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Effect of combined application of herbicides with retardants depending on *Triticum aestivum* L. growth stage

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Abstract. In 2013–2016 the possibility of combined application of herbicides with retardants in winter wheat was investigated in Institute of Soil Science and Plant Cultivation in Wrocław. Two retardants (1. chloromequate chloride, 2. trinexapac-ethyl) were used in tank mixture with fluroxypyr and florasulam + aminopyralide. The mixtures were applied on two dates: 1. at the end of tillering, 2. at the stage of 1–2 nodes (florasulam + aminopyralide) or at the stage of flag leaf (fluroxypyr). Efficacy of weed control was affected only by date of treatment. Irrespective of which herbicide-retardant combination was used, the weed suppression was the lowest on plots treated with the mixtures at a later growth stage. It was reflected in the size of grain yield received from these plots, that was comparable to untreated control. Application of the mixture of fluroxypyr with trinexapac-ethyl in winter wheat tillering and flag leaf growth stages contributed to seed uniformity decrease.

Keywords: fluroxypyr, florasulam + aminopyralide, trinexapac-ethyl, chloromequate chloride, method of application, winter wheat, weed control, plant height

INTRODUCTION

Intensive development of cereal production and increasing competition on EU markets make it imperative to search for new solutions targeted to the reduction of production costs and, simultaneously, ensuring yield stability. It is necessary to look for methods that are efficient, safe for the environment and economically viable (Głazek, Mrówczyński, 1999). Therefore, research on new and effective combinations of agricultural chemicals belonging to different groups is ongoing all over the world (Bhuvaneswari, Krishnam Raju, 2013; Brzozowska et al., 2004; Delchev, 2011; Delchev et al., 2011; Domaradzki, Wróbel, 2012; Miziniak, Piszczek, 2015). Reducing the number of treatment on arable fields result in measurable savings of expenditures and worktime in critical farming period. In order to compile detailed recommendations for the joint application of different agrochemicals, the research that would include combinations of various agents applied at different rates and on different dates ought to be examined.

Protection against lodging and competition from weeds is an important issue in crop production. Plant lodging is connected with bending or falling over of plants to the ground level that results in reduction of photosynthesis rate and, finally in yield decrease and in grain quality deterioration. Application of synthetic retardants is currently a main tool for protection against lodging. Their mode of action consists in inhibition of plant elongation by blocking the synthesis of gibberellins (Rademacher, 2000). Retardants containing trinexapac-ethyl are absorbed by leaves and stems and then they are translocated to the meristem that prevent excessive elongation of internodes (Adams et al., 1992). The most responsive to shortening are the internodes that grow most intensively at the time of the treatment. Additionally, trinexapac-ethyl increases stem diameter, tillering and root mass and, consequently, enhances resistance to drought (Kulczycki et al., 2006; Zagonel et al., 2002). Retardants containing chloromequate chloride show another mode of action. They affect the production of growth hormones that results in the shortening of internodes in the lower part of stem. For this reason, these products should be applied no earlier than at the stage of the first node.

In order to avoid yield loss due to weed competition, chemical weed control is applied worldwide. It can be achieved using wide range of herbicides belonging to different chemical groups and with various modes of action as well as dates of application that makes possible to control weed infestation in agricultural crops. However, there is a discrepancy between the date of use for herbicides and

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retardants. Herbicides should be used early, when weeds are in young growth stages. In turn, retardants are effective when a crop is at a later growth stage. Combined application of herbicides and retardants should be effective in protection of cereals against lodging and against competition from weeds and safe for the crop. Moreover, the compounds of the mixture do not always interact with each other positively which finally results in yield deterioration (Delchev, 2016). For this reason, the priority in studies on joint application of herbicides and retardants is to find the best date of treatment for different combinations of particular compounds.

The objective of this study was the evaluation of joint application of two herbicides with two retardants in winter wheat regarding efficacy in weed control and lodging prevention as well as safety for crop, yield size and yield quality. The study on combined application of herbicides and retardants can be useful to reduce costs of plant production and improve work organization on farm.

Table 1. Growth stages of dominant weed species during early and late treatment (average for 2013–2016).

Weed species	Growth stage (according to BBCH scale)		
	TRZAW 26-29	TRZAW 31-32	TRZAW 39
GALAP	20	25-26	29-30
STEME	50	55	65
THLAR	50	55	65
MATIN	20	31	34
BRSNX	50	55	60
VIOAR	50	55	61
PAPRH	18-20	31	35

TRZAW – *Triticum aestivum* L., GALAP – *Galium aparine* L., STEME – *Stellaria media* (L.) Vill, THLAR – *Thlaspi arvense* L., MATIN – *Matricaria inodora*, BRSNX – *Brassica napus* L. (volunteers), VIOAR – *Viola arvensis* Murr., PAPRH – *Papaver rhoeas* L.

MATERIALS AND METHODS

In 2013–2016 the possibility of combined application of herbicides with retardants in winter wheat was investigated in Department of Weed Science and Tillage Systems of Institute of Soil Science and Plant Cultivation in Wrocław. Two separate experiments were carried out on one arable field that were located in the neighbourhood of Wrocław on brown soil (pH 6.1, $C_{org.}$ 2.48) belonging to good wheat complex. The experiments were designed in a randomized blocks arrangement with four replications. Each of them was one-factor (application method) study with individual plot size of 16 m^2 (2 m × 8 m). In each year of the research, winter wheat followed winter oil seed rape. In 2013/2014 the plots were fertilized with N – 125 kg ha^{-1}, P_2O_5 – 60 kg ha^{-1}, K_2O – 80 kg ha^{-1}, in 2014/2015 with N – 140 kg ha^{-1}, P_2O_5 – 70 kg ha^{-1}, K_2O – 80 kg ha^{-1}, in 2015/2016 with N – 140 kg ha^{-1}, P_2O_5 – 70 kg ha^{-1}, K_2O – 80 kg ha^{-1}.

Retardants containing chloromequate chloride (Antywylegacz Płynny 675 SL at a rate of 2.0 l ha^{-1}) and trinexapac-ethyl (Moddus 250 EC in dose of 0.4 l ha^{-1}) were used in the mixture with fluroxypyr (Starane 250 EC at a rate of 0.8 l ha^{-1}) and with the mixture of florasulam + aminopyralide (Dragon 450 WG in dose of 33.3 g ha^{-1}). Treatments were also included that involved separate application of those products. The mixtures of herbicides and retardants were applied on two dates: early – at the end of tillering (BBCH 29), late – at the 1–2 nodes (BBCH 31-32) for the mixture of florasulam + aminopyralide and at the stage of flag leaf (BBCH 39) for fluroxypyr. Fields with separate application were first treated with herbicide (at the end of tillering) and later retardant was used (BBCH 31-32 or 39). At each time of spraying, particular weed species were in various growth stages (Table 1).

Treatments were carried out using a knapsack sprayer „Gloria" equipped with four flat nozzles TeeJet 11003 VS. The sprayer operated at pressure of 0.25 MPa, producing spray volume 250 l ha^{-1}. At the day of the first spraying weeds were counted on untreated plots. The weeds counting was performed at three points of untreated plots on area of 0.25 m^2. Weed control assessment was made in four weeks after each herbicide treatment by comparison of weed infestation on whole treated vs. untreated plots. Degrees of particular weed species sensitivity to herbicides were determined according to guidelines contained in the Regulation of Minister of Agriculture and Rural Development dated on 04.08.2004, assuming the values as follow: >85% sensitive weed species, 71–85% – semi-sensitive weed species, 60–70% – semi-resistant weed species, <60% – resistant weed species.

Phytotoxicity of the products was evaluated in one week after treatment using a 9-degree scale, where 1 means no crop injury and 9 means total crop destruction. Wheat lodging was estimated on whole treated vs. untreated plots before harvest by the determination of plant bending using 5-degrees scale where: 1 – lack of lodging, 5 – total crop lodging. After wheat heading, the plant height was measured from the ground up to the end of ear. Wheat was harvested at full maturity stage using a harvester Nurserymaster Elite Z 035 and grain yield was determined and adjusted to 14% of grain moisture. At harvest, grain samples were taken for quality analysis. Protein and gluten content was assayed using INSTALAB 600, with near-infrared technique. Weight of hectolitre was measured according to Polish Standard PN-ISO7971-2 and weight of 1000 grain according to Polish Standard PN-R-65950. Seeds were graded for size by sieving them through 2.5 mm × 25 mm mesh sieve and determined for the weight ratio of seeds that did not pass through the sieve to the weight of the to-

Table 2. Meteorological data for the experimental period.

Year	Parameter	Months											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2013	T	-	-	-	-	-	-	-	-	13.9	10.8	5.4	2.7
	P	-	-	-	-	-	-	-	-	96.5	18.8	19.0	12.4
2014	T	0.7	3.1	6.3	10.4	13.5	16.7	21.0	17.5	15.0	11.0	6.1	2.0
	P	35.0	0.5	26.1	30.0	51.8	40.6	80.6	63.4	77.6	40.9	14.2	16.6
2015	T	1.9	2.3	5.4	9.0	13.4	16.8	20.2	22.0	14.8	9.3	6.3	4.7
	P	33.8	9.0	22.5	17.8	30.5	54.8	68.2	45.1	37.6	19.2	32.8	17.0
2016	T	-0.5	3.7	4.8	8.8	15.0	18.0	18.6	19.3	-	-	-	-
	P	16.1	33.3	38.3	34.9	25.7	55.6	89.8	60.2	-	-	-	-

T – temperature [°C], P – precipitation [mm]

tal seed sample. The data were analysed by ANOVA and subjected to Tukey's HSD test to determine significance of differences at $\alpha \leq 0.05$ and homogenous groups were separated. The statistical analysis was performed using Statgraphic 2.0 program.

RESULTS AND DISCUSSION

The experimental period was characterized by similar weather conditions, however some small deviations were noted (Table 2). The autumns were warm and with rainfall sufficient for wheat germination and initial growth. These condition allowed wheat to enter plant dormancy in good status. The winter was relatively mild, except 2016, when some days with temperatures below 0 °C occurred in January. Fortunately, it did not affect plants overwintering negatively. The spring periods distinguished by average precipitation and temperatures that was advantageous for wheat growth and also herbicides and retardants activity. The highest level of rainfall for summer months was noted but it did not cause deterioration of grain quality and delaying in wheat harvest.

The analysis of variance did not show significant differences for plant height, yield and grain parameters between experimental years (Table 3), therefore the results are discussed based on mean data from 3-years period.

The experiment site was similar with respect to dominant weed species composition and abundance in each year of the study. The most numerous weed species were: *Galium aparine* L., *Stellaria media* (L.) Vill., *Viola arvensis* Murr., *Thlaspi arvense* L., *Matricaria maritima* ssp. *inodora* (L.) Dostál, *Papaver rhoeas* L. and volunteers of *Brassica napus* L. The method of fluroxypyr application (separate vs. joint) with retardants did not affect the efficacy in the removal of most weed species (Table 4). Fluroxypyr applied separately as well as in the mixture with retardants efficiently controlled *G. aparine*, *S. media* and *P. rhoeas* regardless of the date of application. *V. arvensis* was

Table 3. Preliminary analysis of variance for the interactions of the herbicides/retardants with experimental years.

Characteristic	Sum of squares	Degree of freedom	Mean squares	F-ratio	p-value
fluroxypyr with retardants					
Plant height	2.42444	12	0.20204	2.72	0.042
Yield	0.11416	12	0.00951	2.30	0.023
WTG	1.55560	12	0.12963	1.61	0.024
Weight of hectolitre	7.51079	12	0.62590	5.92	0.000
Seed uniformity	7.28381	12	0.60698	3.89	0.001
Protein	0.85778	12	0.07148	3.00	0.001
Gluten	14.60650	12	1.21721	6.90	0.000
florasulam + aminopyralide with retardants					
Plant height	5.45841	12	0.45487	3.07	0.004
Yield	0.07696	12	0.00641	2.01	0.047
WTG	3.08508	12	0.25709	2.19	0.031
Weight of hectolitre	2.33206	12	0.20419	1.98	0.041
Seed uniformity	2.48032	12	0.20669	1.22	0.045
Protein	0.93480	12	0.07790	2.45	0.017
Gluten	1.3654	12	0.11378	1.04	0.042

Table 4. The effect of date and application method of fluroxypyr with retardants on weed control in winter wheat (averaged over 2013–2016).

Treatment	Term of treatment (BBCH)	Application method	Weed control [%]						
			GALAP	THLAR	STEME	VIOAR	MATIN	BRSNX	PAPRH
Check#	–	–	10	10	6	14	7	4	4
fluroxypyr + trinexapac-ethyl	29	jointly	94	90	93	73	88	94	100
fluroxypyr + trinexapac-ethyl	39	jointly	87	85	89	68	78	78	97
fluroxypyr + trinexapac-ethyl	29 39	separately	95	90	95	75	87	94	100
fluroxypyr + CCC	29	jointly	96	90	95	77	87	94	97
fluroxypyr + CCC	39	jointly	87	88	87	73	80	83	95
fluroxypyr CCC	29 39	separately	96	90	94	76	87	94	100

number of plant per 1 m^2
Weed name abbreviations see Table 1

Table 5. The effect of date and application method of florasulam + aminopyralide with retardants on weed control in winter wheat (averaged over 2013–2016).

Treatment	Term of treatment (BBCH)	Application method	Weed control [%]						
			GALAP	THLAR	STEME	VIOAR	MATIN	BRSNX	PAPRH
Check#	–	–	13	8	9	9	10	6	4
(florasulam + aminopyralide) + trinexapac-ethyl	29	jointly	92	90	96	87	91	94	100
(florasulam + aminopyralide) + trinexapac-ethyl	31-32	jointly	81	87	88	72	79	82	93
(florasulam + aminopyralide) + trinexapac-ethyl	29 31-32	separately	92	90	97	86	89	94	100
(florasulam + aminopyralide) + CCC	29	jointly	91	90	97	88	93	93	100
(florasulam + aminopyralide) + CCC	31-32	jointly	83	87	89	80	83	82	93
(florasulam + aminopyralide) + CCC	29 31-32	separately	93	90	97	86	91	94	100

number of plants per 1 m^2
Weed name abbreviations see Table 1

Table 6. The effect of date and application method of fluroxypyr with retardants on winter wheat (averaged over 2013–2016).

Treatment	Term of treatment (BBCH)	Application method	Lodging (1:5)	Phytotoxicity (1:9)	Plant height [cm]	Grain yield [t ha^{-1}]	WTG [g]	Weight of hectolitre [kg hl^{-1}]	Seed uniformity [%]	Protein [%]	Gluten [%]
Check	–	–	1.2	1	73.7 a	7.12 b	43.9 a	76.8 b	92.1 a	11.3 a	27.5 ab
fluroxypyr + trinexapac-ethyl	29	jointly	1	1	63.4 b	7.50 a	44.4 a	77.3 a	89.8 b	11.3 a	27.0 b
fluroxypyr + trinexapac-ethyl	39	jointly	1	1	73.9 a	7.24 b	43.5 a	77.6 a	89.6 b	11.8 a	28.1 a
fluroxypyr + trinexapac-ethyl	29 39	separately	1	1	66.8 a	7.58 a	43.8 a	77.0 a	90.7 a	11.7 a	28.0 a
fluroxypyr + CCC	29	jointly	1	1	68.0 a	7.60 a	43.5 a	76.8 b	93.8 a	11.9 a	28.4 a
fluroxypyr + CCC	39	jointly	1	1	65.6 a	7.30 b	44.5 a	77.1 a	94.0 a	11.7 a	28.0 a
fluroxypyr + CCC	29 39	separately	1	1	67.6 a	7.43 a	43.9 a	77.1 a	94.1 a	11.6 a	28.0 a

Values marked by the same letter do not differ significantly within column

Table 7. The effect of date and application method of florasulam + aminopyralide with retardants on winter wheat (averaged over 2013–2016).

Treatment	Term of treatment (BBCH)	Application method	Lodging (1:5)	Phytotoxicity (1:9)	Plant height [cm]	Grain yield [t ha^{-1}]	WTG [g]	Weight of hectolitre [kg hl^{-1}]	Seed uniformity [%]	Protein [%]	Gluten [%]
Check	–	–	1.2	1	75.5 a	7.00 b	44.2 b	77.3 a	93.6 ab	11.5 a	28.2 ab
(florasulam + aminopyralide) + trinexapac-ethyl	29	jointly	1	1	68.2 a	7.58 a	44.0 b	77.6 a	90.2 b	11.7 a	28.6 a
(florasulam + aminopyralide) + trinexapac-ethyl	31-32	jointly	1	1	69.9 a	7.28 b	45.3 a	77.2 a	92.2 b	11.7 a	28.4 a
(florasulam + aminopyralide) + trinexapac-ethyl	29 31-32	separately	1	1	65.7 a	7.46 b	43.3 c	77.5 a	90.3 b	11.9 a	28.1 ab
(florasulam + aminopyralide) + CCC	29	jointly	1	1	66.9 a	7.82 a	45.3 a	76.8 b	93.2 ab	11.8 a	28.5 a
(florasulam + aminopyralide) + CCC	31-32	jointly	1	1	63.6 a	7.30 b	44.1 b	77.0 a	95.6 a	11.7 a	28.0 ab
(florasulam + aminopyralide) + CCC	29 31-32	separately	1	1	67.3 a	7.60 a	45.0 a	77.2 a	94.1 a	11.7 a	27.7 b

Values marked by the same letter do not differ significantly within column semi-sensitive to herbicide applied alone and most of the mixtures tested. The exception is weaker reaction (semi-resistant) to fluroxypyr + trinexapac-ethyl applied in stage of flag leaf. Only the control of *M. maritima* ssp. *inodora*, *T. arvense* and volunteers of *B. napus* was affected by the date of treatment. The application of the tested mixtures in tillering phase controlled these weed species in the range of 87–94%, but at later treatment weed control level decreased to 78–88%.

The joint application of florasulam and aminopyralide with retardants as well as their separate use at the end of tillering were highly efficient against: *G. aparine*, *S. media*, *T. arvense*, *M. maritima* ssp. *inodora*, *P. rhoeas*, *V. arvensis* and volunteers plants of *B. napus* (Table 5). When the mixtures were used later (at the stage of 1–2 nodes), the weed control level was reduced. The only weed species susceptible to the tested mixtures were: *S. media*, *T. arvense* and *P. rhoeas*, while *G. aparine*, *M. inodora*, *V. arvensis* and volunteers of *B. napus* were controlled at a medium level. Likewise, the method of application herbicides and retardants did not affect weed control in the studies performed by Krawczyk (2006), Marczewska-Kolasa, Kieloch (2012) and Grychowski et al. (2018). Additionally, Marczewska-Kolasa and Kieloch (2012) concluded that the date of application and resulting from this weeds growth stage was the only factor affecting weed control, which is important in case of poorer suppression of more competitive weed species, such as *G. aparine* or *M. maritima* ssp. *inodora*. In the separate herbicide-growth retardant treatment, herbicides were used at the stage of tillering resulting in weed control similar to that obtained on plots with joint application at the same growth stage.

An important issue related to joint application of herbicides and retardants is the selectivity of tested mixtures. In this study, both joint as well as separate use of herbicides with CCC or trinexapac-ethyl did not injure winter wheat, regardless of the time of treatment (Table 6, 7). This finding is in accordance with the previous investigations (Krawczyk, 2006; Marczewska-Kolasa, Kieloch, 2009; Miziniak, Praczyk, 2007) that confirm no phytotoxic effect of some combinations on winter wheat. Results to the contrary were obtained in a study on hard wheat where the negative effect of joint application of biostimulants and herbicides on grain germination capacity was demonstrated (Delchev, 2011).

In this study, slight plant lodging in untreated plots was noted (Table 6, 7). Plant bending at the angle of 50° was observed and it was probably caused by infestation with such competitive weeds as *G. aparine* and *M. maritima* ssp. *inodora*. Plant lodging was eliminated at the plots sprayed by retardants, regardless of how the agents were applied (separate vs. joint application).

The examined mixtures reduced plant height depending on how and on what date they were applied. A significant plant height reduction was observed on plots treated with the mixture of fluroxypyr with trinexapac-ethyl at the end of tillering (Table 6). The greatest shortening of wheat stems was obtained after joint application of fluroxypyr with trinexapac-ethyl at the end of wheat tillering while for the mixture of fluroxypyr with CCC the maximum of stalk shortening was observed after application at the stage of flag leaf. The effect of second mixture (florasulam + aminopyralide with trinexapac-ethyl) did not vary with spraying date and with the method of application (Table 7). However, the wheat stem was reduced by 15.8% when the herbicide was applied with CCC at a growth stage of 1–2 nodes.

Different studies on trinexapac-ethyl activity shows diversified effect on wheat growth. Rajala and Peltonen-Sainio (2001) reports that trinexapac-ethyl at the dose of 75 g a.i. ha^{-1} decreased plant height by 20%, while research performed by Matysiak (2006) points out the same degree of stem shortening when the dose of 125 g a.i. ha^{-1} was used and shortening by 11% after 75 g a.i. ha^{-1} was applied. As reported by Miziniak and Praczyk (2007) and Krawczyk (2006), joint application of florasulam with CCC or trinexapac-ethyl as well as the mixture of iodosulfuron methylsodium with CCC reduced wheat stalks to a greater extent than when the two agents were used separately. However, the present study points out the connection between reduction in plant height and the method and the time of treatment.

The main goal for plant protection treatments is save the yield as high as possible. For the joint application of agrochemicals, it is important that the mixture used does not give worse effect than products used separately. Weaker activity of mixtures can be provoke by different factors, e.g. antagonism between mixture compounds, incorrect mixing ratio, inappropriate date of spraying. In this study, the size of saved yield depended on the date of spraying which affected weed infestation. Earlier joint spraying or separate application of herbicides with retardants resulted in significant yield increase in comparison with untreated. The exception is the mixture of florasulam + aminopyralide with trinexapac-ethyl that provide comparable to untreated effect for separate treatment (Table 6, 7). The greatest yield increase as compared to untreated for the mixtures of herbicides with CCC used at the phase of tillering was recorded. Size of saved grain yield recorded for the late treatments (1–2 node or flag leaf stage) attained a comparable effect to the untreated due to poorer weed control. Contrary to present research, previous investigations showed a positive effect of the mixtures of different herbicides with retardants (Krawczyk, 2006; Miziniak, Matysiak, 2019; Miziniak, Praczyk, 2007) on winter wheat yield. However, they only consider differences between various mixtures but not date of application. Moreover, the weed control level was higher than obtained in this study and did not affect grain yield.

In general, there was no evident relationship between grain parameters (weight of 1000 grains, weight of hectolitre, seed uniformity, gluten content) and the way and the date of application of herbicides and retardants (Table 6, 7). Weight of 1000 grains determined in the experiment where the mixtures of fluroxypyr with retardants were tested did not vary between objects. In the second experiment, the decrease of this parameter for grain gained from plots with separate application of herbicide and trinexapac-ethyl was observed. Grain obtained from plots treated with the mixtures of fluroxypyr with trinexapac-ethyl at both wheat growth stages demonstrated less uniformity than those collected from untreated plots and from plots with separate application. On the other hand, weight of hectolitre was grater for wheat treated with the mixture fluroxypyr + trinexapac-ethyl in both dates and fluroxypyr + CCC in flag leaf growth stage. There were no significant decrease in protein and gluten content as compared to untreated.

CONCLUSIONS

1. Weed control was affected by the date of treatment, but not by the method of application of herbicides and retardants. It decreased as compared to early application (the end of tillering) when each of the tested mixtures was used at a growth stage of 1–2 nodes or at the flag leaf stage.
2. When used on the later date, the examined mixtures negatively affected grain yield of winter wheat as compared to earlier application or separate treatment due to weaker weed control.
3. Application of the mixture of fluroxypyr with trinexapac-ethyl in winter wheat tillering and flag leaf growth stages contributed to seed uniformity decrease but it did not disqualify grain as a raw material.

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