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WEED INFESTATION OF A WINTER WHEAT CANOPY UNDER THE CONDITIONS OF APPLICATION OF DIFFERENT HERBICIDE DOSES AND FOLIAR FERTILIZATION

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Abstract

The present study was carried out in the years 2006–2008 in the Bezek Experimental Farm (University of Life Sciences, Lublin). A two-factor field experiment was set up according to a randomized block design, in three replications. The experimental field was situated on medium heavy mixed rendzina developed from chalk rock with medium dusty loam granulometric composition. The soil was characterised by neutral pH, a very high content of P (342.1) and K (278.9) along with a very low level of magnesium (16.0 mg× kg^{-1} of soil) and organic carbon (over 3.5%). The aim of this research was to compare the effect of three herbicide doses and two foliar fertilizers applied in a winter wheat canopy on weed infestation. The herbicides Mustang 306 SE 0.4 l× ha^{-1} and Attribut 70 WG 60 g× ha^{-1} were applied at full recommended doses as well as at doses reduced to 75% and 50%. Foliar fertilizers Insol 3 (1 1× ha^{-1}) and FoliCare (20 kg× ha^{-1}) were applied at full recommended doses twice in the growing season BBCH* development stage 23-25* and 33-35*). The control was not treated with the herbicides and foliar fertilizers. The weed infestation level was determined by means of the quantitative gravimetric method at two dates: the first one 6 weeks after herbicide application and the second one – before harvest. The number of weed individuals was counted; species composition and air-dry biomass of above-ground parts were estimated from randomly selected areas of 1 m× 0.25 m at four sites on each plot. *Galium aparine* and *Apera spica-venti* plants were sampled for molecular analysis 6 weeks after herbicide application (the treatments with the full herbicide dose, a 50% dose and the control without herbicides). The density of weeds and weed air-dry weight were statistically analysed by means of variance analysis, and the mean values were estimated with Tukey's confidence intervals (p=0.05).

It was found that the number of weeds and air-dry weight of weeds in the control treatment were significantly higher in comparison with the herbicide treated plots. The application of different herbicide doses did not differentiate significantly the weed infestation level in the winter wheat canopy. *Galium aparine, Papaver rhoeas, Viola arvensis* and *Apera spica-venti* were dominant weed species in the winter wheat canopy. Foliar application of fertilizers did not influence the weed infestation level in the crop canopy. Molecular analysis showed that herbicide application did not affect genetic variation in the populations of *Galium aparine* and *Apera spica-venti*.

Key words: doses of herbicides, foliar application of fertilizers, winter wheat, weed infestation, DNA analysis RAPD, genetic variation

INTRODUCTION

Reduced herbicide doses used in cereals decrease weed control costs and reduce environmental contamination risks (Domaradzki and Rola 1999, Blackshaw et al. 2006). Domaradzki and Rola (2000, 2004), Domaradzki (2006b), Kraska (2007ab) as well as Kraska and Pałys (2008) indicate the possibility of reducing herbicide rates by as much as 50% without the risk of decreasing grain yields, at the same time maintaining the required weed-killing effectiveness. In addition to the destruction of weeds, herbicide treatments weaken their condition and cause flowering and fruiting disorders (Rola, 1991).

Cleavers belongs to species which are very often found in a winter wheat canopy. This species exhibits a very high tolerance to habitat conditions (Nowicki, 1977). At the same time, it is very prolific. Malicki and Kwiecińska (1999) found that one *G. aparine* plant was capable of producing even 1820 diaspores. Adamczewski and Praczyk (1999), Rola (2002) as well as Kraska (2006, 2007a) indicate the dominant role of cleavers in weed infestation of winter wheat. In practice, *G. aparine* is controlled primarily by the use of herbicides (W e i d e , 1993). A d a m c z e w s k i and P r a c z y k (1999) claim that, among monocotyledonous weeds *Apera spica-venti* poses the greatest threat to winter cereals. K a p e l u s z n y and H a l i n i a r z (2007) point out risks resulting from the possibility of this species becoming resistant to certain active substances of commonly used herbicides.

The aim of the present study was to compare the effectiveness of different herbicide doses and foliar fertilization in the reduction of weed infestation of a winter wheat canopy. At the same time, an attempt was made to verify whether the herbicide rate might affect variation in genetic similarity (DNA changes) of species most frequently found in a winter wheat crop – *Galium aparine* and *Apera spica-venti*.

MATERIALS AND METHODS

The experiment was carried out in the years 2003–2005 in the Bezek Experimental Farm, belonging to the University of Life Sciences in Lublin. The experimental field was located on medium heavy mixed rendzina soil, developed from chalk rock with the granulometric composition of medium silty loam. This soil had a neutral pH, very high content of P – 342.1 and K – 278.9 as well as very low magnesium content – 16.0 (values expressed in $mg \times kg^{-1}$ of soil), very high organic carbon content – over 3.5%, and it was classified as IIIb soil quality class and defective wheat complex.

The experiment was conducted in a randomized block design in three replications. The action of three herbicide doses and two foliar fertilizers was compared in a crop of winter wheat cv. Turnia. The herbicides were applied at full recommended doses, at doses reduced to 75% and reduced by half. The foliar fertilizers were applied at recommended doses. The plots in which neither herbicides nor foliar fertilizers were applied were the control. Tillage was done following generally accepted agricultural practice recommendations. Seeds dressed with the seed dressing agent Panoctine 300 LS (a biologically active substance – guazatine in the form of acetate) were sown at an amount of 500 grains per m^2. Mineral fertilizer doses were as follows: N – 120 $kg \times ha^{-1}$; P_2O_5 – 100 $kg \times ha^{-1}$; K_2O – 120 $kg \times ha^{-1}$. Phosphorus and potassium fertilizers as well as 30 kg $N \times ha^{-1}$ were applied pre-sowing. The remaining part of the nitrogen dose was applied before the start of the growing season at the rate of 60 $kg \times ha^{-1}$ and 30 $kg \times ha^{-1}$ at the shooting stage. In addition, the following plant protection agents were used: the herbicides Mustang 306 SE 0.4 $l \times ha^{-1}$ – basic rate – (BBCH development stage 23-25 *), as well as Attribut 70 WG 60 $g \times ha^{-1}$ – basic rate – (23-25*), Alert 375 SC 1.0 $l \times ha^{-1}$ – (26-29*), Tango 500 SC 0.8 $l \times ha^{-1}$ – (51-56*), Terpal C 460 SL 2.5 $l \times ha^{-1}$ – (32-39*).

The herbicides Mustang 306 SE (containing two active substances: florasulam – a compound from the group of triazolopyrimides – 6.25 $g \times l^{-1}$; 2.4 D EHE – a compound from the group of phenoxyacids – 300 $g \times l^{-1}$ in the form of acid, which corresponds to a content of 452 $g \times l^{-1}$ in the form of 2-ethylhexyl ester) as well as Attribut 70 WG (containing 70% of propoxycarbazone sodium – a compound from the group of sulfonyl-aminocarbonyl triazolinones) were applied jointly. Foliar fertilization was applied twice during the growing period (BBCH stage 23-25 and 33-35) using the following foliar fertilizers: Insol 3 (N-11.5%; Mg-2.84%; B-0.28%; Cu-0.56%; Fe-1.20%; Mn-1.68%; Mo-0.01%; Zn-1.12%) at a dose of 1 $l \times ha^{-1}$ and FoliCare (N-18.0%; P-18.1%; K-18.0%; Mg-1.5%; S-7.2%; B-0.02%; Cu-0.10%; Fe-0.20%; Mn-0.10; Mo-0.01%; Zn-0.02%) at a dose of 20 $kg \times ha^{-1}$. Weed infestation of the crop canopy was determined twice using the quantitative gravimetric method: the first time about 6 weeks after herbicide treatment (BBCH development stage 41-45), the second time before winter wheat harvesting (BBCH stage 89-92). The number of weeds, species composition and air–dry weight of above-ground parts of weeds were determined from the sampling areas surrounded by a frame of 1m × 0.25 m in four randomly selected sites of each plot, in accordance with the recommendations given in the paper of B a d o w s k i et al. (2001). The effectiveness of the action of the herbicides was evaluated by comparing weed infestation of the herbicide-treated plots with the control plots without herbicide application. The obtained results were statistically analysed by means of variance analysis. The mean values were compared by means of the least significant differences using Tukey's test.

In 2008, after six weeks from herbicide application, material for DNA analysis was sampled from the most frequently found species of the dicotyledonous and monocotyledonous classes – *Galium aparine* and *Apera spica-venti*. Samples were collected from the plots in which a full herbicide dose was applied, a dose reduced by half as well as from the control treatment. 8 plant samples were collected from each plot. DNA from the studied species was isolated from leaves using the CTAB method (D o y l e and D o y l e , 1987).

The PCR response was carried out using the modified method of W i l l i a m s et al. (1990). The 15 μl-volume reaction mixture comprised: 1 × PCR buffer (10 mM Tris pH 8.8, 50 mM KCl, 0.08% Nonidet P40) (Fermentas, Lithuania), 160 μM of each dNTP, 5.3 pM of the primer, 1mM $MgCl_2$, 60 ng of genomic DNA, 0.4 U *Taq* DNA Polymerase (Fermentas, Lithuania). Amplification reactions were performed using a T1

Thermocycler (Biometria) for two DNA samples from each genotype, at the same time performing the control reaction without DNA template. The following thermal profile was applied: initial denaturation for 3 min. at 94°C, 44 cycles: 94°C – 45 s, 37°C – 45 s, 72°C – 45 s, with final incubation for 7 min. at 72°C. The reaction products were electrophoresed on a 1.5% agarose gel containing ethidium bromide. The gels were visualised on a transiluminator and photographed using the Poly Doc gel documentation system.

In polymorphism analysis, the presence or absence of a band was treated as a single trait and it was assigned the value of 1 or 0. The pairwise similarity index (SI) for all the investigated forms was estimated according to Dice's formula [N e i and L i , 1979]. Based on the SI matrix, the UPGMA (unweighted pair group method with arithmetic average) analysis was performed using NTSYS-pc 2.10 q software (R o h l f , 2001).

RESULTS

The weed infestation level in the winter wheat canopy, measured by the number of dicotyledonous weeds, total number of weeds and their air-dry weight, was significantly lower in the herbicide-treated plots than in the control plots. Such a correlation was found at both dates of weed infestation estimation. However, the number of monocotyledonous weeds was not significantly differentiated by the herbicide doses applied, at both estimation dates. However, a tendency of the occurrence of a larger number of monocotyledonous taxa in the control treatments was observed (Tab. 1). Foliar fertilization did not have a significant effect on the weed infestation level in the winter wheat canopy (Tab. 2). At the same time, there was no interaction between the herbicide doses applied and foliar fertilization.

Table 1

Weed infestation of a winter wheat canopy per 1 m^2 in dependent on doses of herbicides (means from the years 2006-2008).

Number and dry weight of weeds	Doses of herbicides				$LSD_{0.05}$
	A*	B	C	D	
	1st date				
Number of dicotyledonous weeds	115.3	43.3	43.9	58.9	28.8
Number of monocotyledonous weeds	23.5	17.3	21.6	20.6	**ns
Total number of weeds	138.8	60.6	65.5	79.5	30.9
Air dry weight of weeds in $g \times m^{-2}$	157.5	22.2	24.3	38.3	25.6
	2nd date				
Number of dicotyledonous weeds	31.2	18.4	17.3	18.9	9.8
Number of monocotyledonous weeds	24.0	16.9	22.9	21.2	**ns
Total number of weeds	55.2	35.3	40.2	40.1	18.0
Air dry weight of weeds in $g \times m^{-2}$	296.7	54.9	68.0	67.6	55.8

*A – control (without herbicides)
B – full doses of herbicides
C – 75% doses of herbicides
D – 50% doses of herbicides
**ns – not significant difference

Table 2

Weed infestation of a winter wheat canopy per 1 m^2 in dependent on foliar fertilization application (means from the years 2006-2008).

Number and dry weight of weeds	Foliar fertilizers			$LSD_{0.05}$
	Control	Insol	FoliCare	
	1st date			
Number of dicotyledonous weeds	66.9	65.4	63.6	*ns
Number of monocotyledonous weeds	16.8	23.1	22.4	*ns
Total number of weeds	83.7	88.5	86.0	*ns
Air dry weight of weeds in $g \times m^{-2}$	63.7	59.8	58.2	*ns
	2nd date			
Number of dicotyledonous weeds	20.7	20.3	23.4	*ns
Number of monocotyledonous weeds	16.6	20.6	26.6	*ns
Total number of weeds	37.3	40.9	50.0	*ns
Air dry weight of weeds in $g \times m^{-2}$	105.0	115.8	144.7	*ns

*ns – not significant difference

Table 3
Species composition and number of weeds per 1 m^2 of a winter wheat canopy at the first date of weed infestation estimation (BBCH development stage 41-45) in dependent on doses of herbicides (means from the years 2006-2008).

Species	Doses of herbicides			
	*A	B	C	D
Dicotyledonous				
1. *Galium aparine* L.	65.7	13.7	17.0	23.1
2. *Papaver rhoeas* L.	24.6	2.8	3.7	8.3
3. *Viola arvensis* Murray	9.7	20.7	17.1	19.1
4. *Camelina sativa* (L.) Crantz	6.1	–	–	–
5. *Stellaria media* (L.) Vill.	2.8	0.2	0.4	1.0
6. *Matricaria maritima subsp. inodora* (L.) Dostál	2.3	0.0	0.3	0.4
7. *Veronica persica* Poir.	0.9	3.0	2.5	3.3
8. *Neslia paniculata* (L.) Desv.	0.8	–	–	–
9. *Cerastium holosteoides* Fr. Emend. Hyl.	0.7	–	0.1	0.1
10. *Lamium amplexicaule* L.	0.6	0.6	1.1	1.4
11. *Capsella bursa-pastoris* (L.) Medik.	0.4	–	–	–
12. *Veronica arvensis* L.	0.2	2.0	1.2	1.3
13. *Fumaria officinalis* L.	0.1	0.1	0.2	0.1
14. *Myosotis arvensis* (L.) Hill	0.1			0.1
15. *Cirsium arvense* (L.) Scop.	0.0	0.0	0.0	0.3
16. *Galinsoga parviflora* Cav.	0.1	–	–	–
17. *Sinapis arvensis* L.	0.1	–	–	–
18. *Tussilago farfara* L.	0.1	–	–	–
19. *Geranium pusillum* Burm. f. ex L.	0.0	0.2		
20. *Convolvulus arvensis* L.	–	0.0	0.1	0.2
21. *Fallopia convolvulus* (L.) Á. Löve	–	0.0	–	–
22. *Sonchus arvensis* L.	–		0.3	0.2
Total dicotyledonous	115.3	43.3	43.9	58.9
Number of dicotyledonous species	19	13	13	14
Monocotyledonous**				
23. *Apera spica-venti* (L.) P. Beauv.	23.1	17.0	21.4	20.2
24. *Elymus repens* (L.) Gould	0.4	0.1	0.1	0.3
25. *Poa annua* L.	–	0.1	0.1	0.1
26. *Equisetum arvense* L.	–	0.1	–	0.0
Total monocotyledonous	23.5	17.3	21.6	20.6
Number of monocotyledonous species	2	4	3	4
Total number of weeds	138.8	60.6	65.5	79.5
Number of species	21	17	16	18

0.0 – Species occurring in less than 0.1 per m^2
– Species not occurring
* Explanation as in Table 1
** With *Equisetum arvense* L.

Table 4
Species composition and number of weeds per 1 m^2 of a winter wheat canopy before harvest in dependent on doses of herbicides (means from the years 2006-2008).

Species	Doses of herbicides			
	*A	B	C	D
Dicotyledonous				
1. *Galium aparine* L.	15.5	2.9	4.2	4.6
2. *Viola arvensis* Murray	7.6	10.0	8.5	10.3
3. *Papaver rhoeas* L.	3.2	0.7	0.7	0.5
4. *Matricaria maritima subsp. inodora* (L.) Dostál	2.0	0.5	0.5	0.8
5. *Fallopia convolvulus* (L.) Á. Löve	1.1	1.3	1.0	1.3
6. *Convolvulus arvensis* L.	0.6	1.1	1.0	0.2
7. *Stellaria media* (L.) Vill.	0.6	0.9	–	0.2
8. *Consolida regalis* Gray	0.3	0.3	0.1	0.1
9. *Melandrium album* (Mill.) Garcke	0.2	–	0.2	0.2
10. *Sonchus arvensis* L.	0.1	0.1	0.2	0.3
11. *Cirsium arvense* (L.) Scop.	–	0.2	0.2	0.3
12. *Veronica arvensis* L.	–	0.1	0.3	–
13. *Myosotis arvensis* (L.) Hill	–	0.1	0.1	0.1
14. *Conyza canadensis* (L.) Cronquist	–	0.1	–	–
15. *Chenopodium album* L.	–	0.1	–	–
16. *Anagallis arvensis* L.	–	–	0.2	–
17. *Artemisia vulgaris* L.	–	–	0.1	–
Total dicotyledonous	31.2	18.4	17.3	18.9
Number of dicotyledonous species	10	14	14	12
Monocotyledonous**				
18. *Apera spica-venti* (L.) P. Beauv.	21.4	13.2	19.7	19.1
19. *Elymus repens* (L.) Gould	1.9	1.0	1.0	0.9
20. *Avena fatua* L.	0.6	0.7	0.9	0.7
21. *Setaria pumila* (Poir.) Roem. & Schult.	0.1	1.8	1.1	0.3
22. *Equisetum arvense* L.	–	0.2	0.2	0.2
Total monocotyledonous	24.0	16.9	22.9	21.2
Number of monocotyledonous species	4	5	5	5
Total number of weeds	55.2	35.3	40.2	40.1
Number of species	14	19	19	17

0.0 – Species occurring in less than 0.1 per m^2
– Species not occurring
* Explanation as in Table 1
** With *Equisetum arvense* L.

Table 5

Species composition and number of weeds per 1 m^2 of a winter wheat canopy at the first date of weed infestation estimation (BBCH development stage 41-45) in dependent on foliar fertilization application (means from the years 2006-2008).

Species	Foliar fertilizers		
Dicotyledonous	Control	Insol 3	FoliCare
1. *Galium aparine* L.	34.0	25.4	30.1
2. *Viola arvensis* Murray	15.8	17.8	16.4
3. *Papaver rhoeas* L.	9.4	11.6	8.5
4. *Veronica persica* Poir.	2.5	1.6	3.2
5. *Lamium amplexicaule* L.	1.1	0.3	1.3
6. *Stellaria media* (L.) Vill.	1.3	0.5	1.5
7. *Veronica arvensis* L.	0.8	1.8	0.9
8. *Matricaria maritima subsp. inodora* (L.) Dostál	0.8	0.8	0.7
9. *Cerastium holosteoides* Fr. Emend. Hyl.	0.3	0.2	0.2
10. *Capsella bursa-pastoris* (L.) Medik.	0.3	–	0.1
11. *Neslia paniculata* (L.) Desv.	0.2	0.2	0.2
12. *Fumaria officinalis* L.	0.1	0.1	0.1
13. *Convolvulus arvensis* L.	0.1	0.1	0.0
14. *Myosotis arvensis* (L.) Hill	0.1	0.1	0.0
15. *Galinsoga parviflora* Cav.	0.1	–	–
16. *Tussilago farfara* L.	0.1	–	–
17. *Cirsium arvense* (L.) Scop.	0.0	0.0	0.2
18. *Fallopia convolvulus* (L.) Á. Löve	0.0		–
19. *Camelina sativa* (L.) Crantz	–	4.6	–
20. *Geranium pusillum* Burm. f. ex L.	–	0.1	–
21. *Sonchus arvensis* L.	–	0.2	0.1
22. *Sinapis arvensis* L.	–	–	0.1
Total dicotyledonous	66.9	65.4	63.6
Number of dicotyledonous species	18	17	17
Monocotyledonous*			
23. *Apera spica-venti* (L.) P. Beauv.	16.6	22.9	21.7
24. *Elymus repens* (L.) Gould.	0.1	0.0	0.6
25. *Poa annua* L.	0.1	0.1	0.1
26. *Equisetum arvense* L.	0.0	0.1	0.0
Total monocotyledonous	16.8	23.1	22.4
Number of monocotyledonous species	4	4	4
Total number of weeds	83.7	88.5	86.0
Number of species	22	21	21

0.0 – Species occurring in less than 0.1 per m^2
– Species not occurring
* With *Equisetum arvense* L.

Table 6
Species composition and number of weeds per 1 m^2 of a winter wheat canopy before harvest in dependent on foliar fertilization application (means from the years 2006-2008).

Species	Foliar fertilizers		
Dicotyledonous	Control	Insol 3	FoliCare
1. *Viola arvensis* Murray	8.6	8.5	10.2
2. *Galium aparine* L.	6.9	6.4	7.3
3. *Fallopia convolvulus* (L.) Á. Löve	1.3	0.8	1.4
4. *Papaver rhoeas* L.	1.2	1.2	1.4
5. *Matricaria maritima subsp. inodora* (L.) Dostál	0.8	0.9	1.1
6. *Stellaria media* (L.) Vill.	0.6	0.2	0.4
7. *Convolvulus arvensis* L.	0.5	1.2	0.6
8. *Consolida regalis* Gray	0.2	0.2	0.1
9. *Cirsium arvense* (L.) Scop.	0.1	0.2	0.2
10. *Melandrium album* (Mill.) Garcke	0.1	0.2	0.1
11. *Sonchus arvensis* L.	0.1	0.1	0.3
12. *Chenopodium album* L.	0.1	–	–
13. *Myosotis arvensis* (L.) Hill	0.1	0.1	0.1
14. *Conyza canadensis* (L.) Cronquist	0.1	–	–
15. *Veronica arvensis* L.	–	0.2	0.1
16. *Anagallis arvensis* L.	–	0.1	0.0
17. *Artemisia vulgaris* L.	–	–	0.1
Total dicotyledonous	20.7	20.3	23.4
Number of dicotyledonous species	14	14	15
Monocotyledonous			
18. *Apera spica-venti* (L.) P. Beauv.	14.0	17.2	24.3
19. *Elymus repens* (L.) P. Beauv.	1.2	1.2	1.2
20. *Setaria pumila* (Poir.) Roem. & Schult.	0.8	0.9	0.7
21. *Avena fatua* L.	0.6	1.0	0.4
22. *Equisetum arvense* L.	–	0.3	–
Total monocotyledonous	16.6	20.6	26.6
Number of monocotyledonous species	4	5	4
Total number of weeds	37.3	40.9	50.0
Number of species	18	19	19

0.0 – Species occurring in less than 0.1 per m^2

– Species not occurring

* With *Equisetum arvense* L.

Table 7
Characteristics of RAPD primers selected to estimate polymorphism of *Galium aparine* populations.

Primer	Primer sequence 5'-3'	Number of bands		Range of molecular weight (bp)
		total	polymorphic	
A-05	AGG GGT CTT G	8	8	2500-500 bp
A-07	GAA AAG GGT G	9	7	1500-300 bp
A-11	CAA TCG CCG T	10	9	2000-600 bp
F-05	CCG AAT TCC C	5	5	1300-350 bp
H-17	CAC TCT CCT C	8	8	1600-400 bp
J-05	CTC CAT GGG G	7	7	1500-430 bp
L-02	TGG GCG TCA A	5	4	1500-250 bp
M-07	CCG TGA CTC A	7	7	1200-300 bp
P-06	GTG GGC TGA C	4	4	1000-550 bp
T-01	CGC AGT ACT C	9	7	1200-400 bp
Total		72	66	–
Average per primer		7.2	6.6	–

Table 8
Characteristics of RAPD primers selected to estimate polymorphism of *Apera spica-venti* populations.

Primer	Primer sequence 5'-3'	Number of bands		Range of molecular weight
		total	polymorphic	
A09	GGG TAA CGC C	10	9	1500-320 bp
A12	TCG GCG ATA G	10	9	1600-350 bp
A14	TCT GTG CTG G	9	9	2000-600 bp
M07	CCG TGA CTC A	9	8	1300-350 bp
U136	TAC GTC TTG C	11	10	1600-400 bp
U386	TGT AAG CTC G	7	5	1500-430 bp
X06	TCC GAG TCT G	10	10	1500-400 bp
Total		66	60	–
Average per primer		9.4	8.6	–

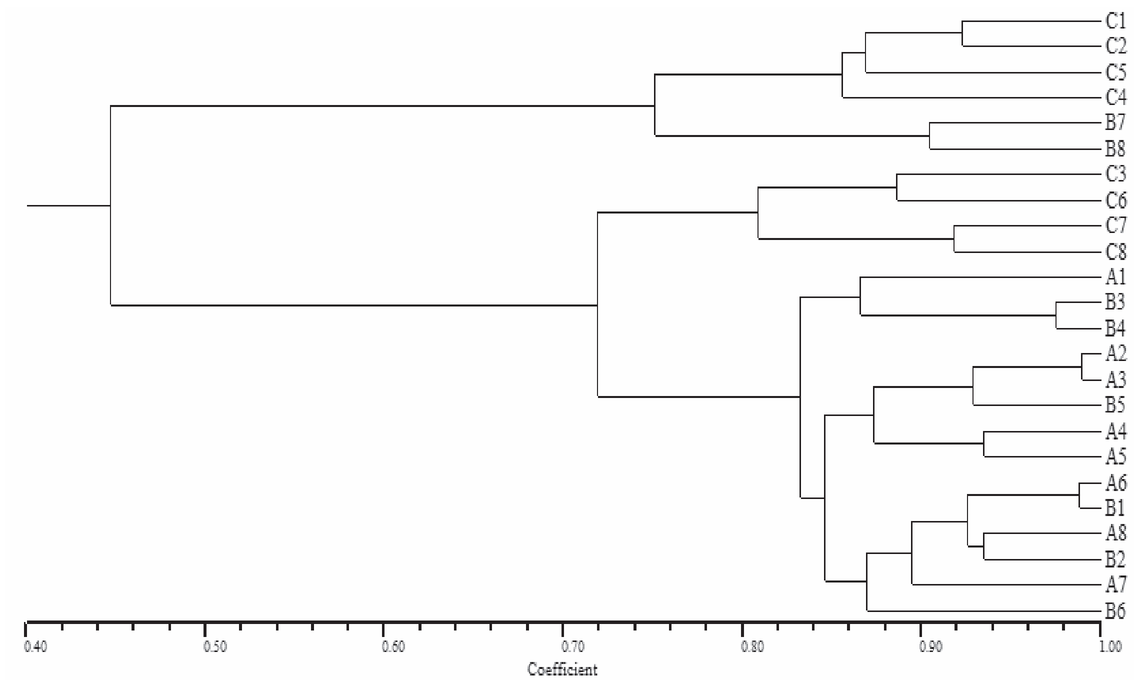

Fig. 1. UPGMA dendrogram of *Galium aparine* forms based on RAPD markers.
A – population from the plot where doses of herbicides were reduced to 50%
B – population growing in the plot where herbicides were applied at full recommended doses
C – control population
Numerals 1-8 mean the numbers of probes from the population

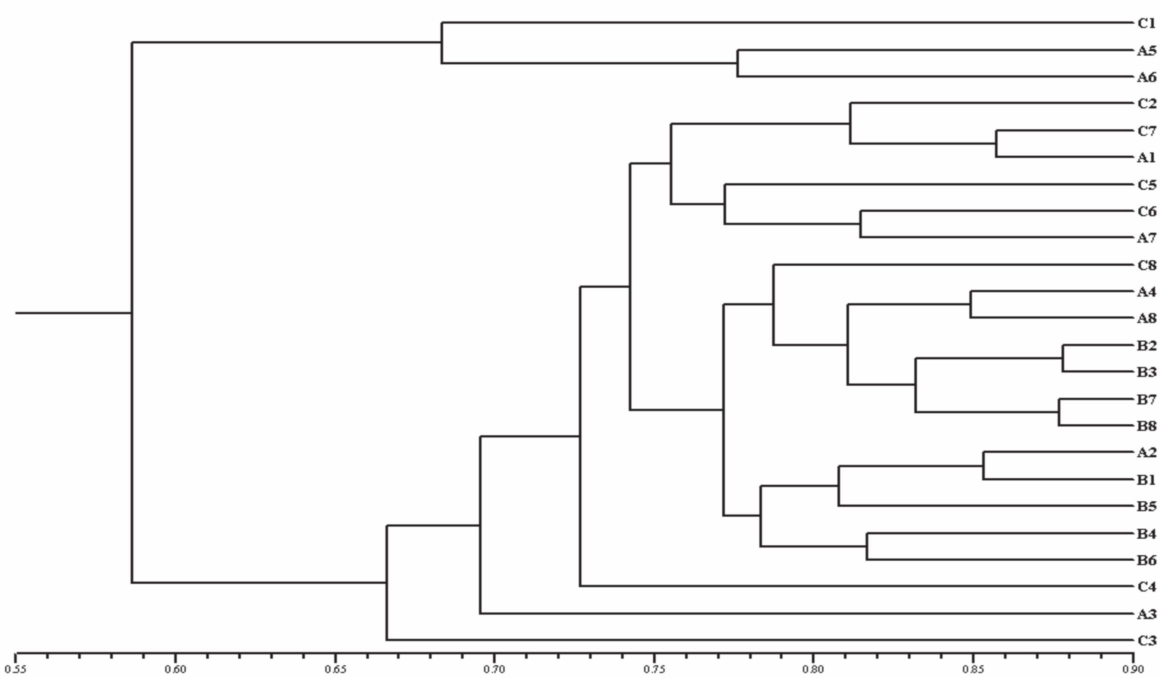

Fig. 2. UPGMA dendrogram of *Apera spica-venti* forms based on RAPD markers.
A – population from the plot where doses of herbicides were reduced to 50%
B – population growing in the plot where herbicides were applied at full recommended doses
C – control population
Numerals 1-8 mean the numbers of probes from the population

In the herbicide-treated plots, compared to the control treatments, a distinctly lower number of weed species was found on the first date of weed infestation estimation. At the same time, at this date the greatest species diversity was found in the variant with the half recommended herbicide dose in the treatments in which the herbicides were applied (Tab. 3).

At the first date of weed infestation estimation, the applied herbicides eliminated, compared to the control with no herbicides: *Camelina sativa, Neslia paniculata, Capsella bursa-pastoris, Galinsoga parviflora, Sinapis arvensis, Tussilago farfara.* At the same time, they significantly reduced the occurrence of *Galium aparine* and *Papaver rhoeas,* but only slightly *Apera spica-venti* (Tab. 3).

At the second date of weed infestation estimation, the largest number of species was found in the treatments with the full recommended herbicide dose and with the dose reduced down to 75% (19 in each). 17 species were found in the treatment with the herbicide rate reduced by half, whereas 14 taxa in the control treatment (Tab. 4). In the group of dicotyledonous species, *Galium aparine, Papaver rhoeas* and *Viola arvensis* were found in greatest numbers at both estimation dates, whereas *Apera spica-venti* from the group of monocotyledonous species (Tabs 1-4).

At the first date of weed infestation estimation, 17 dicotyledonous and 4 monocotyledonous species were found in the foliar fertilization treatments, but in the control treatment 22 species, including 18 dicotyledonous ones (Tab. 5). At the second date of weed infestation estimation, 19 species were found in the foliar fertilization treatments, whereas 18 taxa in the control (Tab. 6).

From among 30 primers tested, 10 primers generating stable and repeatable banding patterns were selected for analysis of the similarity index of *Galium aparine*. The primers amplified a total of 72 fragments, out of which 66 were polymorphic. The number of polymorphic fragments ranged from 4 to 9 for a single primer; on the average there were 6.6 amplicons per primer. The size range of polymorphic bands was from 250 bp to 2500 bp (Tab. 7).

The results of analysis of RAPD marker polymorphism formed the basis for the creation of the Dice similarity index matrix. The similarity index values ranged from 0.446 to 0.984, with the average value being 0.860. Based on the SI matrix, the UPGMA analysis was performed (Fig. 1). 5 groups of clusters were observed on the dendrogram obtained. Genotypes from all the studied populations were grouped together in nearly each group. In the first group of clusters, genotypes from the control population and 2 genotypes from the plot in which the full herbicide dose was applied were clustered together. In the second group, 4 genotypes from the control population were clustered together, and in the third group of clusters, 3 genotypes were located: one from the population growing in the plot treated with the reduced herbicide dose and 2 genotypes from the population growing in the plot treated with the full herbicide dose. In the fourth group of clusters, 4 genotypes were located from the population treated with the reduced herbicide dose and one genotype from the population treated with the full herbicide dose. In the fifth group of clusters, there were 3 genotypes from the population growing in the plot treated with the reduced herbicide dose and 6 genotypes from the plot treated with the full herbicide dose.

7 primers generating stable and repeatable banding patterns were selected for analysis of the similarity index of *Apera spica-venti*. A total of 66 fragments were obtained, out of which 60 were polymorphic. The number of polymorphic fragments ranged from 5 to 10 for a single primer; on the average there were 9.4 amplicons per primer. The size range of polymorphic bands was from 320 bp to 2000 bp (Tab. 8).

The Dice similarity index values estimated for the *Apera spica-venti* population ranged from 0.586 to 0.878, with the average value being 0.709. Based on the SI matrix, the UPGMA analysis was performed (Fig. 2). Two groups of clusters can be distinguished on the dendrogram obtained. In the first group, 2 genotypes were located from the population growing the plot in which the herbicide dose reduced by 50% was applied and one genotype from the control population. 3 subgroups can be distinguished in the second group of clusters. In subgroup A, 4 genotypes from the control population and 2 genotypes from the population treated with the reduced herbicide dose were located. In subgroup B, 4 genotypes from the population growing in the plot treated with the full herbicide dose, 2 genotypes from the population growing in the plot in which the reduced herbicide dose was applied and one genotype from the control group were located. In subgroup C, 4 genotypes from the population treated with the full herbicide dose and one genotype from the population treated with the reduced herbicide dose were located. On the edges of the second group of clusters, there were 2 genotypes from the control group and one genotype from the population growing in the plot treated with the reduced herbicide dose.

DISCUSSION

At the first date of weed infestation estimation, the applied doses of the herbicides Mustang 306 SE (florasulam and 2.4 D EHE) and Attribut 70 WG (propoxycarbazone sodium) reduced the number of weeds from 42.7% to 56.3%, whereas their air-dry weight from 75.7% to 85.9%. Before harvest, the number of weeds in the treatments in which the herbicides were applied was lower, compared to the control, from 27.2% to 36.1%, whereas in the case of air-dry weight from 77.1% to 81.5%. When applying in a winter wheat crop the herbicide Atlantis 04 WG (containing two active substances: mesosulfuron-methyl 30 gxkg^{-1} + iodosulfuron-methyl-sodium 6 g× kg^{-1} and as a safener mefenpyr-diethyl 90 g× kg^{-1}) and Factor 365 EC (containing 5 g× l^{-1} of metosulam and 360 g× l^{-1} 2.4 D), Kraska (2006) achieved a greater reduction in the number (from 67.0% to 80.5%) and dry weight of weeds (from 81.4% to 92.5%) at the first date of estimation, and at the second date, the number of weeds decreased from 81.8% to 86.3% and air-dry weight of weeds – from 81.8% to 90.6%.

The effectiveness in the reduction of weed species occurring in greatest numbers in the wheat canopy, in particular *Galium aparine* and *Apera spica-venti*, decreased in the treatments with the reduced herbicide dose. Kraska (2006, 2007a) obtained similar correlations with regard to the same species on the same soil type. Whiting et al. (1991) found that it was possible to reduce the occurrence of dicotyledonous species and *Apera spica-venti* in cereals by the application of herbicides at reduced rates from 30 up to 60%. On the other hand, Davies and Whiting (1989), when applying herbicides containing isoproturon, did not find any difference in the effectiveness of control of *Apera spica-venti* between the full dose and the dose reduced down to 50%. Similarly, Kraska (2006) achieved high effectiveness of control of *Apera spica-venti* from 95.1% to 97.5% by applying mesosulfuron-methyl + iodosulfuron-methyl-sodium and metosulam + 2.4 D at full doses and doses reduced by half. In the authors' study, the effectiveness of control of *Apera spica-venti* was much lower and it was, at the first date of weed infestation estimation, from 7.4% to 26.4%, whereas at the second date from 7.9% to 38.3%. High effectiveness in the control of *Galium aparine* (96-98%) in spring cereals was achieved by Domaradzki (2006a) with respect to the mixture 2.4 D + florasulam, but only when it was applied at a full rate and a rate reduced by 25%. Slightly lower effectiveness in the reduction in the numbers of this species was achieved in the authors' study. At the first date of weed infestation estimation, it was from 64.8% to 79.1%, whereas at the second date from 70.3% to 81.3%.

The obtained results indicate the possibility of reducing herbicide doses in a winter wheat crop grown on rędzina without the risk of increased weed infestation. Davies and Whiting (1989), Spandl et al. (1997), Domaradzki (2006b) as well as Kraska (2006) also showed that it was possible to reduce herbicide doses from 20% to 50% without a significant reduction in winter and spring cereal yields, maintaining the required weed-killing effectiveness.

Herbicide application in a crop canopy limits its weed infestation, but at the same time it may decrease the number of species composing a weed community (Adamiak and Zawiślak, 1992; Jędruszczak, 1998). As a consequence, it may lead to the development of communities with several species strongly competitive to a crop plant (Rola, 1991; Stupnicka-Rodzynkiewicz et al. 1988). It found confirmation in the present study, but only at the first date of weed infestation estimation, at which the number of species in the treatments where herbicides were applied was lower than in the control treatment. But before harvest, a completely reverse situation was found, notably, the control treatment proved to be the least floristically diverse. Probably, the applied herbicide doses, while limiting weed infestation with the dominant species, in particular, *Galium aparine* and *Apera spica-venti*, enabled, in the second part of the growing period, germination and growth of the species which were smothered by strong dominants in the control plot. Kraska (2007a) found a similar situation in another study on the same soil type. Pawłowski and Wesołowski (1982) as well as Rola (1991) found that a single dominant species was more harmful for a crop plant than a multi-species community.

Foliar fertilization used in the present experiment did not have any effect on the weed infestation level in the winter wheat crop.

The RAPD method has been used many times to estimate genetic differentiation of many plant species (Martins-Lopes et al. 2007; Martinsa et al. 2006; Kant et al. 2006). Hübner et al. (2003) analysed cleavers populations from different countries and they found a low correlation between genetic differentiation of the studied populations and their geographic origin. Ernst (2003) analysed genetic variation of cleavers populations based on RAPD markers. Out of 40 RAPD primers analysed, the author selected 17 primers generating polymorphic banding patterns. In the presented study, 10 RAPD primers generating repeatable and polymorphic banding patterns were selected for analysis of polymorphism of the studied *Galium aparine* populations, whereas 7 such primers were selected for analysis of *Apera spica-venti*. Similarly to the present study, the author found that the *Galium aparine* population in question was genetically diverse. In addition, the author did not find any correlation between the results of molecular analysis and herbicide sensitivity tests. Also in the authors' study, no correlation was found between the application of different herbicide doses and variation in genetic similarity of the studied populations of *Galium aparine* and *Apera spica-venti.*

CONCLUSIONS

1. Weed infestation of the winter wheat canopy was not significantly differentiated by the application of the full and reduced, by 25% and 50%, doses of the herbicides Mustang 306 SE and Attribut 70 WG. It indicates the possibility of reducing herbicide rates in a winter wheat crop without the risk of increased weed infestation.
2. 6 weeks after herbicide application (the first date of weed infestation estimation), the most diverse floristic composition was found in the control treatment. But when estimating weed infestation before harvest, the number of weed species found in the treatments in which herbicides were applied was by far larger than in no-herbicide treatments.
3. The species dominant in the winter wheat canopy were *Galium aparine, Papaver rhoeas, Viola arvensis* and *Apera spica-venti*.
4. At both date of weed infestation estimation, the applied herbicides limited to the greatest degree the occurrence of *Galium aparine* and *Papaver rhoeas,* whereas *Apera spica-venti* only slightly.
5. The application of foliar fertilization did not have a significant effect on the weed infestation level in the winter wheat canopy.
6. The *Galium aparine* and *Apera spica-venti* populations in question, herbicide-treated or untreated, were characterised by high genetic similarity. The herbicide doses applied did not affect genetic variation of the weed population in question.

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Zachwaszczenie łanu pszenicy ozimej w warunkach stosowania zróżnicowanych dawek herbicydów oraz nawożenia dolistnego

Streszczenie

Badania przeprowadzono w latach 2006-2008 w Gospodarstwie Doświadczalnym Bezek niedaleko Chełma. W dwuczynnikowym doświadczeniu przeprowadzonym w układzie bloków losowanych porównywano działanie trzech dawek herbicydów oraz dwóch nawozów dolistnych w łanie pszenicy ozimej odmiany Turnia uprawianej w monokulturze. Herbicydy były stosowane w pełnych zalecanych dawkach, zredukowanych do 75% i do 50%. Nawozy dolistne Insol 3 (N-11,5%; Mg-2,84%; B-0,28%; Cu-0,56%; Fe-1,20%; Mn-1,68%; Mo-0,01%; Zn-1,12%) i FoliCare (N-18,0%; P-18,1%; K-18,0%; Mg-1,5%; S-7,2%; B-0,02%; Cu-0,10%; Fe-0,20%; Mn-0,10; Mo-0,01%; Zn-0,02%) stosowano dwukrotnie w okresie wegetacji. Kontrolę stanowiły poletka na których nie stosowano zarówno herbicydów jak i nawozów dolistnych. W pracy oceniono poziom zachwaszczenia łanu (liczba osobników, skład gatunkowy i powietrznie sucha masa) pszenicy ozimej w 6 tygodni po zastosowaniu herbicydów Mustang 306 SE (florasulam – 6,25g$\times$l^{-1}; 2,4-D EHE – 300g$\times$l^{-1}) i Attribut 70 WG (70% propoksykarbazonu sodowego) oraz przed zbiorem. Jednocześnie podjęto próbę sprawdzenia czy wielkość dawki herbicydu może wpływać na zmiany DNA gatunków dominujących w łanie pszenicy ozimej – *Galium aparine* i *Apera spica-venti*.

Poziom zachwaszczenia łanu pszenicy ozimej mierzony zarówno liczbą chwastów, jak i powietrznie suchą masą nie był istotnie różnicowany przez zastosowane dawki herbicydów. Uzyskane wyniki wskazują na możliwość obniżenia dawek herbicydów

w łanie pszenicy ozimej bez ryzyka wzrostu poziomu zachwaszczenia. Nawożenie dolistne nie zmieniało poziomu zachwaszczenia łanu. Gatunkami dominującymi w łanie pszenicy ozimej w 6 tygodni po zastosowaniu herbicydów oraz przed zbiorem były *Galium aparine, Papaver rhoeas* oraz *Viola arvensis,* natomiast z jednoliściennych *Apera spica-venti*. Analiza molekularna nie wykazała, aby zastosowane dawki herbicydów wpłynęły na zróżnicowanie genetyczne *Galium aparine* i *Apera spica-venti*.