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ORIGINAL PAPER

Effect of a set of herbicides and the dates of their application to *Triticum aestivum* L. cultivars on the content and yield of raw protein depending on the date of sowing

Margarita Nankova[1] • Georgi Sabev[1] • Zornitsa Petrova[1] • Genoveva Bankova - Atanassova[1]

[1]Dobrudzha Agricultural Institute – General Toshevo, 9521, General Toshevo, Bulgaria

Corresponding Author: Margarita Nankova, E-mail: nankova_margo@abv.bg

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Abstract

Nankova, M., Sabev, G., Petrova, Z., Bankova – Atanassova, G. (2021). Effect of a set of herbicides and the dates of their application to Triticum aestivum L. cultivars on the content and yield of raw protein depending on the date of sowing. Field Crops Studies, XIV(2-3-4), 27-42.

The investigation was carried out at DAI – General Toshevo during 2012 – 2014 in four-field crop rotation (wheat-maize-wheat-sunflower). Cultivars Aglika and Enola were sown on two dates – optimal (1st – 15th October) and late (after 20th November). The following set of herbicides was tested: Derby super VG – 3,3 g/da; Palace 75WG + oil – 25+100 g/ml/da; Granstar 75DF – 1,5 g/da; Lintur 70VG – 15 g/da; Husar Max OD – 100 ml/da; Secator OD – 10 ml/da and Mustang 306,25CK – 80 ml/da. The herbicides were applied on three dates: I date A – stage 29 according to Zadoks ; II date B – stage 32 according to Zadoks and III date C – stage 37 according to Zadoks. The experiment included two check variants K_1 – weed-free check (manually weeded from the emergence of the first weeds till heading of wheat), and K_2 (*nil*) – a check infested till harvesting of the crop. A high level of significance was determined for the tested herbicides on the values of raw protein in grain and protein yield from the tested cultivars under the conditions of the experiment. The years of investigation had highest effect on the values of the tested characteristics. Best results were obtained in 2012. At the late date of

herbicide application, the amount of protein decreased in both cultivars. Averaged for the investigated period, the size of protein yield of cultivar Enola was with 18.7 % higher than the yield of cultivar Aglika.

In all three years of the investigation, highest yield of raw protein was obtained from the manually weed-free check (K_1) and lowest – from the infested check (K_2). Averaged for the tested variants of the trial, only after the application of the herbicides Derby super VG and Secator OD the raw protein yield remained at the level of the weed-free check (K_1). There was a strong positive correlation of grain yield with raw protein yield. Averaged for the period of investigation, the sowing date of cultivar Aglika did not show significant effect on the values of the correlation coefficients. In cultivar Enola, the late sowing date lead to high increase of the correlation. This tendency was best expressed in harvest year 2014 (r=,875**).

Key words: Wheat, Cultivars, Dates of sowing, Herbicides, Stages of application

Introduction

The content of protein in wheat grain as influenced by different factors has been studied for more than 100 years (Pierre, 1869). Protein is the principal nitrogen-containing constituent in the wheat kernel. Therefore, soil and climatic factors that affect the N nutrition of the wheat plant will cause differences in wheat grain protein (Smika and Greb, 1973). Herbicides can affect wheat protein levels by directly affecting the nitrogen uptake by the roots or the N metabolism and protein synthesis within the plant (Hance, 1981; Martin at al, 1990; Pederson et al, 1994). Kumar (2012) reported that herbicides were able to reduce the carbohydrate and protein content gradually from lower to higher concentration of herbicides.

The complex methods for control of weeds in the cereal crops include various agronomy practices for restriction of their distribution and for ensuring better conditions for the growth and development of the cultural plants (Kalinova, S., 2002). Under the conventional technologies for growing of common wheat, the control of the weed vegetation is a significant part of the entire agro technology of the crop.

Without efficient control of the weed vegetation, higher yields and quality production are unthinkable (Heard et al. 2003; Lyubenov, 1996 and 1996[a]; Stoynev, 2004). The development of weeds may be critical for the quality of the produce (Nankov et al., 2005).

Delchev and Petrova (2012) reported that there are insufficient investigations on the effect of the herbicides on grain quality in Bulgaria, and most of these investigations are focused on durum wheat (Delchev and Deneva, 2001; Delchev, 2009; Kolev et al., 2011). The authors concluded that the information worldwide was also scarce (Liu et al., 1994; Camele and Rana, 1995).

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Mungova et al. (2013) found out considerable increase of the technological quality of wheat kernel when comparing a untreated check variant to variants with combined application of Akurat 60 VG at dose 1 g/dka, Impact 25 SC at dose 50 ml/dka and various fertilizers for foliar treatment – Amalgerol, Vertex H 34, Foliar extra, etc.

The aim of this investigation was to evaluate the effect from the use of a set of herbicides at different stages of the development of cultivars Aglika and Enola (*Triticum aestivum* L.) on the protein content and the raw protein yield after two dates of sowing.

Material and methods

The investigation was carried out at DAI – General Toshevo during 2012 – 2014 in four-field crop rotation (wheat-maize-wheat-sunflower). Cultivars Aglika and Enola were sown in Haplic Chernozems on two dates – within the optimal (1st – 15th October) and late (after 20th October). The mineral fertilization of wheat was done with conventional fertilizers at norm $N_{12}P_{10}K_{0}$.

The tested herbicides were from the group of sulfonylureas characterized with two types of impact: auxin type and inhibitors of acetolactate synthase.

The auxin type herbicides (hormone-like preparations) inhibit the transportation of the auxin phyto hormone in plants. They case yellowing and wilting of leaves, epinastic deformations of petioles, stems, leaves and tumor growth on stems. The commercial preparations used were Lintur 70 VG and Mustang 306,25CK.

Table 1. Characteristics of the tested herbicides

Name	Active substance	Applied norm	Action against
Derby super VG	150,2 g/kg florasulam +300,5 g/kg aminopyralid-potassium	3,3 g/da	Broad-leaved weeds
Palace 75 WG + oil	75 g/kg pyroxulam	25+100 g/ml/da	Broad-leaved + grass weeds
Granstar 75 DF	750g/kg tribenuron-metil	1,5 g/da	Broad-leaved weeds
Lintur 70 VG	4,1% triasulfuron +65,9% dicamba	15 g/da	Broad-leaved weeds
Husar Max OD	8 g/l mesosulfuron +8 g/l iodosulfuron +23 g/l antidot	100 ml/da	Broad-leaved + grass weeds
Secator OD	106 g/l amidosulfuron + 25 g/l iodosulfuron	10 ml/da	Broad-leaved weeds
Mustang 306,25CK	6,25 g/l florasulam +300 g/l 2-4D - ester	80 ml/da	Broad-leaved weeds

The inhibitors of acetolactate synthase cause the perishing of weeds within several weeks, and stunted growth, dwarfing, necrosis and chlorosis of the shoots are observed. These symptoms are caused by insufficient amino acids (leucine and isoleucine) leading to exhaustion and perishing of weeds. The following used commercial preparations belong to this group: Derby super VG, Granstar 75DF, Secator OD, Palace 75WG and Husar Max OD (Table 1).

The herbicides were applied on three dates: I date A – stage 29 according to Zadoks; II date B – stage 32 according to Zadoks and III date C – stage 37 according to Zadoks (Zadoks et al, 1974). Two check variants were involved in the experiment: K_1 – weed-free check (manual weeding from emergence of the first weeds till heading of wheat) and K_2 (*nil*) – infested check till harvesting of the crop.

Total nitrogen in grain was determined using Kjeldahl method and protein (N x 5,7) - BDS ISO 1871.

The data were analyzed as completely randomized design using analysis of variance (ANOVA) test. All statistical analysis was carried out using the SPSS Inc. computer program, at 0.05 level of significance.

The main meteorological elements, especially precipitation, were in all three years of the investigation considerably variable in comparison to the mean values of the long-term period (1954 – 2014) (Figure 1). The growth season of wheat during harvest year 2014 was characterized with the highest sum of rainfalls – 674.7 mm, which exceeded the mean long-term value with 52.9 % and was almost equal to the sum of autumn-and-winter rainfalls and the rainfalls during April – July. In this respect, this was the most humid year apart from 2010 for the 60-year period of meteorological observations in the region.

Harvest year 2012 was also characterized with more rainfalls during the growth season as compared to the mean long-term values – with 14.7 %. The autumn-and-winter rainfalls were with 26.8 % higher than the April – July rainfalls. Harvest year 2013 was with the lowest sum of precipitation during the growth season of the crop – 386.0 mm. The vegetation rainfalls during April – July were 136.2 mm, which resulted in drought at the critical stages of wheat development in May and June.

Averaged for the three years, higher mean temperatures during the growth season of wheat were measured in 2013 (10.7° C) and in 2014 (10.2° C) due to the higher mean monthly temperatures during the autumn-and-winter period. During the autumn-and-winter period of 2012, the mean air temperatures were lower than the mean long-term values. This year of the investigation, however, was characterized with higher mean monthly temperatures during the growth season of wheat from April to July in comparison to both 2013 and 2014, and to the mean values of the long-term period.

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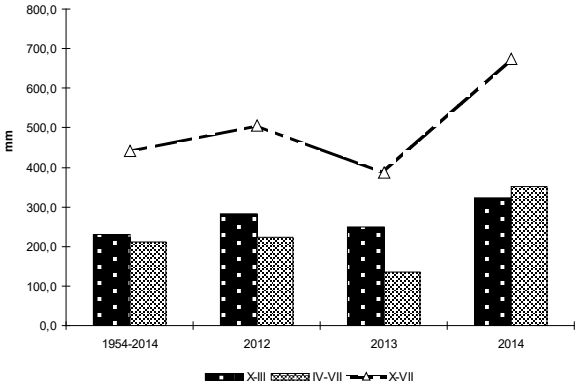
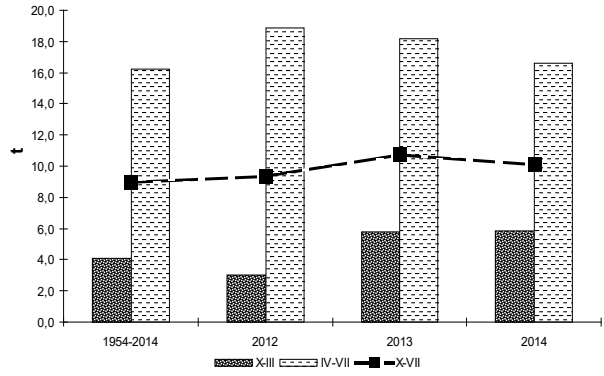


Rainfalls



Air temperatures

Figure 1. Characteristics of the meteorological elements

Results and discussion

The analysis of the variances for protein content in grain of both cultivars revealed strongly expressed correlations of the values of the index with the independent and combined effect of the factors tested in the experiment (Table 2). Undoubtedly, most expressed was the effect of the meteorological conditions during the years of investigation. At the late date of sowing, the combination of the meteorological components had higher influence on the concentration of protein in the grain of both cultivars in comparison to the optimal date of sowing.

The analysis demonstrated that the protein in the grain of cultivar Enola was affected to a higher degree by the meteorological factor than cultivar Aglika. The strength of this effect was 68.39 % at the optimal date of sowing, and 76.49 % at the late date of sowing (Figure 2). The combination of the meteorological components in cultivar Aglika when sown on the optimal date was also of primary importance, but the strength of their independent influence on the concentration of protein in

grain was only 35.01 %, which resulted in higher effect of the other factors and their combined interaction. Thus the date of herbicide application was the factor second in strength of effect on the concentration of nitrogen in grain, followed by the set of the tested herbicides.

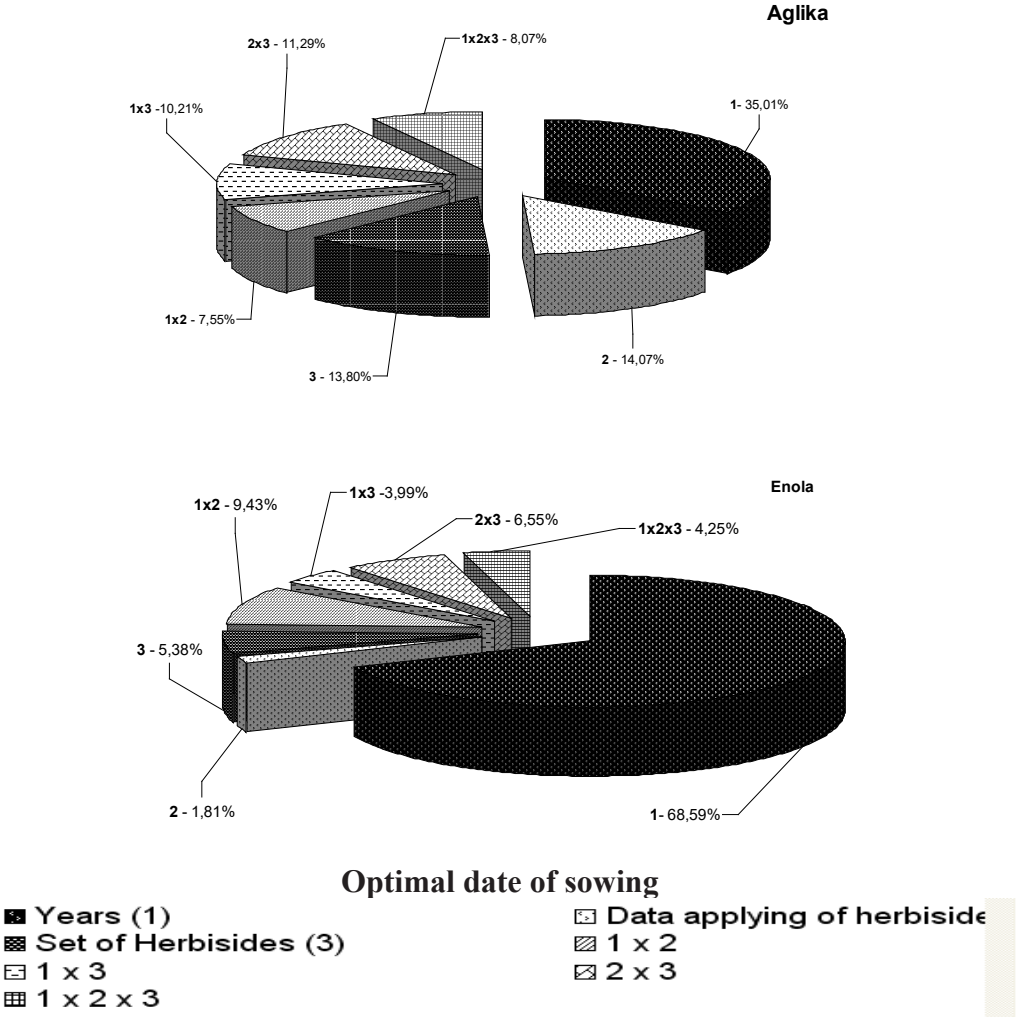


Figure 2. Strength of the factors' effect on grain protein content

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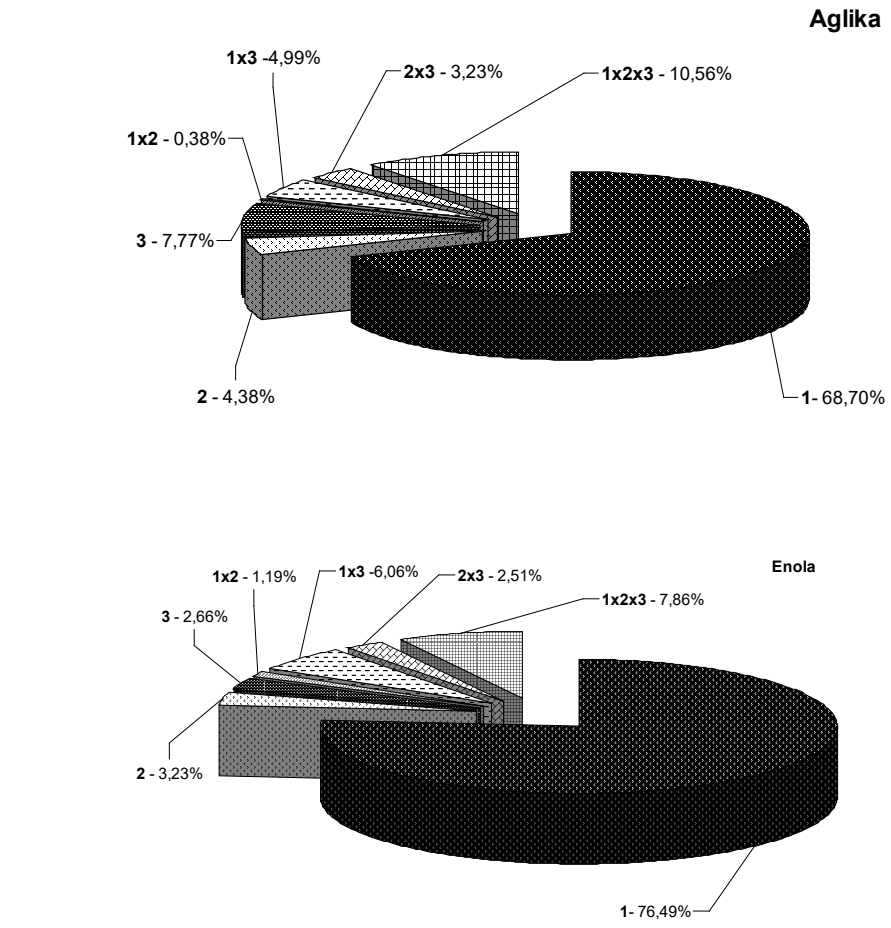

Figure 2. (continued) Strength of the factors' effect on grain protein content

Table 2. Protein content - analysis of variances (*p – 0,05*).

Source	df	AGLIKA				ENOLA			
		Optimal date of sowing		Late date of sowing		Optimal date of sowing		Late date of sowing	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.
Years (1)	2	3802,7	0	7307,6	0	9912,5	0	5961,6	0
Date of herbicide application (2)	2	1528,1	0	465,8	0	262,9	0	251,9	0
Set of herbicides (3)	8	374,6	0	206,7	0	194,3	0	51,8	0
1 x 2	4	410,2	0	20,1	0	681,4	0	46,4	0
1 x 3	16	138,6	0	66,3	0	72	0	59	0
2 x 3	16	153,2	0	42,9	0	118,4	0	24,4	0
1 x 2 x 3	32	54,8	0	70,2	0	38,4	0	38,3	0

Table 3. Protein yield - analysis of variances (*p – 0,05*)

Source	df	AGLIKA				ENOLA			
		Optimal date of sowing		Late date of sowing		Optimal date of sowing		Late date of sowing	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.
Years (1)	2	15023,7	0	10214,1	0	9397,9	0	5285,9	0
Date of herbicide application (2)	2	914,4	0	852,7	0	2332,1	0	1290,6	0
Set of herbicides (3)	8	1096,1	0	1484,6	0	919,5	0	351,7	0
1 x 2	4	1026,1	0	399,3	0	356,5	0	55,1	0
1 x 3	16	226,3	0	163,5	0	120,8	0	42,2	0
2 x 3	16	445,1	0	259,2	0	173,8	0	81,2	0
1 x 2 x 3	32	134,2	0	107,2	0	51,4	0	28,6	0

The set of tested herbicides had higher effect on the protein in grain regardless of the date of sowing in comparison to cultivar Enola. Although the effect was significant, in cultivar Enola the date of herbicide application had lower influence on the values of protein in comparison to cultivar Aglika. The strength of the combined interaction of the factors revealed considerable differentiation according to the cultivar and the date of its sowing. A permanent tendency was observed

toward greater strength of the interaction between the three factors on the values of protein after the late date of sowing in comparison to the optimal date, this effect being better expressed in cultivar Aglika than in cultivar Enola.

The raw protein yield was significantly influenced by the level of the tested factors and their combined interactions (Table 3). The meteorological conditions of the year were the determining factor for this index. Their effect was better expressed on cultivar Aglika than on cultivar Enola. A well expressed differentiation was observed in the independent and combinative interaction between the factors in the respective cultivars according to the stage of application of the herbicides.

In both cultivars the set of tested herbicides was the factor second in strength of effect (Figure 3). Its influence at the late date of sowing was stronger on cultivar Aglika (25.93 %) in comparison to cultivar Enola (14.75 %). At the optimal date of sowing the set of herbicides had higher effect on the raw protein yield of cultivar Enola (19.06 %). The independent effect of the stage of application of the herbicides, although significant, was comparatively weaker on cultivar Aglika at both dates of sowing – from 3 to 4 %.

In cultivar Enola the strength of effect of this factor increased more than three times, with a tendency towards higher strength at the late date of sowing. Among the combined interactions, best expressed was the interaction *date of application of herbicides x type of herbicides*. This interaction had stronger influence on the values of the index in cultivar Aglika in comparison to Enola. The full combinative interaction between the factors also had higher effect on the raw protein yield from cultivar Aglika than Enola, without significant differences between the two dates of sowing of the cultivars.

The grain from the two cultivars in 2013 was with lower concentration of protein in comparison to the other years of the investigation (Table 3). Most probably the higher air temperatures in May and June, accompanied with strongly expressed deficiency of soil moisture, had negative influence on its concentration in kernel. At the same time the raw protein yield, averaged for the cultivars, was 65.33 kg/da and was less than the yield obtained in 2012 with only 3.32 g/da. Regardless of the fact that in 2014 the highest values of raw protein content were obtained, averaged for the cultivars (12.90 %), the mean yield of raw protein was lowest for the investigated period – 48.03 kg/da. It was lower than the average yield for 2012 – 2014 (60.67 kg/da) with 20.83 % , and lower than the yield of 2012 968.65 kg/da) with 30.04 %.

In all three years of investigation cultivar Enola exceeded cultivar Aglika with averagely 18 % by size of the raw protein yield. In both cultivars the late date of sowing lead to slight decrease of the values of the index in comparison to the optimal date of sowing.

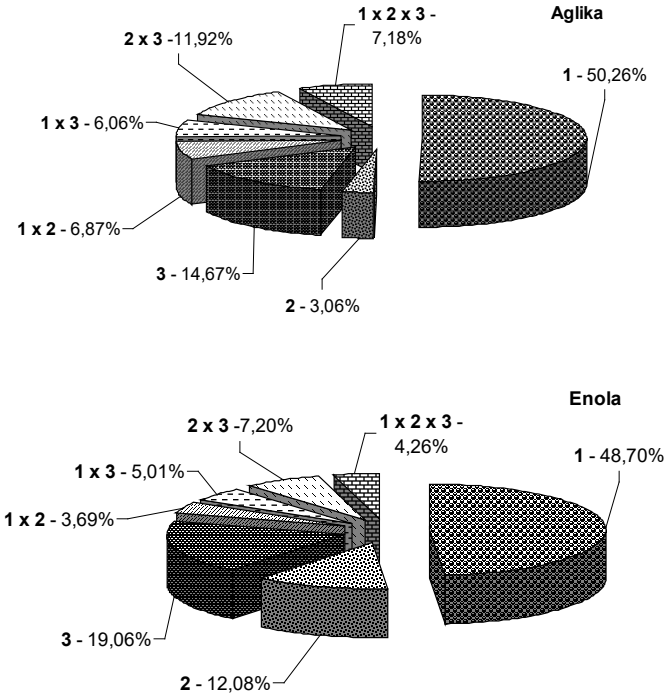

Figure 3. Strength of the factors' effect on grain protein yield

Table 3. Protein content and protein yield according to years of investigation

Years of investigation	AGLIKA				ENOLA			
	Optimal date of sowing		Late date of sowing		Optimal date of sowing		Late date of sowing	
	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da
2012	10,15 b	60,6 b	11,32 b	60,6 c	12,01 b	76,2 c	12,63 b	77,2 c
2013	8,87 a	63,8 c	9,09 a	59,5 b	9,10 a	69,0 b	9,71 a	69,0 b
2014	11,72 c	43,7 a	13,02 c	44,7 a	13,06 c	54,6 a	13,81 c	49,1 a

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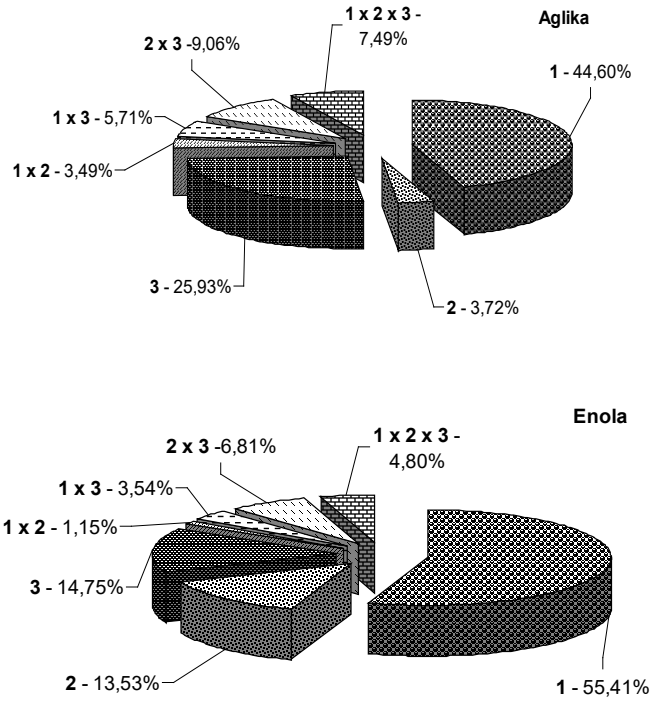

Figure 3. (continued) Strength of the factors' effect on grain protein yield

As already commented above, the stage of application of the tested herbicides lead to significant variations of raw protein content in grain and the obtained yields from it. Grain quality, as a polygenically dependant trait, is a combination of biological, physical and chemical, technological and utility characteristics, depending on a number of factors (Kolev et al, 2011).

The use of herbicides during the growth season is one of those factors with considerable importance for the expression of the biological potential of plants. The results from our investigation showed that in cultivar Aglika, regardless of the date of sowing, a tendency was observed toward some increase of the raw protein content after the late application of herbicides at stage 37 (Zadoks) – emergence of flag leaf (Table 4). In cultivar Enola the late application of herbicides on both

sowing dates had the opposite effect – slight decrease of the raw protein content. Both cultivars, regardless of the stage of herbicide treatment, formed grain with higher concentration of protein after the late date of sowing as compared to the optimal one. In cultivar Aglika this mean increase was with 8.8 % for all variants of the experiment, and in cultivar Enola – with 5.6 %.

Table 4. Protein content and protein yield according to the date of herbicides application and stages of wheat development (Zadoks).

Stage of development	AGLIKA				ENOLA			
	Optimal date of sowing		Late date of sowing		Optimal date of sowing		Late date of sowing	
	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da
Stage 29	9,2 a	54,1 a	10,6 a	53,4 a	11,6 b	71,0 c	12,5 c	72,1 c
Stage 32	10,6 b	59,1 c	11,4 b	57,9 b	11,6 b	68,3 b	11,9 b	65,3 b
Stage 37	11,0 c	54,9 b	11,5 b	53,5 a	11,0 a	60,4 a	11,7 a	57,8 a

The row protein yield as a function of two values (concentration of protein in grain and grain yield) is subjected to differentiation according to the date of sowing, the stage of herbicide application and the type of cultivar. The obtained mean results for the wheat crop showed that the use of the herbicides at a late stage of its development (emergence of flag leaf) caused significant decrease of the yield from raw protein – with about 10 % in comparison to the application of herbicides at tillering stage (29 Zadoks).

The genotypic differentiation in the response of the cultivars was very strong. For cultivar Enola the late application of herbicides after tillering stage decreased the protein yield at both sowing dates of the cultivar. This decrease reached 20 % after late sowing. This is indicative of high susceptibility with regard to grain yield since the decrease of protein in grain was not so strongly expressed. In relation to the obtained mean values of raw protein yield, cultivar Aglika was with well expressed tolerance to the stages of herbicide application. In practice, there was no decrease in the values of the index at the late date, and after their application at stage 32 Zadoks, a mean increase of raw protein yield with 9 % was determined in comparison to herbicide application at tillering. Regardless of this fact, averaged for the period of investigation, cultivar Enola exceeded cultivar Aglika with 18.6 % by raw protein yield. This tendency was present in all stages of application of the herbicides on both sowing dates of the cultivars, highest exceeding being determined after their use at tillering stage (29 Zadoks) – averagely with 33.1%.

The herbicides tested in the trial, as well as the two check variants, differentiated the mean values of raw protein content in grain of the investigated cultivars from

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10.7% in K_1 to 12.4% in Lintur 70 VG (Table 5). The infested check (K_2) and all tested herbicides increased the concentration of protein in grain in both cultivars after the two dates of their sowing. According to Peltzer and Bowran (1996) some of the herbicides increased the grain protein of wheat in the absence of weeds provided there was adequate available nitrogen. These tended to belong to specific mode of action groups; acetolactate inhibitors (Group A), inhibitors of photosynthesis (Group C) and the phenoxyalkanoic acids of Group I.

Highest differentiation with regard to the values of raw protein yield was found between the two check variants. Averaged for the period, the yield from the infested check was the lowest obtained and conceded to the manually weeded variant with 18.5 kg/da.

Averaged for the dates of sowing, cultivar Aglika reached a mean maximum of raw protein yield after the use of Secator OD – 60.0 kg/da, followed by K_1 – 59.3 kg/da. In cultivar Enola, the highest values of the index were obtained from the variants with Derby super VG – 71.6 kg/da, K_1 – 71.3 kg/da and Secator OD – 70.4 kg/da. The herbicides inhibitors of acetolactate synthase with action against broad-leaved and grass weeds (Palace 75 WG and Husar Max OD) lead to the lowest mean yields of raw protein obtained from both cultivars. They, however, significantly exceeded the infested check.

Table 5. Protein content and protein yield according to the set of herbicides.

Variants of treatment with herbicides	AGLIKA				ENOLA			
	Optimal date of sowing		Late date of sowing		Optimal date of sowing		Late date of sowing	
	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da	Protein content, %	Protein yield, kg/da
K_1 - check	9,4 a	58,4 e	11,0 c	60,2 j	10,7 a	70,9 f	11,5 a	71,6 f
K_2 - check	9,8 bc	44,0 a	10,9 b	40,5 a	11,3 cd	53,0 a	12,0 c	49,7 a
Derby super VG	9,8 c	57,5 d	11,1 cd	58,4 e	11,8 g	72,8 g	12,3 e	70,3 e
Palace 75 WG	9,7 b	52,5 b	11,0 c	54,6 c	11,1 b	63,4 b	12,2 e	65,3 cd
Granstar 75 DF	10,4 e	57,5 d	10,8 b	54,4 c	11,7 f	69,7 e	11,7 b	64,8 c
Lintur 70 VG	12,0 g	60,2 f	12,6 f	56,6 d	12,4 h	67,0 d	12,7 f	63,3 b
Husar Max OD	10,4 e	56,7 c	10,6 a	53,7 b	11,2 bc	65,1 c	12,0 c	64,7 c
Secator OD	10,6 f	60,6 j	11,2 e	59,3 f	11,3 d	70,9 f	12,2 de	69,8 e
Mustang 306,25CK	10,2 d	56,7 c	11,1 c	56,9 d	11,2 b	66,5 d	12,1 d	66,2 d

The genotypic specificity in the response of the two cultivars, averaged for the tested herbicides and the check variants, was very well expressed (Figure 4). The mean raw protein yields were among those obtained from the two check variants,

tending towards the yields obtained from the manually free-weed check (K_1). This fact is an indication that regardless of the various effects of the tested set of herbicides, they all have positive influence to different extent on the productivity of wheat with regard to protein.

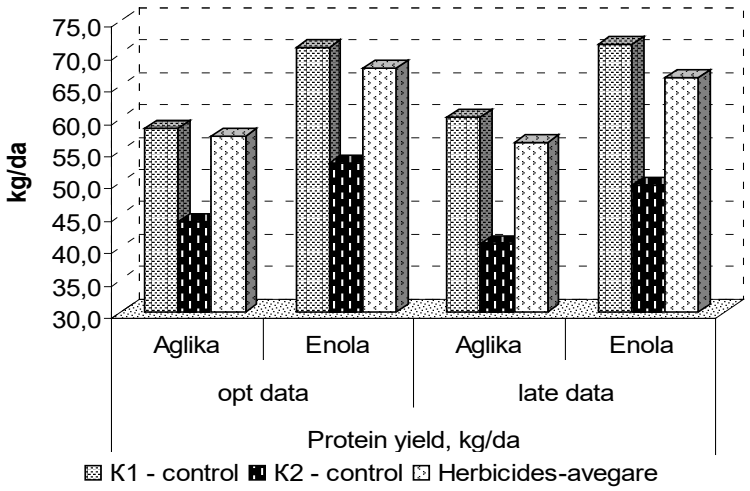

Figure 4. Raw protein yield averaged for the tested herbicides in comparison to the check variants during 2012-2014

Table 6. Correlations between grain yields and raw protein yields (Pearson Correlation)

Years	Aglika		Enola	
	Optimal date of sowing	Optimal date of sowing	Optimal date of sowing	Optimal date of sowing
2012	0,761(**)	0,756(**)	0,723(**)	0,820(**)
2013	0,438(**)	0,586(**)	0,807(**)	0,863(**)
2014	0,510(**)	0,839(**)	0,781(**)	0,875(**)

The correlation of grain yield with raw protein yield was significant for both cultivars (Table 6). The values of the correlation coefficients varied considerably depending on the year and the date of sowing. In cultivar Aglika, these values were lower in 2013 (a mean of ,512**), and in cultivar Enola – in 2012 (a mean of ,772**). The late date of sowing increased the correlation of grain yield with raw protein yield in both cultivars. The investigation showed that the mean values of this correlation were ,648** in cultivar Aglika, and ,812** in cultivar Enola. In years with high sum of the autumn and winter rainfalls and high sum of the rainfalls during the growth season (2014), the correlation of grain yield with raw protein

yield was also stronger.

Conclusions

A high level of statistical significance was found for the effect of the tested herbicides on the values of raw protein in grain and the grain yield from the studied cultivars under the conditions of the experiment. The years of investigation had the highest influence on the values of the tested characteristics. Best results were obtained in 2012.

At the late stage of herbicide treatment, the amount of protein decreased in both cultivars. Averaged for the investigated period, the size of protein yield from cultivar Enola was with 18.7 % higher than the yield from cultivar Aglika. In all three years of study, highest raw protein yield was obtained from the manually weed-free check (K_1), while the lowest was from the infested check variant (K_2). Averaged for the tested variants of the trial, only after treatment with herbicides Derby super VG and Secator OD the raw protein yield remained at the level of the weed-free check (K_1).

There was a strong positive correlation of grain yield with raw protein yield. Averaged for the period of investigation, the date of sowing of cultivar Aglika did not significantly affect the values of the correlation coefficients. In cultivar Enola, the late date of sowing caused sharp increase of the correlation. This tendency was best expressed in harvest year 2014 (r= ,875**).

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