



**Full Length Article**

## Barnyardgrass Shows Sensitivity to Reduced Doses of Topramezone at Different Growth Stages

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### Abstract

Using less than the labelled dose of herbicides depends mostly on weed spectrum and growth stage of target weeds. The aim of the greenhouse experiment was to determine the efficacy of recommended (67.2 g a.i. ha<sup>-1</sup>) and reduced doses (44.8, 33.6, 22.4, 16.8 and 8.4 g a.i. ha<sup>-1</sup>) of topramezone combined with adjuvants (NIS and MSO) on barnyardgrass (*Echinochloa crus-galli* [L.] P. Beauv.) at two different growth stages: BBCH 13 and BBCH 21–22. The results showed unsatisfactory efficacy of topramezone applied alone at the recommended dose on both growth stages (>50%). When applied with adjuvants (MSO or NIS) at BBCH 13, topramezone 100% visually damaged barnyardgrass and reduced biomass >90%, at each applied dose. In contrast, at BBCH 21–22 only topramezone double dose achieved 73% visual damage. Reduced topramezone doses applied with adjuvants gave insufficient barnyardgrass control at tillering stage (< 40%). Results indicate the possibility of reducing the topramezone dose to more than 87% by adding adjuvants (MSO or NIS) but only when applied at early growth stage of barnyardgrass (BBCH 13). At BBCH 21–22 satisfactory barnyardgrass control cannot be achieved by using topramezone either alone or with adjuvants. © 2019 Friends Science Publishers

**Keywords:** Reduce dose; Adjuvants; Growth stage; Bleaching; Three leaves; Tillering

### Introduction

Reduced dose of some post-emergence herbicides (POST) can effectively control weeds in row crops. Doses reduced 50 to 80% of the rate recommended by manufacturers have already been applied in maize on more than 50% or 80% of areas in the Netherlands, Denmark, Germany and France (Meissle *et al.*, 2010). Depending on the weed spectrum to be controlled, it is important to keep efficacy close to 100% in order to avoid the selection of resistant traits (Kudsk, 2008). In Croatia, reduced dose of herbicide gave satisfactory results in maize and sugar beet (Galzina *et al.*, 2007; Goršić *et al.*, 2008; Šćepanović *et al.*, 2011) but always when applied with adjuvants in the tank mix and in early weed growth stage. Most adjuvants used are surfactants (NIS) and methylated seed oils (MSO) but selecting an appropriate adjuvant depends mostly on physicochemical characteristics of the specific herbicide. Furthermore, for an effective POST control a proper application time is fundamental. Weeds at a more advanced growth stage can become less susceptible to herbicides and are not controlled, causing a negative effect on crop yield.

Barnyardgrass is the most frequent monocotyledon

weed species in 91% maize fields in Croatia (Šarić *et al.*, 2011) and rice fields in Asia (Irshad *et al.*, 2008). Maximum maize yield loss ranged from 26 to 50% depending on barnyardgrass density and crop growth stage (Kropff *et al.*, 1984; Bosnic and Swanton, 1997). In POST application, using reduced doses is likely to miss the effective control of grasses at later growth stage. Poor to excellent barnyardgrass control were recorded in previous maize field researches, using a reduced dose of the newly developed herbicide topramezone (Goršić *et al.*, 2008; Šoštarić *et al.*, 2015; Liu *et al.*, 2017) applied at different growth stages and different adjuvants. Topramezone is an HPPD-inhibiting herbicide indirectly inhibiting carotenoid biosynthesis in susceptible plants. Very different efficacy on barnyardgrass was recorded with topramezone at a rate of 50 g a.i. ha<sup>-1</sup> tank-mixed with MSO. In the location where barnyardgrass had 4–6 leaves at application, efficacy was 100%, contrary to the location with adult plants where efficacy was only 48% (Goršić *et al.*, 2008).

Although the best efficacy can be expected when herbicides are applied at early growth stages, in field conditions plants are very rarely at the same development

stage. Knowing herbicide efficacy at different growth stages is therefore extremely important when farmers use a reduced dose strategy. The study was to evaluate the efficacy of herbicide topramezone in various rates with and without adjuvants on two different growth stages of barnyardgrass.

## Material and Methods

### Plant Material

Seeds of barnyardgrass (HPPD sensitive) were hand-collected in September 2013 on the experimental station of the Faculty of Agriculture, University of Zagreb, Sasinovecki Lug, Croatia (45°52'N, 16°10'E).

### Laboratory and Greenhouse Experiment

Experiment was conducted in 2014, at the experimental farm of the University of Padova, Legnaro, Italy (45°21'N, 11°58'E). In order to stimulate uniform germination, seeds were soaked in concentrated sulfuric acid (90%) for 15 min and rinsed with deionized water. Seeds were then planted in plastic pots (10 × 7 cm) containing humus substrate. As soon as seedlings had one fully developed true leaf they were transplanted into plastic pots (15 × 15 × 10 cm) filled with a standard potting mix (60% silty loam soil, 15% sand, 15% perlite, 10% peat) and placed in an outdoor greenhouse covered with a plastic net. The experimental unit was one pot with five seedlings. Topramezone (CLIO® 33.6 SC; BASF) and adjuvants (DASH® HC, BREAK® THRU; BASF) were applied using a precision bench sprayer with a boom equipped with three flat-fan (extended range) hydraulic nozzles with dose spray volume of 300 L ha<sup>-1</sup>, pressure of 215 kPa, speed of 0.75 m s<sup>-1</sup> and using TeeJet® nozzles XR11002-VK. Topramezone was applied alone at seven different doses including increased (134.4 g a.i. ha<sup>-1</sup>), recommended (67.2 g a.i. ha<sup>-1</sup>) and reduced doses (44.8, 33.6, 22.4, 16.8, 8.4 g a.i. ha<sup>-1</sup>) and the same doses were tanked-mix with two adjuvants MSO at 0.25% (v/v) and NIS at 0.1% (v/v). Each dose was applied with just one adjuvant. Adjuvants were also applied without topramezone and an untreated control was included in experiment. Application times were at the three-leaves stage (BBCH 13) (10 July) and one-two tiller stage (BBCH 21–22) (23 July) of barnyardgrass.

### Measurements

Spray mixture pH was measured for each treatment using a Beckham pH meter to determine the influence of topramezone alone and in combination with MSO or NIS on the pH of the herbicide solution.

Barnyardgrass plants were visually evaluated for percentage of damage at 3, 7, 14 days after treatment (DAT) at BBCH 13, and 3, 7, 14, 21, 26 DAT at BBCH 21–22. Aboveground barnyardgrass biomass was harvested from each pot at 14 DAT for BBCH 13 and 26 DAT for BBCH 21–22 and then dried at 105°C for 36 h.

### Statistical Analysis

Analysis of variance was used to study the influence of herbicide and adjuvant treatments and was performed for visual evaluation and biomass reduction of barnyardgrass plants. Data obtained were subjected to ANOVA, and the Fisher's LSD test ( $P > 0.05$ ) was calculated to detect differences between treatment means (SAS Institute, 1997).

### Weather Data

Since the experiment was conducted in an outdoor greenhouse covered by permeable mesh, minimum and maximum mean temperature, mean precipitation and relative humidity values were recorded for the period after herbicide application for each barnyardgrass growth stage (Table 1).

## Results

### Solution pH

Herbicide solution at all studied doses and combinations was about neutral, ranging from pH 6.14 to 7.70 depending on the rate and adjuvant. Decreasing herbicide solution pH was observed with increasing of topramezone dose (7.33 – 6.72), in almost all cases (Table 2). Moreover, lower pH of the solution was observed in all combinations with MSO (6.14 – 6.63) when compared to topramezone applied alone (6.72 – 7.33) or in combination with NIS (6.86 – 7.70).

### Visual Damage of Barnyardgrass at BBCH 13 and 21–22

Treatment-by-dose interaction was detected ( $P < 0.05$ ) for all herbicide and adjuvant combinations at both growth stages (data not shown). Visual assessments always showed progression of the symptoms with increasing DAT in plants at BBCH 13, while at BBCH 21–22 even some regression of symptoms was detected (Table 3). At BBCH 13 some initial symptoms were observed at first assessment (3 DAT), with a maximum of 32% bleached plants when the lowest topramezone dose (1/8R) was applied with MSO. At BBCH 21–22, plants also showed some phytotoxic symptoms 3 DAT (data not shown), although in some treatments lack of efficacy was observed even 26 DAT. Generally, at both barnyardgrass growth stages, phytotoxicity declined with the decreasing amount of a.i., applied without adjuvants. Moreover, topramezone showed completely lack of efficacy with recommended and reduced doses (> 67.2 g a.i.) even applied at BBCH 13. So, only topramezone applied alone at double dose (134 g a.i.) achieved 95% barnyardgrass reduction. At BBCH 21–22 even a double dose of topramezone did not bleach barnyardgrass (73%) significantly. At BBCH 21–22 the addition of adjuvants

**Table 1:** Minimum and maximum mean temperature, precipitation and relative humidity after herbicide application at greenhouse

Growth stage	Period after herbicide applications	Temperature (°C)		Rainfall (mm)	Humidity (%)	
		min	max		min	max
BBCH 13	10/7 -24/7 2014	17.3	28.2	4.6	42.3	99.4
BBCH 21–22	23/7 – 20/8 2014	17.6	27.6	4.6	49.4	99.8

**Table 2:** pH values of spray mixtures

Adjuvants	Topramezone (g a.i. ha <sup>-1</sup> )							
	0	8.4	16.8	22.4	33.6	44.8	67.2	134.4
-	7.32	7.33	7.24	7.18	7.12	7.07	6.96	6.72
MSO	6.14	6.63	6.56	6.55	6.52	6.48	6.50	6.38
NIS	7.70	7.48	7.41	7.33	7.25	7.23	7.06	6.86

**Table 3:** Visual bleaching of barnyardgrass at BBCH 13 and BBCH 21-22

Treatments	Application rate (g. a. i. ha <sup>-1</sup> )	Visual bleaching (VB)					
		BBCH 13		BBCH 21-22			
		3 DAT <sup>a</sup>	7 DAT	14 DAT	14 DAT	21 DAT	26 DAT
		-----%					
Untreated		0 e	0 f	0 e	0 h	0 h	0 g
MSO <sup>b</sup>		0 e	0 f	0 e	0 h	0 h	0 g
NIS <sup>c</sup>		0 e	0 f	0 e	0 h	0 h	0 g
2 R <sup>d</sup>	134.4	5 de	92.5 b	95 b	30 b	10 g	73.3 a
topramezone		20 b	100 a	100 a	40 a	60 ab	73.3 a
topramezone + MSO		6.25 d	95 ab	100 a	33.3 b	55 bc	73.3 a
topramezone + NIS							
R	67.2	2.5 de	47.5 d	48.7 c	10 e	50 cd	10 e
topramezone		20 b	100 a	100 a	30 b	40 e	60 b
topramezone + MSO		5 de	90 b	100 a	40 a	66.7 a	73.3 a
topramezone + NIS							
2/3 R	44.8	0 e	0 f	0 e	6.7 ef	1.6 h	3.3 fg
topramezone		13.7 c	100 a	100 a	30 b	43.3 de	40 c
topramezone + MSO		6.25 d	95 ab	100 a	20 c	10 g	20 d
topramezone + NIS							
1/2 R	33.6	7.5 d	13.7 e	32.5 d	0 h	1.7 h	3.3 fg
topramezone		3.5 de	100 a	100 a	20 c	33.4 f	20 d
topramezone + MSO		5 de	90 b	100 a	15 d	5 gh	0 g
topramezone + NIS							
1/3 R	22.4	0 e	7.5 e	0 e	0 h	0 h	0 g
topramezone		13.7 c	100 a	100 a	21.7 c	10 g	5 ef
topramezone + MSO		5 de	90 b	100 a	5 fg	0 h	0 g
topramezone + NIS							
1/4 R	16.8	0 e	0 f	0 e	0 h	0 h	0 g
topramezone		18.7 bc	100 a	100 a	10 e	10 g	10 e
topramezone + MSO		6 d	90 b	100 a	3.3 b	1.7 h	0 g
topramezone + NIS							
1/8 R	8.4	0 e	0 f	0 e	0 h	0 h	0 g
topramezone		32.5 a	100 a	100 a	0 h	0 h	10 e
topramezone + MSO		7.5 d	82.5 c	100 a	0 h	0 h	0 g
topramezone + NIS							

<sup>a</sup>Abbreviation: DAT, days after treatment<sup>b</sup>Methylated seed oil (MSO) at 0.25% (v/v)<sup>c</sup>Nonionic surfactant (NIS) at 0.1% (v/v)<sup>d</sup>R – the producer recommended dose (67.2 g. a. i. ha<sup>-1</sup>)

Means followed by the same letter within a column are not significantly different according to Fisher's protected LSD at P, 0.05:

BBCH 13: LSD<sub>3DAT</sub> = 5.13; LSD<sub>7DAT</sub> = 6.85; LSD<sub>14DAT</sub> = 2.16BBCH 21-22: LSD<sub>14DAT</sub> = 4.26; LSD<sub>21DAT</sub> = 7.33; LSD<sub>26DAT</sub> = 4.18

also did not improve control, with a maximum of 73% bleached plants (2R + adjuvants and R + NIS).

On the contrary, addition of adjuvants to topramezone significantly improved control at BBCH 13. All applications with adjuvants achieved excellent efficacy on barnyardgrass (100%) 14 DAT, regardless of the herbicide rate and type of adjuvant.

### Biomass Reduction

Biomass reduction data support the visual control observations at 14 (BBCH 13) and 26 DAT (BBCH 21–22)

and suggest a similar phytotoxic level. Excellent barnyardgrass dry weight reduction at BBCH 13 was achieved only when topramezone was applied with adjuvants, regardless of topramezone rate or type of adjuvant. When applied alone, only double topramezone dose achieved 86.6% biomass reduction, all other applied doses gave very poor biomass reduction at both growth stages (Table 4). At recommended dose applied without adjuvants topramezone gave only 45.4% barnyardgrass reduction.

Barnyardgrass biomass reduction at treatments applied with adjuvant ranged from 91.5 to 96.7% at BBCH 13.

**Table 4:** Biomass dry weight reduction of barnyardgrass 14 DAT at BBCH 13 and 26 DAT at BBCH 21–22

Treatments	Application rate (g a.i. ha <sup>-1</sup> )	Dry weight (%)	
		BBCH 13	BBCH 21-22
Untreated	-	0.0 e	0.0 d
MSO <sup>a</sup>		0.0 e	0.0 d
NIS <sup>b</sup>		12.3 d	0.0 d
2 R <sup>c</sup> Topramezone	134.4	86.6 b	61.0 a
topramezone + MSO		95.6 a	62.3 a
topramezone + NIS		96.7 a	55.7 a
R topramezone	67.2	45.4 c	14.9 bc
topramezone + MSO		93.9 a	19.8 b
topramezone + NIS		93.9 a	55.7 a
2/3 R topramezone	44.8	2.0 e	0.0 d
topramezone + MSO		95.1 a	19.1 b
topramezone + NIS		94.3 a	7.4 bc
1/2 R topramezone	33.6	0.4 e	0.0 d
topramezone + MSO		95.0 a	17.3 bc
topramezone + NIS		92.9 a	0.0 d
1/3 R topramezone	22.4	0.0 e	0.0 d
topramezone + MSO		96.3 a	23.6 b
topramezone + NIS		94.5 a	0.0 d
1/4 R topramezone	16.8	0.0 e	0.0 d
topramezone + MSO		96.5 a	1.7 cd
topramezone + NIS		91.4 a	0.0 d
1/8 R topramezone	8.4	0.0 e	0.0 d
topramezone + MSO		94.9 a	0.0 d
topramezone + NIS		94.4 a	0.0 d

<sup>a</sup>Methylated seed oil (MSO) at 0.25% (v/v)

<sup>b</sup>Nonionic surfactant (NIS) at 0.1% (v/v)

<sup>c</sup>R – manufacturer's recommended dose (67.2 g a.i. ha<sup>-1</sup>)

Means followed by the same letter within a column are not significantly different according to Fisher's protected LSD at P, 0.05 –

LSD<sub>BBCH 13</sub> = 5.33; LSD<sub>BBCH 21-22</sub> = 16.25

Instead, efficacy on barnyardgrass biomass reduction was poor at BBCH 21–22.

## Discussion

Topramezone application at BBCH 13 provided adequate control of barnyardgrass only by adding MSO or NIS. Even at the recommended dose topramezone applied alone could not provide adequate control of this weed species. The latter had also been found in the field experiment of Šoštarčić *et al.* (2015) where recommended topramezone dose applied alone showed only 17.5% efficacy on barnyardgrass. This supports the manufacturer's recommendation to apply topramezone always in combination with an adjuvant. Moreover, when topramezone was used alone at the BBCH 21-22, only double dose provided good efficacy. The effect of weed growth stage on herbicide efficacy is documented in the literature. A studied population of *Echinochloa crus-galli* sp. showed highest sensitivity to topramezone at BBCH 13, with efficacy decreasing even at BBCH 14 (Cauwer *et al.*, 2012). Decreased sensitivity of *Panicum* sp. to topramezone with increasing number of true leaves was also reported. Cauwer *et al.* (2014) noted that plants were two to eleven fold less sensitive to topramezone when treated at BBCH 14 than at BBCH 11 or 12. Lack of efficacy could be due to: plant morphology and physiology

(reduced herbicide adsorption and translocation), physiochemical properties of the a.i., ability of grasses to regenerate. For example, Brosnan *et al.* (2011) determinate mechanism allowing *Cynodon dactylon* to recover from topramezone or tembotrione injury by increasing concentration of zeaxanthin β-antheraxanthin in the total xanthophyll pigment pool. Still, prior to tissue regeneration, the a.i. has to be absorbed and translocated into plant tissue. Herbicide absorption depends mostly on plant morphology and physiochemical properties of the a.i. Leaves of barnyardgrass are covered with epicuticular wax whose content increases with the growth stage of the plant. The amount of waxes prevents leaf wetting because of lipophilic properties of the cuticle structure (McWhorter, 1993). If it is taken into consideration that the reduced amount of applied herbicide after absorption could perhaps not be successfully translocated to all plant leaves, low efficacy at decreased herbicide doses can be explained, as shown in present research. However, reaching the herbicide target could be increased by adding adjuvants to the spray mixture, as the results of this experiment show except in the case of tillering stage. Comparing two types of adjuvants, MSO enhanced faster development of symptoms, which is connected to adjuvant properties. MSO has lipophilic components enabling its faster penetration to lipophilic leaf cuticle as well as lowering the drop tension (Xu *et al.*, 2010). NIS instead provides only decreasing of the contact angle between droplet and leaf but without enabling easier passage through the lipophilic cuticle (Ryckaert *et al.*, 2007). Moreover, results showed decreasing pH in the herbicide solution when topramezone is combined with the MSO which could result with better absorption of a.i. (Table 2). The results of relevant research studies on weak acid herbicides and their behaviour in different carrier water pH are rather conflicting. Grossmann and Ehrhardt (2007) and Xie *et al.* (2011) reported increased mesotrione and topramezone efficacy while using ammonium nitrate and other pH-reducing acid adjuvants. However, Liu (2002) explained herbicide (bentazone) behaviour at different pH values by taking into consideration not only herbicide pKa but also herbicide solubility. For instance, topramezone is a weak acid with 4.06 pKa at 25°C with high solubility in water at 20°C (100 000 mg/L). Bentazone used in author's research is also weak acid with 3.51 pKa at 25°C but its solubility is 7112 mg/L which is still high but lower when compared with topramezone. According to the author when an herbicide has a low concentration and solubility is not a limiting factor, uptake is greater at low pH. However, when the concentration increases and solubility is limiting, then the higher pH increases uptake. Moreover, Hartzler (2003) also reported that the reducing spray mixture pH often increases weak acid herbicide uptake, because a higher proportion of the herbicide molecules will be non-ionic and will thus diffuse faster into the cuticle. In contrary, increasing pH makes the herbicide ionic and thus more difficult to penetrate through the lipophilic cuticle. This has

greater effect at later growth stages of the plant, and on the older leaves as seen also in this study (Table 3).

## Conclusion

In conclusion, using less than the labelled dose of topramezone is only effective at BBCH 13 of barnyardgrass and only in combination with adjuvants. Considering all factors that may influence topramezone efficacy at BBCH 21-22, the use of topramezone at this growth stage is not recommended.

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