

## Effects of selective herbicide treatment on weed community in cereal crop rotation

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### ABSTRACT

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The efficacy of different frequently used selective herbicides and their impact on weed community were assessed in the field experiment with cereal crop rotation in 2013–2016. All herbicide treatments provided effective weed control, but community composition changed after 4 years. The density of many weed species increased on the untreated plots. Annual grasses *Apera spica-venti* and *Alopecurus myosuroides* had temporal increase at plots treated with 2,4-D, florasulam and aminopyralid, but decline at plots treated with pyroxsulam and/or chlorsulfuron. Significant treatment effect was confirmed only for Shannon's index ( $H'$ ), where the highest  $H'$  was found in untreated controls (1.83) and the smallest in plots treated with 2,4-D + florasulam + aminopyralid (1.61). The pyroxsulam + florasulam + aminopyralid showed the greatest efficacy on a broad spectrum of weeds while maintaining reasonable species diversity.

**Keywords:** chemical weed management; herbicide efficacy; field trial; weed diversity

Weed communities are an integral part of agroecosystems. Individual weed species differ in their ecological function and competitive ability towards the crop (Marshall et al. 2003). Besides their negative effect on crop yield and quality (Hance and Holly 1990), many weed species have an important ecological function in maintaining farmland biodiversity (Storkey and Westbury 2007). Weed species diversity has declined in agricultural landscape during recent decades mainly due to agricultural intensification (Murphy and Lemerle 2006, Storkey et al. 2012) accompanied by simplification of crop rotations (Stoate et al. 2001, Meyer 2013) and excessive herbicide and fertiliser use (Storkey et al. 2010, Salonen et al. 2013). A new approach to biodiversity-friendly weed management requires finding a balance between sufficient crop production and maintaining satisfactory species diversity

(Marshall et al. 2003). This goal is an effective control of highly competitive problem species while sustaining beneficial species at economically acceptable levels (Storkey and Westbury 2007). Studies by Ulber et al. (2010) and Jones and Smith (2007) indicate that treatment by selective herbicides can provide considerable weed control with retention of beneficial species and species diversity.

The aim of this experiment is to evaluate the efficacy of different frequently used selective herbicides and their impact on weed species diversity. Herein, we focused on weed management in winter cereal rotation because the majority of current temperate conventional cropping systems are characterised by monocultures or simple rotations with a limited number of crops. Our goal is to determine the effect of a low cost strategy using cheaper herbicides which have been

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in use for a long time and have a narrower efficacy spectrum than the modern products that contain 3 active ingredients and hence a broader efficacy spectrum. High weed control efficiency in cereal crops has been attributed to acetolactate synthase (ALS) inhibitors which target a broad spectrum of broadleaf and grass weeds (Hyvönen et al. 2003). We therefore tested selective herbicides with active ingredients in this group. Our hypothesis is that the use of herbicides with sub-optimal effect leads to a shift in hard-to-control weed species and negatively affects some parameters of species diversity.

## MATERIAL AND METHODS

**Experimental site.** The field experiment was established in 2009 in the experimental area of the Crop Research Institute in Prague-Ruzyně. The site characteristics are listed in Table 1. The trial area was split into 20 randomised plots with 5 different herbicide treatments in 4 replications. Herbicide treatments differed in the herbicide target – only dicots and dicots + annual grasses; and in intensity of weed control – narrower spectrum and broader spectrum (Table 2). The area of each plot was 100 m<sup>2</sup> and the 10 by 10 m plots were separated from field boundaries and from each other by 2 m on all sides in order to eliminate interaction between plots. Herbicides were applied post emergency in spring from the tillering crop stage to the beginning of stem elongation (BBCH 21–31) by the Agrio-Napa 12 sprayer. A sequence of winter wheat (2014–2016) and winter barley (2013) was grown in the experimental field. Barley was sown

Table 1. Experimental site characteristics

Longitude	14°30'E
Latitude	50°08'N
Altitude (m a.s.l.)	345
Average annual temperature (°C)	7.9
Average annual precipitation total (mm)	472
FAO Classification	Haplic Luvisols, on loess
pH <sub>KCl</sub>	6.3
Organic carbon	2.4
Clay content (%)	31.3

on 11 September 2012, wheat on 9 October 2013, 3 October 2014 and 12 October 2015. Uniform cropping practice was carried out in the entire study area: mouldboard ploughing, 0.20–0.25 m deep, seedbed preparation with power harrow and mineral fertilisation before sowing with 45 kg N/ha, 20 kg P/ha, 37 kg K/ha; and sowing at 350 seeds/m<sup>2</sup>. Fungicides were used before heading in case of infection pressure.

**Assessment.** Weed species composition and weed density were assessed from 2013 to 2016. Weed data was recorded in spring at the 4 to 6-leaf crop stage before herbicide application and 4 weeks after herbicide application. Individual plants in each plot were counted in five 0.25 m<sup>2</sup> random sampling squares (0.5 m by 0.5 m) and densities of these five squares were averaged and converted to 1 m<sup>2</sup> samples. The weed species composition at trial commencement is listed in Table 3. The following species prevailed in April 2013: *Papaver*

Table 2. Summary of herbicides and active ingredients used in the trial. Classification group by herbicide resistance action committee (HRAC)

Herbicide	Dose	Formulation	Active ingredient	Content of a.i.	HRAC group	Target weeds
Esteron	0.8 L/ha	EC	2,4-D	600 g/L	O	dicot, narrower spectrum
Esteron	0.8 L/ha	EC	2,4-D	600 g/L	O	annual
Glean 75 PX	15 g/ha	WG	chlorsulfuron	750 g/L	B	grasses + dicot, narrower spectrum
Mustang Forte	1 L/ha	SE	aminopyralid	10 g/L	O	dicot broader spectrum
			2,4-D	180 g/L	O	
			florasulam	5 g/L	B	
Hurricane	200 g/ha	WG	aminopyralid	50 g/L	O	annual grasses + dicot, broader spectrum
			florasulam	25 g/L	B	
			pyroxsulam	50 g/L	B	
Untreated control						

Table 3. Weed species composition assessed in April 2013

Weed species	Number of plants		(%)	
	avg.	SE	avg.	SE
<i>Papaver</i> spp. L.	52.6	22.9	30.9	17
<i>Veronica</i> spp. L.	20.1	15.5	15.1	9.7
<i>Galium aparine</i> L.	16.1	11.8	11.9	9.1
<i>Apera spica-venti</i> (L.) P. Beauv.	15.3	10.3	10.5	
<i>Stellaria media</i> (L.) Vill.	5.7	5.1	6.1	5.8
<i>Fumaria officinalis</i> L.	4.5	4.1	4.1	3.3
<i>Viola arvensis</i> Murray	3.5	2.1	2.9	2.5
<i>Thlaspi arvense</i> L.	3.3	2.5	2.7	1.9
<i>Consolida</i> spp. (DC) Gray	3.2	3	4	3.8
<i>Lamium</i> spp. L.	2.4	2.1	1.9	1.5
<i>Euphorbia helioscopia</i> L.	1.8	1.1	1.3	0.8
<i>Centaurea cyanus</i> L.	1	0.9	1.2	1
<i>Tripleurospermum inodorum</i> (L.) Schultz-Bip.	1	0.8	1.2	0.9
<i>Silene noctiflora</i> L.	1	0.9	1	1
<i>Polygonum aviculare</i> L.	< 1		< 1	
<i>Erodium cicutarium</i> (L.) L`Hér.	< 1		< 1	
<i>Sonchus arvensis</i> L.	< 1		< 1	
<i>Agrostemma githago</i> L.	< 1		< 1	
<i>Alopecurus myosuroides</i> Huds.	< 1		< 1	

Average (avg.) number of plants/m<sup>2</sup> ± standard errors (SE); proportion of individual species in the dataset (%)

spp. L., *Veronica* spp. L., *Galium aparine* L., *Apera spica-venti* (L.) P. Beauv., *Stellaria media* (L.) Vill. and *Fumaria officinalis* L. A total of 35 different species were identified over the experimental period. Weed cover (%) and herbicide efficacy (%) were estimated 4 weeks after herbicide application. Headlands and plot edges were excluded from sampling. Weed species were identified at the species level whenever possible and some species, such as *Vicia* spp. L., were identified at genus level. Botanical nomenclature followed Kubát et al. (2002).

Efficacy evaluation of herbicides was performed in EPPO (European and Mediterranean Plant Protection Organisation) standard PP1/152(4) and PP1/93(3) (Bulletin OEPP/EPPO 2012). We used the subjective estimation method which compares % weed cover at treated plots with un-

treated control and evaluates weed damage due to herbicide application. Efficacy of the studied herbicides was recorded in range 0–100% (0% – no weed control; 100% – full weed control with no weed survival). For weed species on which a lower efficacy than 90% was observed, the population density was also recorded.

Special attention was paid to weed species considered beneficial for biodiversity and those providing resources for invertebrates and seed-eating birds (Marshall et al. 2003, Storkey 2006). These are marked \* in the text.

**Statistical analysis.** Weed community species diversity was calculated for each plot repetition by Simpson's dominance ( $D = \sum_i p_i^2$ ), Shannon's diversity ( $H' = - \sum_i p_i \ln(p_i)$ ) and evenness ( $E = H/\ln S$ ) indices; where  $p_i$  is the proportion of individuals of  $i^{\text{th}}$  species in the total number of individuals ( $S$ ) in the sample quadrat (Pielou 1966). The effects of treatment and year on diversity indices, weed density and herbicide treatment efficacy were tested by ANOVA, and Tukey's honestly significant difference (*HSD*) multiple comparison test at  $\alpha = 0.05$  determined homogenous groups. Analysis was conducted in Statistica 13.3 software (TIBCO Software Inc., USA).

Multivariate data analysis in CANOCO 5 software (ter Braak and Šmilauer 2012) provided data exploration. Data logarithmic transformation preceded analyses and the optimal redundancy analysis (RDA) ordination method was used because of the gradient length on the first canonical axis in compositional turnover in detrended correspondence analysis (DCA). Year and treatment explanatory variables were compiled, and the gross effects were tested using separate RDAs with single explanatory variables. Net effects were then tested using partial RDAs with a single explanatory variable and the other variable as covariate. Both effects of explanatory variables on weed species composition were tested by Monte-Carlo permutation tests for 999 permutations at  $P = 0.05$  significance level; as in Lososová et al. (2004). Finally, the ratio of particular canonical eigenvalues to the sum of all eigenvalues measured the proportion of explained variation.

## RESULTS AND DISCUSSION

**Species diversity and weed community composition.** Three diversity indices were used to de-

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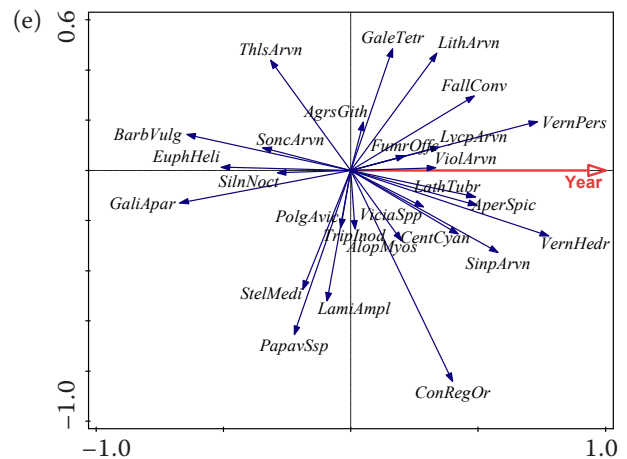
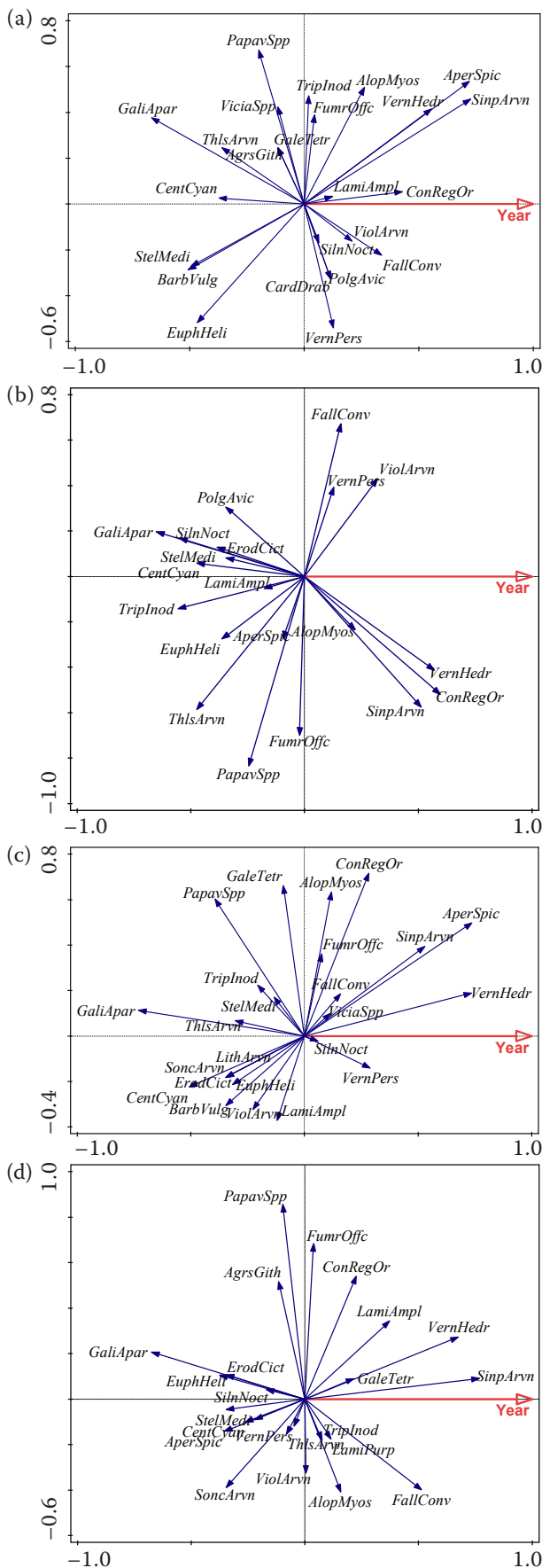


Figure 1. Ordination diagrams of partial redundancy analysis (RDA) of weed species composition in different treatments: (a) 2,4-D; (b) 2,4-D + chlorsulfuron; (c) aminopyralid + 2,4-D + florasulam; (d) aminopyralid + florasulam + pyroxsulam; (e) untreated control; year as explanatory variable and repetition as covariate. AgrsGith – *Agrostemma githago*; AlopMyos – *Alopecurus myosuroides*; AperSpic – *Apera spica-venti*; BarbVulg – *Barbarea vulgaris*; CardDrab – *Cardaria draba*; CentCyan – *Centaurea cyanus*; ConRegOr – *Consolida regia* and *C. orientalis*; ErodCict – *Erodium cicutarium*; EuphHeli – *Euphorbia helioscopia*; FallConv – *Fallopia convolvulus*; FumrOffc – *Fumaria officinalis*; GaleTetr – *Galeopsis tetrahit*; GaliApar – *Galium aparine*; LamiAmpl – *Lamium amplexicaule*; LamiPurp – *Lamium purpureum*; LathTuber – *Lathyrus tuberosus*; LithArvn – *Lithospermum arvense*; LycpArvn – *Lycopsis arvensis*; PapavSpp – *Papaver* spp.; PolgAvic – *Polygonum aviculare*; SilnNoct – *Silene noctiflorum*; SinpArvn – *Sinapis arvensis*; SoncArvn – *Sonchus arvensis*; StelMedi – *Stellaria media*; ThlsArvn – *Thlaspi arvense*; TripInod – *Tripleurospermum inodorum*; VernHedr – *Veronica hederifolia*; VernPers – *Veronica persica*; ViciaSpp – *Vicia* spp.; ViolArvn – *Viola arvensis*

scribe different diversity aspects. All indices were influenced by year, and diversity was significantly higher in 2015 and 2016 than at trial commencement (Table 4). Significant herbicide treatment effect was confirmed only by Shannon's index ( $H'$ ) where the highest  $H'$  was found in untreated control and the smallest at plots treated with 2,4-D + florasulam + aminopyralid. Other herbicide treatments showed the same effect (Table 4) and did not significantly differ compared to untreated control. Although the treatment effect was insignificant for Simpson's dominance ( $D$ )

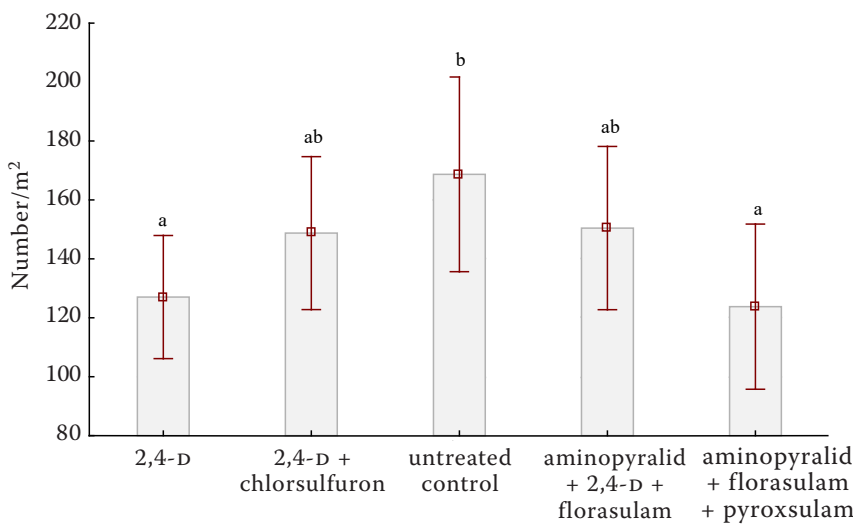


Figure 2. Effect of treatment on average weed density for 2013–2016. Different letters indicate significant differences at  $\alpha = 0.05$  by Tukey *HSD* (honestly significant difference) test, vertical columns show 0.95 confidence interval

and evenness (E) indices, treatment with 2,4-D + florasulam + aminopyralid had the highest D and the smallest E.

Decrease in species diversity due to herbicide treatment is supported by other studies. Edesi et al. (2012) showed decreasing Shannon’s index tendency from herbicide use in a five-year trial comparing conventional and organic farming. Jones and Smith (2007) also confirmed the highest species richness at untreated plots and the smallest at herbicide treated plots in a short-term trial, and also higher species richness in single-component herbicide application than in sequential application of multiple herbicides. In contrast, we found no species diversity difference between the application of products with one active ingredient

and those with three active ingredients. Pawlonka and Rymuza (2014) confirmed significant species richness decrease after 6-years chlorsulfuron application; and Zengin (2001) reported that the abundance of many weed species in spring wheat decreased after 3 or 4 years repeated 2,4-D applications. On the contrary, Derksen et al. (1995) and Ulber et al. (2010) recorded no significant effect of herbicide treatment on species richness.

Both explanatory variables (time and treatment) together explained 27.3% of total variation in weed species data. Partial RDA detected that species data variation explained by net effect of time was 20.4% and by herbicide treatment it was 6.9%. This is partly supported by Streit et al. (2003) who confirmed the significant effect of herbicide

Table 4. The average values of indices (avg.) ± standard errors (SE) in monitored years and treatments

		Shannon’s index		Shannon evenness		Simpson index	
		avg.	SE	avg.	SE	avg.	SE
Year	2013	1.62 <sup>a</sup>	0.06	0.67 <sup>ab</sup>	0.03	0.31 <sup>b</sup>	0.03
	2014	1.55 <sup>a</sup>	0.04	0.62 <sup>a</sup>	0.02	0.34 <sup>b</sup>	0.02
	2015	1.90 <sup>b</sup>	0.05	0.75 <sup>c</sup>	0.02	0.21 <sup>a</sup>	0.01
	2016	1.88 <sup>b</sup>	0.03	0.73 <sup>bc</sup>	0.01	0.22 <sup>a</sup>	0.01
Treatment	2,4-D	1.75 <sup>ab</sup>	0.04	0.7 <sup>a</sup>	0.02	0.26 <sup>a</sup>	0.02
	2,4-D + chlorsulfuron	1.71 <sup>ab</sup>	0.05	0.7 <sup>a</sup>	0.02	0.27 <sup>a</sup>	0.02
	aminopyralid + 2,4-D + florasulam	1.61 <sup>a</sup>	0.07	0.65 <sup>a</sup>	0.02	0.3 <sup>a</sup>	0.03
	aminopyralid + florasulam + pyroxsulam	1.79 <sup>ab</sup>	0.07	0.71 <sup>a</sup>	0.03	0.26 <sup>a</sup>	0.03
	untreated control	1.83 <sup>b</sup>	0.08	0.7 <sup>a</sup>	0.02	0.25 <sup>a</sup>	0.03

The averages marked by the same letter in individual columns did not significantly differ at  $\alpha = 0.05$  (Tukey *HSD* test)

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Table 5. Average (avg.) herbicide efficacy on weeds in % ± standard error (SE) for 2013–2016

Herbicide	Dicot		Grasses	
	avg.	SE	avg.	SE
2,4-D	95.8 <sup>ab</sup>	0.26	0 <sup>a</sup>	–
2,4-D + chlorsulfuron	95.4 <sup>a</sup>	0.19	89 <sup>b</sup>	2.6
Aminopyralid + 2,4-D + florasulam	95.6 <sup>a</sup>	0.23	95 <sup>c</sup>	0.33
Aminopyralid + florasulam + pyroxsulam	96.5 <sup>b</sup>	0.16	97.8 <sup>c</sup>	0.17

The averages marked by the same letter in individual columns did not significantly differ at  $\alpha = 0.05$  (Tukey *HSD* (honestly significant difference) test)

application on weed communities. In contrast to our findings, de Mol et al. (2015) recorded that year-factor made only a small contribution to weed species composition variation and de Mol et al. (2012) did not find a correlation between weed species composition and herbicide treatments. While long-term herbicide application affects both the composition of above ground weed communities and the soil seed bank (Bàrberi et al. 1997), a dominant short-term effect of herbicide application on above-ground weed species richness and abundance is confirmed in study of Hald (1999).

Ordination diagrams of partial RDA (Figure 1) show shifts in weed species composition under different herbicide treatment regimes. As expected, the abundance of many weed species increased in untreated control. These comprised common species such as *Veronica hederifolia* L., *V. persica* Poir., *Sinapis arvensis* L., *Viola arvensis* Murray and *Centaurea cyanus* L. and less common species including *Lathyrus tuberosus* L., *Lycopsis arvensis* (L.) M. Bieb., *Consolida regalis* Gray and *Consolida orientalis* (Gr. et Godr.) Schrödinger. Plots treated with 2,4-D + chlorsulfuron caused the biggest time

shifts in weed species (Figure 1b) and the pyroxsulam + florasulam + aminopyralid showed smaller time shifts than other treatments (Figure 1d). In addition, the *Apera spica-venti* and *Alopecurus myosuroides* Huds. annual grasses showed time increase at plots treated with 2,4-D and florasulam and decrease at plots treated with the pyroxsulam and chlorsulfuron. *V. persica* and *V. hederifolia* abundance increased at all plots, while *Galium aparine* and *Papaver* spp. abundance decreased. Some species with high biodiversity value, such as *Sinapis arvensis* L.\* , *Lamium amplexicaule* L.\* , and *Fallopia convolvulus* (L.) Á. Löve\* increased at plots treated with the pyroxsulam + florasulam + aminopyralid and 2,4-D, but the density of other beneficial species, including *Centaurea cyanus*\* , *Polygonum aviculare* L.\* , and *Stellaria media*\* decreased at all herbicide treated plots.

**Weed density and treatment efficacy.** We established significant impact of herbicide treatment on weed density before herbicide application (Figure 2), where the highest average weed density was at untreated control plots (168 plants/m<sup>2</sup>) and the smallest at plots treated with the pyroxsulam + florasulam + aminopyralid and 2,4-D (124 and 127/m<sup>2</sup>, respectively); and treatment with 2,4-D + florasulam and the 2,4-D + chlorsulfuron provided the same effect.

The Tukey *HSD* test proved significant differences in herbicide efficacy (Table 5). The pyroxsulam + florasulam + aminopyralid showed the highest efficacy on dicot and grass weeds (96% and 98%, respectively) and the 2,4-D + chlorsulfuron showed the least efficacy (95.4% and 89%). Lower efficacy of all herbicide treatments (90–95%) were recorded for the following species: *Consolida regalis*; *C. orientalis*; *Galium aparine*; *Papaver* spp.\*; *Fumaria officinalis* L.\* and *Fallopia convolvulus*\*. The *Alopecurus myosuroides* density on herbicide treated plots did not differ significantly from untreated control plots; with the average number of plants ranging from 0.7

Table 6. Average weed number/m<sup>2</sup> ± standard error after treatment of selected weed species

Weed species/ treatment	2,4-D	2,4-D + chlorsulfuron	Aminopyralid + 2,4-D + florasulam	Aminopyralid + florasulam + pyroxsulam	Untreated control
<i>Veronica</i> spp.	4.5 ± 0.3 <sup>b</sup>	4.3 ± 0.3 <sup>b</sup>	4.7 ± 0.4 <sup>b</sup>	2.3 ± 0.2 <sup>a</sup>	3.9 ± 0.4 <sup>b</sup>
<i>Viola arvensis</i> *	1.3 ± 0.2 <sup>c</sup>	1.7 ± 0.3 <sup>c</sup>	0.3 ± 0.7 <sup>a</sup>	0.5 ± 0.1 <sup>ab</sup>	1.1 ± 0.2 <sup>bc</sup>
<i>Stellaria media</i> *	0.3 ± 0.09 <sup>ab</sup>	0.1 ± 0.05 <sup>a</sup>	0.1 ± 0.04 <sup>a</sup>	0.5 ± 0.1 <sup>b</sup>	1.2 ± 0.1 <sup>c</sup>
<i>Apera spica venti</i>	8.8 ± 0.7 <sup>cd</sup>	7 ± 0.5 <sup>bc</sup>	6.4 ± 0.5 <sup>b</sup>	2.7 ± 0.3 <sup>a</sup>	9.6 ± 0.7 <sup>d</sup>

\*weed species with high biodiversity value (Marshall et al. 2003, Storkey 2006)

to 1.6/m<sup>2</sup>. Keller et al. (2015) also found an increase in this weed's frequency after three decades of field experiment and ascribed this to a high proportion of winter cereals in the crop rotation.

The *Veronica* spp. density on untreated plots was comparable with herbicide treated plots, except in plots treated with the pyroxsulam + florasulam + aminopyralid and 2,4-D where *Veronica* spp. density was significantly lower. *Apera spica-venti* was best controlled by the pyroxsulam + florasulam + aminopyralid and we recorded the highest abundance of this weed after other tested herbicide treatments (Table 6). Pawlonka and Rymuza (2014) recorded an increasing density of this species after chlorsulfuron applications.

In conclusion, the treatment with all tested herbicides provided effective weed control. Modern products containing 3 active ingredients had slightly better efficacy than older products containing 1–2 active ingredients which also caused bigger weed species time shifts. Hurricane (pyroxsulam + florasulam + aminopyralid) proved the best tested product in winter cereal rotation for efficacy on a broad spectrum of weeds while maintaining reasonable species diversity. Hurricane treatment enabled survival of some species with high biodiversity value, such as *Sinapis arvensis*\*, *Lamium amplexicaule*\* and *Fallopia convolvulus*\*; other beneficial species, including *Centaurea cyanus*\*, *Polygonum aviculare*\* and *Stellaria media*\*, were suppressed at all herbicide treated plots. Mustang Forte (2,4-D + florasulam + aminopyralid) is another modern herbicide with a broader spectrum (only dicot) and high efficacy, but it left poorest weed community with low species diversity. Treatment only 2,4-D effectively suppressed some dicotyledonous weeds without significant decrease in diversity; thus this product can be recommended for fields with a low density of grass weeds.

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