Influence of soil pH, rainfall, dosage, and application timing of herbicide Merlin 750 WG (isoxaflutole) on phytotoxicity level in maize (*Zea mays* L.)

J. Soukup, M. Jursík, P. Hamouz, J. Holec, J. Krupka

Czech University of Agriculture in Prague, Czech Republic

ABSTRACT

Pot trials and field studies were carried out to describe the influence of soil pH and rainfall on the phytotoxic effect of the herbicide Merlin 750 WG (isoxaflutole) in maize. Symptoms as bleaching, and root and shoot weight reduction in early growth of maize were found. In pot trials, a statistically significant crop injury in early growth of maize was found only at the herbicide dose of 100 and 130 g/ha followed by 30 mm precipitation directly after herbicide application in soils with pH 6.5 and 7.2. Bleaching and significant weight reduction of maize up to growth stage BBCH 13 were observed in field trials at treatments with early post-emergence application of Merlin and 20 mm precipitations. Bleaching symptoms recovered up to BBCH 19. Significant differences were found in maize shoot weight and cob yield between treatments with and without watering (20 mm irrigation), and between pre- and post-emergence application of Merlin under field conditions. No significant differences were found between herbicide doses tested.

Keywords: herbicide; isoxaflutole; phytotoxicity; crop injury; maize; pH, rainfall

Merlin is a pre-emergence maize herbicide which controls both grass and broadleaf weeds (Hornuf et al. 1998). Greenhouse and field experiments to determine the response of maize and weeds to herbicide RPA 201772 (IFT code before registration) were conducted by Bhowmik et al. (1999). The weeds were controlled depending on dose – velvetleaf (*Abutilon theophrasti*) was the most susceptible, followed by common lambsquarters (*Chenopodium album*), large crabgrass (*Digitaria sanguinalis*), barnyardgrass (*Echinochloa crus-galli*), and yellow foxtail (*Setaria glauca*). The bleaching injury to maize less than 10% were found in the field. This injury was temporary and plants recovered within 2 to 3 weeks. $GR_{(80)}$ value was found 435 g/ha in the greenhouse. In experiences of Hornuf and Souche (2000) Merlin was selective until BBCH 12 stage of the maize. Occasionally visible chloroses regrew rapidly and had no negative yield impact. They warn that Merlin cannot be used in tank mixtures with EC-formulations. Very good efficacy of Merlin against the newly spreading weed the prickly lettuce (*Lactuca serriola*) in the Czech Republic was found by Mikulka and Chodová (2003).

Active ingredient isoxaflutole [(5-cyclopropyl-1, 2-oxazol-4-yl alpha alpha alpha-trifluoro-2-mesyl-p-tolyl ketone, (IFT)] described by Luscombe et al. (1995) and Créange et al. (1998) belongs to a new family of herbicides referred to as isoxazoles. The herbicide causes the inhibition of important enzyme hydroxyphenyl-pyruvate deshydrogenase (HPPD). With trough inhibition this enzyme follows the destruction of chlorophylle and chloroplasts in weeds. This new mode of action has good prospects from the point of view of herbicide resistance in maize growing weeds (Créange et al. 1998, Mikulka and Chodová 2000).

IFT undergoes rapid conversion to herbicide active diketonitrile derivative (DKN) in soil. Further degradation of the DKN metabolite produces a non-biologically active benzoic acid (BA) metabolite, and ultimately CO_2. The methods to determine the transport and fate of IFT and its metabolites in the environment were developed by Lin et al. (2002).

Isoxaflutole hydrolysis increased with increasing pH and temperature. The half-life of IFT is very short (< 24 h), but the half-life of DKN is much longer; hence, DKN remains for an extended time in soil. The sum of the concentrations of IFT and DKN disappear in the 0–10 cm surface soil layer, and the soil half-lives of this sum were comprised between 45 and 65 days (Rouchaud et al. 2002). In this study, metabolites were not detected in the 10–15 and 15–20 cm surface soil layers, which explain the degradation and sorption processes.

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Table 1. Experimental treatments in pot trials

Levels/ factor	Herbicide dose (g/ha)	Simulated rainfall (mm)	pH
1	70	0	5.5
2	100	10 (immediately after herbicide application)	6.5
3	130	30 (immediately after herbicide application)	7.2
4		30 (t maize emergence)	

Chemical degradation of isoxaflutole into diketonitrile was studied by Beltran et al. (2000, 2001). In organic buffers at different pH values was found the dependence on pH and temperature. Isoxaflutole hydrolysis increased with increasing pH and temperature: at pH 9.3 the rate of degradation was 100-fold faster than at pH 3.8, but the degradation of isoxaflutole in organic buffers (RCOOH/RCOONa) was considerably faster than in inorganic ones, depending on the number and the position of the CH_4 groups as the substituents. DKN, is more polar than IFT, and considerably more stable. It binds tightly to soils with higher organic matter content and greater proportion of DKN was retained by those soils. Sorption and desorption studies were conducted by Mitra et al. (1999–2001) in different soil varying in chemical and physical properties. Isotherms of isoxaflutole using Freundlich's model were non-linear in all the soils, indicating differential distribution of sorption site energies in various soils. Sorption of IFT decreased as the soil pH increased from 4.5 to 8.5, which was depicted by the reduction of K_F values. Clay content of the soils did not have a high correlation with K_F. The sorption of isoxaflutole was not influenced by the Ca^{2+} concentration in the soil solution. A high correlation between the desorption coefficient and the organic matter content of soils for desorption was found. While the clay content did not greatly influence the desorption of DKN. Although the sorption of DKN was generally reversible, a sorption-desorption hysteresis was apparent in all soils.

During the registration field studies conducted at our department and also after registration of herbicide Merlin in the Czech Republic, the crop injury (bleaching and growth retardation) in some cases occurred. The aim of this study is to specify the effect of risk factors, which can influence the

Table 2. Experimental treatments in field trials

Treatment	Herbicide	Dosage (g/ha)	Active ingredient	Dose of a.i. (g/ha)	Timing	Simulated rainfall (mm)
1	Trophy + Gesaprim 90 WG	2.5 l/ha + 1.5 l/ha	acetochlor + dichlormid + atrazine	1920 + 320 + 750	PRE	–
2	Merlin 750 WG	100	isoxaflutole	75	PRE	–
3	Merlin 750 WG	130	isoxaflutole	97.5	PRE	–
4	Merlin 750 WG	100	isoxaflutole	75	POST	–
5	Merlin 750 WG	130	isoxaflutole	97.5	POST	–
6	Trophy + Gesaprim 90 WG	2.0 l/ha + 1.5 l/ha	acetochlor + dichlormid + atrazine	1536 + 256 + 750	POST	–
7	Merlin 750 WG	100	isoxaflutole	75	PRE	20 mm
8	Merlin 750 WG	130	isoxaflutole	97.5	PRE	20 mm
9	Merlin 750 WG	100	isoxaflutole	75	POST	20 mm
10	Merlin 750 WG	130	isoxaflutole	97.5	POST	20 mm
11	control plot		untreated	–	–	–

Table 3. Weather conditions during field experiments

Month		4.	5.	6.	7.	8.	9.
Precipitations (mm)	1961–1990	38.8	72.1	75.0	71.1	83.1	50.0
	2001	64.7	36.6	80.4	90.2	134.4	127.9
	2002	37.1	15.2	58.8	73.1	108.6	48.7
Temperatures (°C)	1961–1990	8.4	13.5	16.7	18.1	17.6	13.9
	2001	7.7	15.2	15.1	18.9	18.7	12.4
	2002	8.8	17.0	18.4	19.9	20.1	12.9

phytotoxicity level resulting in crop injury and (or) yield decrease.

MATERIAL AND METHODS

The experiments were carried out during 2000 and 2001 in pots. Obtained data were verified and completed in 2001 and 2002 in field experiments (Tables 1–6).

Pot experiments. The pot experiments were carried out to verify preliminary the influence of all tested factors together in one testing site. Cambisol with pH 5.5, clay content 24.5%, C_{ox} 3.6%, KVK 154 mmol(+).kg was used. Nutrient content was 31 ppm P, 321 ppm K, 1166 ppm Ca, and 86 ppm Mg. The pots were 14 × 14 × 14 cm large, filled with homogenized soil, prepared 3 weeks before start of experiment on different pH using $CaCO_3$. In every pot 6 maize seeds (Dumas) were sowed at 4 cm depth. Five plants were retaining in each pot after emergence. Herbicide Merlin 750 WG (75% isoxaflutole, Aventis Crop Science) was used. The herbicide treatment was carried out pre-mergence with laboratory sprayer, using the Lurmark nozzles. After the treatment the pots were placed in greenhouse. The rainfall simulation was made using a laboratory sprayer (Table 1). The following irrigation was equal for all pots, according to plant requirements. Total water amount used during the experiment was 62 mm.

Experimental evaluations. Injury symptoms according to EPPO 135 (SRS, 1997). Root and shoot fresh weight of 5 plants/pot at maize growth stage BBCH 13.

Field trials. Because a high injury level was found in the pot trial, if neutral and basic soils were used, hence, such experimental fields were selected in Central Bohemia, Kraliky for the field studies. Altitude was 278 m above sea. The soil type was luvisol, clay content 36%, soil pH (KCl) 6.3, KVK 233 mmol(+). Nutrient content was 48 ppm P, 273 ppm K, 280 ppm Mg, 2360 ppm Ca. There were multi-factorial field trials carried out in 2001 and 2002. The plots were in randomised blocks, with four repetitions, each plot 21 m^2 (3.5 × 6 m). Commercial herbicides were used. Tank-mix of Trophy (768 g/l acetochlor, 128 g/l dichlormid, from Dow AgroSciences) and Gesaprim 90 WG (900 g/kg atrazine, from Novartis) was used as common standard treatment. The isoxaflutole treatments were made with Merlin 750 WG (750 g/kg isoxaflutole, Aventis). The treatments (Table 2) were carried out with small plot sprayer ZEMS (Domanínek, CZ). Applied water volume was 300 l/ha; nozzles Lurmark, application pressure 2.0 MPa. The maize was sowed in Mai 9 (2001) respectively April 26 (2002). Pre-emergence herbicide application was made immediately after sowing. The early post-emergence herbicide application was made at the growth stage BBCH 10-11, 16 days (2001), and 14 days (2002) after sowing. The irrigation dose was applied by watering can during one day immediately after herbicide treatment for both pre- and post-timings. Natural precipitations were recorded (Table 4). The weed control was well on all treated plots; the weed competition did not influence the experiment.

Experimental evaluations. Phytotoxicity (bleaching) in early growth of maize (in %), according

Table 4. Natural precipitations within 4 decades after pre- emergence herbicide treatment

Year	DAT 1–10 (mm)	DAT 11–20 (mm)	DAT 21–30 (mm)	DAT 31–40 (mm)
2001	5.1	7.5	23.0	46.8
2002	5.3	0.5	0.7	9.5

DAT = days after treatment

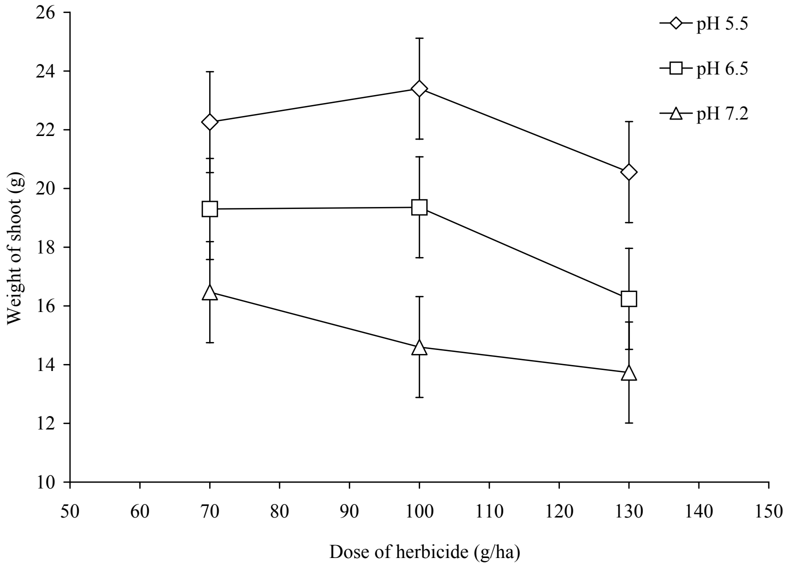

Figure 1. Maize shoot weight at BBCH 13 in pot trial (interactions with herbicide dose and pH)

to EPPO 135. Root and shoot fresh weight in early growth of maize (BBCH 13) – each plot had 8 plants. Shoot and cob weight at the time of maize harvest (BBCH 85) – each plot had 8 plants. Experimental data were evaluated by software-package Statgraphics Plus using Analysis of Variance. The contrasts between treatments were tested by the Tukey-test.

RESULTS AND DISCUSSION

In pot trials, the crop injury was found especially in soils with pH 6.5 and 7.2, and by 30 mm watering. Increased dosage of the herbicide caused the decrease in maize shoot weight (Figure 1, Table 5). The shoot weights after the highest dose of isoxaflutole differs significantly from both lower 75.0, and 52.5 g/ha doses. The root weight decreased significantly at doses 75 g/ha; further dose increase had no significant influence. The watering influenced most distinct all assessed characteristics. The maize was very sensitive to volume and timing of watering. The irrigation with 30 mm water immediately after herbicide treatment caused strong shoot retardation, which differs significant from treatments with a lower amount of watering or later application. Similar effect was found at the roots,

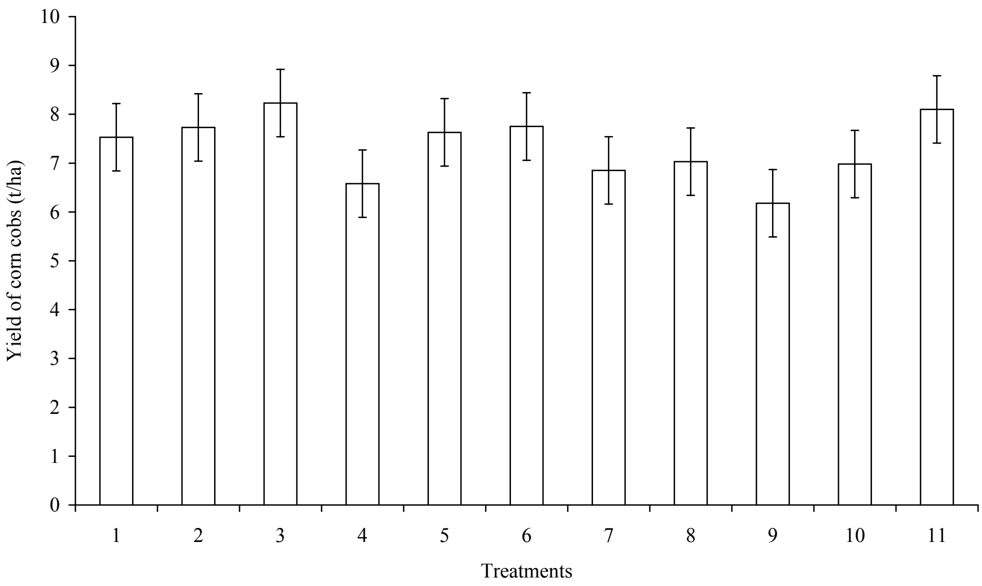

Figure 2. Yield of corn cobs in field trial

Table 5. Maize injury in pot trials at BBCH 13

		Weight of shoots (g/pot)		Weight of roots (g/pot)		Injury (%)		Number of injured plants	
		mean	homogenous groups	mean	homogenous groups	mean	homogenous groups	mean	homogenous groups
Dosage	70	19.34	B	14.41	B	2.73	A	0.71	A
	100	19.12	B	13.06	A B	6.32	A B	1.13	A B
	130	16.84	A	13.93	A B	8.45	B	1.52	B
	D_{min} ($\alpha = 0.05$)	1.64		1.32		3.76		0.47	
Rainfall	0	18.36	A B	14.21	B	0.56	A	0.22	A
	10	19.98	B	16.00	C	1.85	A	0.61	A B
	30	16.64	A	11.37	A	17.53	B	2.75	C
	30 POST	18.76	B	13.65	B	3.39	A	0.89	B
	D_{min} ($\alpha = 0.05$)	2.08		1.67		4.78		0.60	
pH	5.5	22.07	C	14.65	B	2.77	A	0.58	A
	6.5	18.30	B	14.10	B	9.08	B	1.50	B
	7.2	14.93	A	12.65	A	5.66	A B	1.27	B
	D_{min} ($\alpha = 0.05$)	1.64		1.32		3.76		0.47	

where the application of 30 mm water resulted in decreasing weight, that significant differs from all others watering doses and timings. Watering influenced the degree (%) of maize injury very strongly. The 30 mm water dose applied immediately after herbicide application differs from all others water treatments. Later watering at the growth stage of maize emergence had no more phytotoxic effect and was comparable to the treatment without watering. The reason probably is the transport of isoxaflutole from the soil surface into the sphere of maize seeds germination and they are its de-

Table 6. Maize shoot weight and yield in field study

Treatment	Treatment level	Weight of shoots BBCH 13			Yield of corn cobs		
		g	compared to untreated (%)	compared to untreated homogenous groups	t/ha	compared to untreated (%)	compared to untreated homogenous groups
Untreated		27.98	100.00		7.48	100.00	
Dosage	100	26.75	95.54	A	6.83	91.08	A
	130	21.91	78.23	A	7.46	99.50	B
Rainfall	0	29.31	104.68	B	7.54	100.50	B
	20	19.34	69.09	A	6.76	90.08	A
Timing	PRE	28.41	101.47	B	7.46	99.42	B
	POST	20.25	72.30	A	6.84	91.17	A
D_{min} ($\alpha = 0.05$)			19.91			5.46	

composition into herbicide active diketonitrile. A high concentration of DKN close to germinating seeds and first roots lead to a fast increase of DNK concentration within maize plants. Fast translocation trough xylem part of vascular system into leaf margins and tips (Creange et al. 1998) can cause maize plants to not be able to metabolise these high concentrations sufficiently. Slow activity of the degradation enzyme, e.g. P-450 monoxigenase (Hock et al. 1995) in young maize plants can lead to inhibition its HPPD and bleaching symptoms. If the inhibition remains longer, a weight reduction in early growth appears. Soil reaction influenced significantly the shoot weight, which decreased according to increasing pH. Observed differences were significant between all different soil pH tested. Reduction of root weight was found with increased pH value as well, but significant differences were found only between pH 7.2 and both lower pH values tested. Highest injury (in %) was found likewise at pH 6.5 and 7.2. The higher phytotoxicity can be due to the lower sorption of isoxaflutole in the soil. In the results of Mitra et al. (1999) there is a high correlation between soil pH and sorption – sorption of isoxaflutole decreased as the soil pH increased from 4.5 to 8.5. Desorption of IFT at higher pH values followed its hydrolysis and the increased availability of DKN for maize plants can be the reason of phytotoxicity in our experiments.

Under field conditions, the phytotoxicity after irrigation was seen in both experimental years. Bleaching symptoms on maize leaves as in the studies of Bhowmik et al. (1999) was recorded in early growth of the maize. The yellow coloration remains on leaf borders, and the oldest leaves had a reduction in growth. Significant differences were found in shoot weight at the growth stage BBCH 13 (Table 6). The watering in the amount of 20 mm after herbicide treatment caused the shoot reduction by 30%, as well as the post-emergence compared to pre-emergence application. No significant differences were found between herbicide dosages used. The bleaching on injured plants persists up to growth stage BBCH 18–19 for the period of 4–6 weeks. At harvest, the best corn cob yield was achieved on handy weeded plots, and early post-emergence applied atrazine + acetochlor (Figure 2). The isoxaflutole treatments varied in yield. Higher doses of herbicide (130 g/ha) influenced positively the yield of corn cobs but significant differences were found between pre- and post-emergence application, and also between treatments with and without watering. Post-emergence application caused a higher phytotoxicity. The post-emergence application of isoxaflutole is not registered in the Czech Republic, but the probability of maize injury is very high in its practice, because the maize emerges quickly and the herbicides are often applied later as recommended.

In both pot and field studies was the phytotoxicity of isoxaflutole obtained under specific experimental conditions. These results documented show expected risk of crop injury, when isoxaflutole containing herbicides are used in neutral and basic soils, and/or under conditions that accelerate both desorption and transport processes (high soil moisture, low organic matter content). To eliminate the risk of injury, the dose from 50 to 75 g/ha isoxaflutole is recommend in these areas, combining with other herbicide partners to force the herbicide activity at those lower doses.

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ABSTRAKT

Vliv pH půdy, srážek, dávkování a termínu aplikace na úroveň fytotoxicity herbicidu Merlin 750 WG (isoxaflutol) v kukuřici

V nádobových a polních pokusech byl sledován vliv pH a srážek na fytotoxicitu herbicidu Merlin 750 WG (isoxaflutol) v kukuřici. Byly zjištěny symptomy poškození, které se projevovalo vybělením listů a poklesem hmotnosti kořenů a nadzemní hmoty v raných růstových fázích kukuřice. V nádobových pokusech bylo poškození pozorováno při použití dávky 100 a 130 g/ha, pokud následovala po aplikaci herbicidu závlaha ve výši 30 mm při pH půdy 6.5 a 7.2. Vybělení listů a redukce hmotnosti rostlin byla pozorována v polních pokusech při časně postemergentní aplikaci a závlahové dávce 20 mm. Symptomy vybělení odezněly do fáze BBCH 19. Statisticky významné rozdíly byly zjištěny v polních podmínkách v hmotnosti nadzemní biomasy a ve výnosu palic mezi zavlažovanými a nezavlažovanými parcelami a mezi pre- a postemergentní aplikací. Mezi testovanými dávkami herbicidu nebyl shledán statisticky významný rozdíl.

Klíčová slova: herbicid; isoxaflutole; fytotoxicita; poškození plodiny; kukuřice; pH; srážky

Corresponding author:

Ing. Josef Soukup, CSc., Česká zemědělská univerzita v Praze, 165 21 Praha 6-Suchdol, Česká republika
phone: + 420 224 382 780, fax: + 420 224 382 535, e-mail: soukup@af.czu.cz