ISSN 1392-3196
Zemdirbyste-Agriculture, vol. 95, No. 3 (2008), p. 259–265
UDK 633.11“324”:631.559:632.952

THE EFFECT OF AMISTAR FUNGICIDE ON GRAIN YIELD AND PROTEIN COMPOSITIONS IN WINTER WHEAT

Grażyna PODOLSKA

Institute of Soil Science and Plant Cultivation-National Research Institute
Czartoryskich 8, Pulawy, Poland
E-mail: grazyna.podolska@iung.pulawy.pl

Abstract

A microplot experiment was carried out in Pulawy, Poland in 2005 and 2006. The aim of the research was to analyze the grain yield, yield components and some quality characteristics in two cultivars of winter wheat under Amistar 250 S.C. fungicide application. The fungicide was applied at two different growth stages a) flag leaves (39 BBCH), and b) milk maturity growth stage (73 BBCH). Plots without fungicides were used as a control. Amistar fungicide application term had a substantial effect on most of the quality characteristics but had no effect on grain yield and yield components namely, 1000 kernel weight, number of heads per area unit, number of kernels per head. Administration of Amistar 250 S.C. fungicide in ‘Tonacja’ cultivar at flag leaf stage increased gluten content (data based on the comparison with the control). In ‘Sukces’ and ‘Tobacja’ cultivars application of investigated fungicide at the beginning of grain formation stage, decreased the gluten content. Use of fungicide affected the protein compositions like content of albumins and globulins, gliadins and glutenins. In ‘Tonacja’ cv. after fungicide application in flag leaf, the content of albumins and glutenins, gliadins and glutenins increased compared with the control. In ‘Sukces’ cv. the composition of proteins like gliadins and glutenins increased when fungicide had been applied at the beginning of seeds formation stage.

Key words: winter wheat, fungicide, yield, yield components, protein composition, quality.

Introduction

It is well know that environmental factors, especially temperature during kernel formation, nitrogen supply, cultivars, fungicide and herbicide as well as water stress influence grain yield and yield components /Rachoń, 1991; Ozturk, Aydin, 2004; Podolska et al., 2004/. Additionally, the baking quality highly depends on genotypic and environmental factors and it is recognized that variation in protein content and composition significantly modifies wheat quality /Borghi et al., 1995/. Protein fractions: albumins and globulins, gliadins and globulins are called protein compositions. Many studies have been conducted in an attempt to explain wheat quality variation as a function of genetic variation in protein composition /Triboi et al., 2000; Daniel, Triboi, 2000; Konopka et al., 2007/. Gliadins and glutenins are the major storage proteins in wheat. The genetic control and the relationship between gluten protein composition and quality characteristics become increasingly better understood. According to our knowledge, there are no papers in which the influence of fungicide on protein

composition has been reported. The aim of present research was to analyze the grain yield, yield components, some quality characteristics and protein composition in two cultivars of winter wheat under application of Amiastar 250 S.C. fungicide.

Materials and Methods

The effect of the fungicide was studied in a microplot experiment in 2004/2005 and 2005/2006 in Pulawy, Poland (51^{o} 25' N, 21^{o} 58' E). A two-factor experiment was done in split-plot design in four replications. Two cultivars of winter wheat namely, 'Tonacja' and 'Sukces' were used as the first experimental factor. The second factor was related to the 2 different winter wheat phenological growth stages at which the fungicide was applied: flag leaf growth stage (39 BBCH), milk maturity growth stage (73 BBCH), respectively. The plots without the fungicide application were used as the control. The area of each plot was 1x1 m. The crop was sown in September 2004 and September 2005, at a seed rate of 450 seeds m^{-2} and harvested in October 2005 and October 2006. Fifty kg N ha^{-1} was applied at the start of vegetative growth followed by 40 kg N ha^{-1} at shooting stage. The plants were protected against weeds by manual weeding. At the crop harvest, grain yield per m^2 and field components (number of plants and heads m^{-2}, 1000 kernel weight, productive tillering, grain yield per plant and per head, number of grains per plant and per head) were determined.

The seed qualities: total protein content (calculated after determining N content, using Dumas method, x 5.7), gluten content, sedimentation index, and falling number by Hagberg-Perten were determined after harvesting. Determination was performed according to ICC standard methods.

Protein extraction and analysis

Before analyses, grain was milled in a laboratory mill type IKA A10 (Labortechnik, Germany). Proteins were extracted using the solvent system developed by Wieser et al. (1998). Albumins plus globulins were twice extracted with 1 mL of 0.4 mol/L of NaCl with 0.067 of mol/L $HKNaPO_4$ (pH 7.6); gliadins were extracted with 1 mL of 60% ethanol (three-fold extraction), and glutenins were twice extracted with 1 mL of 50% 1-propanol, 2mol/L of urea, 0.05 mol/L of Tris-HCl (pH 7.5), 1% DTT, under nitrogen. The chromatographic separation was carried out on a Hewlett-Packard apparatus series 1050: RP-18 Vydac 218TP54 column with 5μm bead size and 300 Å pore size, 250 x 4.6 mm; a Zorbax 300SB-C18 pre-column, 4.6 x 12.5 mm; a column temperature of 45^{o} C, a mobile phase flow rate of 1 ml/min, and an injection volume of 20 μl. A two-component gradient was used. A component: 0 min 75%, 5 min 65%, 10 min 50%, 17 min 25%, 18 min 15% and 19 min 75%. The first component (A) was water with 0.1% of TFA, and the second (B) was ACN with 0.1% of TFA. The spectra were determined by a diode-array detector (HP 1050). Quantification of proteins was performed by UV absorbance at 210 nm. The identification of gliadins was based on the second derivative of their UV spectra according to the method developed by Dziuba et al. (2007).

Statistics

The data were subjected to the analysis of variance with Tukeya tests at a significance level of $p<0.05$.

Results and Discussion

Yield and yield components

In both growing seasons, there was no significant effect of Amistar 250 S,C fungicide spraying, on grain yield and yield components (number of plants and heads m^{-2}, 1000 kernel weight, productive tillering, grain yield per plant and head, number of grains per plant and per head) (Tables 1 and 2).

Table 1. Yield and yield components of winter wheat depending on the term of fungicide application in 2005

Trait	Term of fungicide application			LSD
	Flag leaf	Milk maturity	Control	
Grain yield kg m^{-2}	1.24	1.34	1.24	n. s.
Number of plants m^{-2}	403	396	419	n. s.
Number of heads m^{-2}	696	731	687	n. s.
1000 kernel weight	50.8	50.7	51.0	n. s.
Productive tillering	1.6	1.7	1.9	n. s.
Grain yield per plant (g)	1.78	1.83	1.80	n. s.
Grain yield per head (g)	1.03	1.00	1.11	n. s.
Number of grains per plant	35.4	35.1	36.0	n. s.
Number of grains per head	2.4	19.6	21.8	n. s.

Note. n. s. – no statistical differences

Table 2. Yield and yield components of winter wheat depending on the term of fungicide application in 2006

Trait	Term of fungicide application			LSD
	Flag leaf	Milk maturity	Control	
Grain yield kg m^{-2}	1.24	1.22	1.21	n. s.
Number of plants m^{-2}	400	395	392	n. s.
Number of heads m^{-2}	794	767	758	n. s.
1000 kernel weight	50.4	50.4	50.6	n. s.
Productive tillering	2.0	2.0	1.9	n. s.
Grain yield per plant (g)	3.1	3.1	3.1	n. s.
Grain yield per head (g)	1.56	1.59	1.60	n. s.
Number of grains per plant	62.6	61.3	60.6	n. s.
Number of grains per head	32.0	31.2	30.8	n. s.

No significant effect phenomenon was connected with marginal appearance of fungal disease in 2005 and 2006. Yield increase in 2005 on the plots sprayed with Amistar fungicide at milk maturity stage in comparison to the control, was approximately 8%. In contrast, fungicide application at flag leaf growth stage, gave a yield increase of approximately 2%. Jończyk (1999) claims that fungicide application, against leaf and head diseases, three times during vegetation season in winter wheat caused 14%

yield increase. The increase of winter wheat yield after fungicide spraying was also observed by another researcher; Golinowskiej (2001), Brzozowskiej (1997) and Jaczewska-Kalicka (2001).

Spraying with Amistar fungicide affected the quality parameters of winter wheat grain. In 2005, no effect of spraying application at various terms was observed on gluten content and gluten index (Tables 3–6). The protein content was affected by the interaction between cultivars and term of fungicide spraying. In cv. 'Sukces' the highest protein content was found in the control compared with the experiment conducted under application of fungicide at flag leaf growth stage. The cv. 'Sukces' gave higher protein content compared with 'Tonacja' but the differences were not significant. In 2006, the protein content was higher than in 2005 by approximately 1.38%. (Table 3)

Table 3. The effect of fungicide application, in relation to different cultivars, on protein content in winter wheat in 2005 and 2006

Year	2005			2006		
Term of fungicide application	Cultivar			Cultivar		
	Sukces	Tonacja	Average	Sukces	Tonacja	Average
Control	11.06	10.37	10.41	12.0	12.0	12.0
Flag leaf	10.58	10.25	10.75	11.5	12.0	11.8
Milk maturity	10.87	10.62	10.72	12.0	12.2	12.1
Average	10.83	10.41	10.62	11.8	12.1	12.0
LSD for						
Fungicide		n. s.			n. s.	
Cultivars		n. s.			n. s.	
Fungicide x cultivar		0.403			n. s.	

In 2005, the gluten content was lower than in 2006 by approximately 3.3%. There was no significant effect of experiment factors on gluten content in 2005. In 2006, the cv. 'Tonacja' gave higher gluten content compared with the cv 'Sukces'. The interaction between the term of fungicide application and type of cultivar was found. Under fungicide application at milk maturity stage, the cv. 'Sukces' gave the highest gluten content in comparison with the control (Table 4).

The gluten index was affected by experimental factors only in 2006. Application of fungicide at milk maturity stage in cv 'Sukces' resulted in lower gluten index detection than in the control experiment (Table 5).

In 2005, spraying with fungicide at flag leaf growth stage, in both cultivars, increased falling number compared with spraying at milk maturity stage. In 2006, only 'Tonacja' resulted in lower falling number in the case of spraying at milk maturity stage (Table 6).

Table 4. The effect of fungicide application, in relation to different cultivars, on gluten content in winter wheat in 2005 and 2006

Year	2005			2006		
Term of fungicide application	Cultivar			Cultivar		
	Sukces	Tonacja	Average	Sukces	Tonacja	Average
Control	23.5	22.4	23.0	25.55	27.75	26.65
Flag leaf	22.0	22.5	22.3	24.85	28.15	26.50
Milk maturity	22.5	23.0	22.8	23.45	26.75	25.10
Average	22.7	22.6	22.7	24.61	27.55	26.0
LSD for						
Fungicide		n. s.			n. s.	
Cultivars		n. s.			1.17	
Fungicide x cultivar		n. s.			2.03	

Table 5. The effect of fungicide application, in relation to different cultivars, on gluten index in winter wheat in 2005 and 2006

Year	2005			2006		
Term of fungicide application	Cultivar			Cultivar		
	Sukces	Tonacja	Average	Sukces	Tonacja	Average
Control	68	64	66	67	56	62
Flag leaf	67	65	66	57	63	60
Milk maturity	70	63	66	54	65	59
Average	68	64		59	61	
LSD for						
Fungicide		n. s.			n. s.	
Cultivars		n. s.			n. s.	
Fungicide x cultivar		n. s.			3.93	

Table 6. The effect of fungicide application, in relation to different cultivars, on falling number in winter wheat in 2005 and 2006

Year	2005			2006		
Term of fungicide application	Cultivar			Cultivar		
	Sukces	Tonacja	Average	Sukces	Tonacja	Average
Control	317	261	289	356	305	331
Flag leaf	336	305	320	349	330	339
Milk maturity	305	254	280	312	275	343
Average	319	273		372	303	
LSD for						
Fungicide		n. s.			n. s.	
Cultivars		36.21			33.14	
Fungicide x cultivar		48.61			55.75	

The analysis of chromatograms indicates a typical arrangement of protein fractions and a lack of qualitative differences between control and experimental factors (Table 7). Proteins soluble in 0.4 M NaCl (albumins and globulins) consist of 21% of the total protein content in the control kernels (Table 7). Wheat kernels obtained from the cv 'Tonacja'. were the richest in proteins. The gliadin content was differentiated in individual cultivars. 'Tonacja' cultivar had the highest amount of gliadins, while the 'Sukces' had the lowest. In both cultivars, the smallest gliadins sub-fraction were ϖ gliadins (about 3% of the total protein content in the case of 'Sukces' cultivar and 6% in the case of 'Tonacja'). The largest gliadin fraction was – α/β subunits fraction, consisting of 30% and 25% of total protein content in 'Sukces' and 'Tonacja', respectively. Glutenins were found to be the main group of storage proteins in both cultivars.

Table 7. Protein content of grain samples (mAU per kernel)

Cultivar	Treatment	Albumins + globulins	Gliadins		Glutenins			Sum
			ϖ	α/β	γ	HMW	LMW	
Sukces	Control	17362	2087	23340	12425	5035	18752	79001
	Flag leaf	16834	1928	24225	12405	5050	19230	79672
	Milk maturity	17217	2191	27512	12778	5291	19107	84095
Tonacja	Control	24419	6026	23652	10764	5715	25154	95731
	Flag leaf	26889	6939	28127	12269	6029	27633	107885
	Milk maturity	24953	6654	26196	11729	5779	26811	102123

In cultivars, the application of the fungicide at flag leaf growth stage decreased the content of albumins and globulins, while increasing the content of α/β gliadins and LMW glutenins. An average decrease was established at the level of approximately 4% for albumins. Increase at the level of approximately 4% for α/β gliadins and 3% for LMW. The most significant changes were observed in 'Tonacja' kernels. Spraying of fungicide at the flag leaf stage caused the increase of albumins and globulins by approximately 10% an increase of α/β gliadins by approximately 16% (Table 7).

The evaluation of using fungicides in winter wheat cultivation and their influence on seed and flour quality has not been described to the wide extend. Results obtained in this field are not consistent. For example, Rachon (1991) reported the increase of protein concentration in wheat kernels after using fungicide. Goodling however, reported that only test weight was increased. In contrast, Podolska (2004) has shown that there is an interaction between the weather conditions or protection against pests and grain quality formation.

Many papers reported significant effect on winter wheat protein compositions, depending on water stress, environmental conditions and nitrogen supply. These papers indicate that each factor influences protein content and protein composition in winter wheat in different, factor specific manner. For example, Daniel and Triboi claim that the temperature and nitrogen supply differently influenced the gliadin composition. The present studies conducted by Podolska indicate that protein composition in winter wheat kernels, clearly depends on cultivar and term of fungicide application.

Conclusions

1. The results presented in this study showed that the fungicide Amistar considerably affected seed quality and protein content and composition.

The effect was associated with weather conditions during the vegetation season (year), term of fungicide application and cultivar of winter wheat.

Quantitative effect was the most important one. In this study, the cultivar and term of Amistar application essentially affected the change in protein composition in grain.

2. In the cultivar 'Sukces', the application of the fungicide at flag leaf growth stage decreased the content of albumins and globulins and increased α/β gliadins sub fraction and LMW glutenins. 'Tonacja' responded differently from 'Sukces' to fungicide application at flag leaf stage resulting in an increase in albumins and globulins and an increase in α/β gliadins content.

Received 2008-07-31
Accepted 2008-08-19

REFERENCES

1. Blumenthal C. S., Barlow E. W. R., Wrigley C.W. Growth environment and wheat quality: The effect of heat stress on drought properties and gluten proteins; critical review article // Journal of Cereal Science. – 1993, vol. 18, p. 3–21

2. Borghi B., Giordani G., Corbellini M. et al. Influence of crop rotation, manure and fertilizers on bread making quality of wheat (*Triticum aestivum L*) // European Journal of Agronomy. – 1995, vol. 4, p. 37–45

3. Daniel C., Triboi E. Effect of temperature and nitrogen nutrition on the grain composition of winter wheat: effects on gliadin content and composition // Journal of Cereal Science. – 2000, vol. 32, p. 45–56

4. Gooding M. J., Dimmock J. P. R. E., Ruske R. et al. The effect of fungicides on the yield and quality of wheat grain // VII Congress ESA Book of Proceedings. Cordoba. – 2002, p. 441–443

5. Jaczewska-Kalicka A. Yield losses of winter wheat caused by fungal diseases // Prog. In Plant. Prot. – 2005, vol. 45, p. 722–724

6. Jończyk K. Efektywność chemicznego zwalczania chorób grzybowych w uprawie pszenicy ozimej i żyta // Pam. Puł. – 1999, vol. 114, p. 151–158

7. Konopka I., Tańska M., Pszczółkowska A. et al. The effect of water stress on wheat kernel size, color and protein composition // Polish Yournal of Natural Science (in press).

8. Ozturk A., Aydin F. Effect of water stress AT various growth stages on some quality characteristics of Winter wheat // J. Agron. Crop. Sc. – 2004, vol. 190, p. 93–99

9. Podolska G., Stypuła G., Stankowski S. Plonowanie i wartość technologiczna ziarna pszenicy ozimej w zależności od intensywności ochrony zasiewów // Annales Universitates Mariae Curie-Skłodowska Lublin-Polonia. – 2004, vol. LIX, Nr. 1, Sec. E, 59, 1 p. 269–276

10. Rachoń L. Plonowanie kilku odmian pszenicy ozimej w warunkach stosowania fungicydu i retardanta // Fragm. Agron. – 1991, vol. 3, p. 35–41

11. Triboi E., Abad A., Michelena A. et al. Environmental effects on the quality of two wheat genotypes;1.quantitative and qualitative variation of storage proteins // European Journal of Agronomy. – 2000, vol. 13, p. 47–64