each species was evaluated according to the methods of EPPO (2007b) and it is given in percentage (0% = no weed control, 100% =full weed control) of the untreated weedy control.

All data were subjected to ANOVA. Means were separated with Student-Newman-Keuls test at $P \leq 0.05$.

Results and Discussion

Phytotoxicity tests

Rates of 150 g ha^{-1}, 400 g ha^{-1}, and 800 g ha^{-1} of cycloxydim did not cause visual damages on CT plants in 2010 and 2011. The treatments did not result in significant alterations compared to the grain yield of 8,100 kg ha^{-1} and 10,290 kg ha^{-1} measured on the control plots in 2010 and 2011, respectively (Table 2).

The rate of quizalofop-p-tefuril used to control annual weeds induced low phytotoxicity (10%) only in the second year, reaching a grain yield of 7,990 kg ha^{-1} and 10,380 kg ha^{-1} on the plots in 2010 and 2011, respectively. The higher herbicide dose expressed obvious damages. In 2010 both higher rates resulted in significant decrease in yields giving 6,570 kg ha^{-1} and 5,920 kg ha^{-1} compared to the control and the treatment with cycloxydim, respectively. In 2011 similar alteration was observed (8,950 kg ha^{-1} with 120 g ha^{-1}), however, significantly justified difference was obtained only with 240 g ha^{-1}, thus a grain yield of 3,490 kg ha^{-1} was harvested (Table 2).

Using 55 g ha^{-1} of haloxyfop-r-methyl ester caused no grain yield losses in 2010. In 2011 a lower grain yield, 9,140 kg ha^{-1} was harvested compared to the treatment with cycloxydim which was however not significant. The higher doses expressed severe plant damages. With the application of 215 g ha^{-1} the yield dropped to its half – 4,270 kg ha^{-1} (2010), 3,490 kg ha^{-1} (2011) –, while the use of 430 g ha^{-1} resulted in almost complete death of the crops in addition to a phytotoxicity of 93% (2010) and 92% (2011) (Table 2). Less damages were caused by doses of propaquizalofop. Applications of 75 g ha^{-1}, 150 g ha^{-1}, and 300 g ha^{-1} resulted in 11%, 11%, and 15% phytotoxicity, respectively. Significant difference in grain yield was induced by only the latter treatment reaching 7,220 kg ha^{-1} (Table 2).

The increase of fluazifop doses to 150 g ha^{-1}, 375 g ha^{-1}, and 750 g ha^{-1} caused ever increasing damages, reducing the grain yield to 9,310 kg ha^{-1}, 4,070 kg ha^{-1}, and 2,160 kg ha^{-1}, respectively (Table 2).

The above results confirm that CT maize plants have great resistance to cycloxydim (cf. Zivojinovic et al, 2009; Széll et al, 2010). It is therefore possible to use a combination of a herbicide and a product to control dicots, because no phytotoxicity is expected as it was the case with ST maize (Dotray et al, 1993). In addition, cycloxydim can be applied in higher doses (e.g. 2x 300 g ha^{-1}, 600 g ha^{-1}) in certain cases.

Under field conditions, the CT-maize has unreliable, low resistance to various APP graminicides. Complete plant death did not occur following any

Table 2 - The effect of herbicide treatments on maize in the two years of research (2010, 2011).

Treatments		Maize injury (Phytotoxicity%)		Maize grain yield (kg ha^{-1})	
Herbicide (ai.)	Dosage (g ha^{-1})	2010	2011	2010	2011
cycloxydim[1]	150	0a	0a	7,920a	10,520a
	400	0a	0a	7,840a	10,490a
	800	0a	0a	8,040a	10,550a
quizalofop-p-tefuril[1]	40	0a	10b	7,990a	10,380a
	120	31b	26e	6,570b	8,950a
	240	73d	86g	5,920c	3,490c
haloxyfop-r-methyl ester[1]	55	0a	11b	8,170a	9,140a
	215	68c	75f	4,240d	3,740c
	430	93e	92g	1,430e	1,770d
propaquizafop[1]	75	-	11a	-	10,130a
	150	-	11a	-	8,960a
	300	-	15c	-	7,220b
fluazifop-p-butyl	150	-	19d	-	9,310a
	375	-	76f	-	4,070c
	750	-	92g	-	2,160d
control[2]		0a	0a	8,100a	10,290a

Values of control within a column followed by the same letter are not significant different at $P \leq 0.05$ according to Student-Newman-Keuls test. [1]Adjuvants was mixed to the herbicides. [2]The control plots were treated by dicamba + topramezone.

treatments, though damages were observed the extent of which greatly depended on the herbicides and doses used. In our experiments the lower rates of graminicides did not reduce the grain yield, while application of higher dosage did. This type of resistance is similar to that Shukla et al (1997) found with giant foxtail (*Setaria faberi*) and green foxtail (high and low resistance to CHD products and APP products, respectively). Therefore it is excluded that other graminicides could be safely used for weed control in tolerant maize. These results are similar to those stated by Vangessel et al (1997) for ST maize. It can not be proposed that the treatments not producing significant yield loss in the trials (quizalofop-p-tefuril 40 g ha^{-1}, haloxyfop-r-methyl ester 55 g ha^{-1}, propaquizalofop 75 g ha^{-1}) should be used under farm conditions, as it was not studied what biotic or abiotic stress may induce severe damages in CT maize.

Herbicide efficacy examination

Experiences obtained with weed control in CT maize confirm that control of dicot and monocot species should be separated. Treatment to control dicot species shall be made at maize three leaf stage (BBCH 13) in order to free the crop from the mass competitiveness of weeds. Bentazone + dicamba showed excellent efficacy.

From the monocots only the annual species were present in the area, thus treatment with 150 g ha^{-1} cycloxydim was evaluated. Post-emergent treatment to control monocots shall not be made too early because some species germinating later (e.g. wild-proso millet, foxtail species) shall not be in contact with the herbicide. Most monocots are developed at treatment time, in the stage of tillering, however application of 150 g ha^{-1} cycloxydim killed them two weeks after treatment, and no regrowth was observed with any species (Table 3).

Good efficacy of cycloxydim was observed in controlling developed annual monocots, and no phytotoxicity develops even in case of later application due to the great tolerance of maize. Combination of bentazone + dicamba complements well the pest management program thus the maize field will be free from both monocot and dicot species (Table 3).

The most serious and difficult to control weeds of maize are undoubtedly the perennial monocots. Their rapid and intensive spreading can be observed (see Introduction). Increasing sowing area of maize in Europe gives a good opportunity to the intensive spreading of such grass weed species. Therefore super selective monocot weed control in CT maize hybrids is a great of importance.

Growing cycloxydim-tolerant maize may result in efficacious post-emergent weed control similar to that obtained in glyphosate-tolerant maize (Johnson et al, 2000). It may be a reliable option in Europe for the genetically modified HT maize. The size of acreage shall probably increase with the development of hybrids of high productivity.

Table 3 - Average cover of major weed species in the untreated control and treated areas and efficiency of treatments in controlling major weed species in the two years of research (2010, 2011).

Treatments	Weed species	Non-treated areas 2010	Non-treated areas 2011	Treated areas 2010	Treated areas 2011	Treated areas 2010	Treated areas 2011
		Cover in%				Efficacy%	
	Echinochloa crus-galli	52	10.5	2	0.03	98bc	99a
cycloxydim[1]	*Setaria verticillata*	11.5	3.2	0.8	0.05	97c	100a
	Panicum miliaceum	5.2	4.5	0.03	0	100a	100a
	Amaranthus retroflexus	1.5	9.5	0.03	0.03	99abc	99a
dicamba + bentazone	*Chenopodium album*	6.8	43.8	0.1	0.1	99abc	99a
	Datura stramonium	3.8	6.5	0	0.03	100a	99a
	Other dicots	5.5	7.3	-	-	-	-

Values of control within a column followed by the same letter are not significant different at $P \leq 0.05$ according to Student-Newman-Keuls determined by ANOVA. [1]The dose of cycloxydim was 150 g ha^{-1}, adjuvants was mixed to the herbicides.

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