Chapter 3. PLANT PROTECTION FOR FOOD SAFETY

ISSN 1392-3196
Zemdirbyste-Agriculture, vol. 95, No. 3 (2008), p. 242–250
UDK 633.11“324”:631.576

EFFICACY OF FUNGICIDE TREATMENTS ON THE WINTER WHEAT SENESCENCE, GRAIN YIELD, PROTEIN CONCENTRATION AND YIELD

Bronislava BUTKUTĖ, Vanda PAPLAUSKIENĖ, Irena GAURILČIKIENĖ

Lithuanian Institute of Agriculture (LIA)
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania
E-mail: brone@lzi.lt, vanda@lzi.lt, irena@lzi.lt

Abstract

Field experiments were conducted over 3 years to assess the effects of fungicides (F) containing strobilurins and triazoles on grain protein concentration, grain and protein yield of the winter wheat cv. ‘Zentos’. The efficacy of F use on the parameters tested depended on the weather conditions of the harvest year and on the F applied. F use prolonged retention of green canopy of wheat plants in 2003–2004. Chlorophyll concentrations in flag leaves at the end of plant vegetation, i. e. in the fifth week after F treatment, were higher in the plots treated with strobilurins compared with those treated with triazole propiconazole and untreated plots. F application significantly increased grain yield in all F-treated plots in 2003 and 2004, and in most plots in 2002. F use only insignificantly affected protein concentration in grain, which amounted to 12.6–14.8% DM in the plots treated with strobilurins, 13.0–14.6% DM – with triazoles and 12.8–14.5% DM in untreated plots. Protein yield was higher for the strobilurin-treated plots (with a small exception) than for those treated with propiconazole.

Key words: strobilurin, triazole, leaf chlorophylls, grain protein concentration, grain yield, protein yield.

Introduction

Wheat is subject to more diseases than other grains, and, in some seasons, especially in wet ones, heavier losses are sustained from those diseases than are in other cereal crops. Yield losses due to diseases vary between crops and regions and often amount to 10–20% /Hewitt, 2000/. Fungicides (F) are available to control most foliar diseases of wheat. F can be classified in a number of ways – one of them is mode of action or chemistry. The modes of actions of the F components, used in this study, are diverse. The strobilurins bind to one specific site, the quinol oxidation (Q_0) site, of cytochrome b and thereby stop energy production in the fungus which results in its death, while triazoles and morpholines prevent production of sterols – key components of fungal cell membranes /Yamaguchi, Fujimura, 2005; FRAC, 2007/. Strobilurins, with a chemistry based on a natural product from a mushroom, are F of new generation and proved to be quite effective, protective, eradicant and potential broad-spectrum substances against foliar diseases of winter wheat. They have low mammalian toxicity and are environmentally safe. In addition to disease control, strobilurins have useful non-fungicidal physiological effects: they improve nitrogen metabolism and inhibit ethylene biosynthesis /Grossmann, Retzlaff, 1997; Bertelsen et al., 2001 /. This latter effect is responsible for the greening effect which results in delayed senescence with higher amount of chlorophylls /Leandro et al., 1997; Habermeyer et al., 1998/ and index of photosynthesis /Häuser-Hahn et al., 2004; Oerke et al., 2004/.

F composed of several active ingredients are characterised by a wider mode of action, provide a versatile and lasting protection against fungal diseases, moreover, they prevent development of pathogen resistance /Kendal, Hollomon, 1994/. Conclusions of the research on leaf disease incidence and F use effects on grain quality are diverse and sometimes somewhat contradictory /Ruske et al., 2001; Clarck, 2003/. Fungicidal effect on protein concentration in grain is inconsistent and depends on the weather conditions of the year and varietal peculiarities / Ruske et al., 2003; Everts et al., 2005/.

The objective of the present study was to assess the effects of strobilurine and triazole F on the senescence of winter wheat leaves, protein concentration in grain, grain and protein yields.

Materials and Methods

The trials were conducted during 2002–2004 at the Lithuanian Institute of Agriculture in Dotnuva. The winter wheat cv. ʽZentos' was sown at a rate of 4.5 million seed per ha at 12 cm row spacings. Each plot had a surface area of 22 m^2 (2.2 m wide and 10 m long). The experimental design was a randomised block with four replicates. The treatments tested are listed in Table 1. F. were applied at the BBCH 47 (flag leaf sheath was opened) – BBCH 55 (middle of heading) stages. The plots were harvested by a harvester Sampo.

Table 1. Experimental design

Treatment, commercial product	Name of active ingredient (a. i.)	Rate of a. i. g ha^{-1}
1. Control	No fungicides	–
2. Allegro Plus	Kresoxim-methyl (S*) + epoxiconazole (T*) + fenpropimorph (M*)	125 + 125 + 150
3. Rombus	Trifloxystrobin (S)+ propiconazole (T)	125 + 125
4. Amistar	Azoxystrobin (S)	250
5. Opera	Pyraclostrobin (S)+epoxiconazole (T)	200 + 75
6. Acanto	Picoxystrobin (S)	250
7. Opus	Epoxiconazole (T)	125
8. Tilt	Propiconazole (T)	125

* Chemical group of active ingredient: S – strobilurins, T – triazoles, M – morpholines

Measurements of the concentrations of chlorophylls, total nitrogen in flag leaves of F – treated plants were started on the third day after treatment and were repeated weekly until the end of vegetation (5–6 weeks). Chlorophyll concentration were determined according to Ermakov /Ермаков и др., 1987/. Samples for the total nitrogen concentration determination in flag leaves were dried, ground by a mill with 1 mm sieve and analysed by the Kjeldahl method, using FOSS Tecator Kjeltec system with 1002 Distilling Unit. Pigment and nitrogen analyses were not done on the vegetative mass of the treatments that had lost greenness. Protein concentration in grain was calculated by multiplying the total nitrogen concentration, measured after Kjeldahl method, by the coefficient 5.7 (LST 1523).

The data were statistically processed by a software package ANOVA /Tarakanovas, Raudonius, 2003/.

The year 2002 was exceptional with warm and dry weather. Abundant rainfalls occurred only at the end of June and beginning of July. Although the air temperature was high, soil moisture was satisfactory. Due to the drought, the germination of the winter wheat sown in the autumn of 2002 was protracted and at the onset of winter the plants were at differed stages of development. In 2003 the spring was cool. A spell of warm weather occurred at the end of April. Before the middle of June there was a shortage of moisture not only at the 0–20 cm, but also at deeper (to 50 cm) soil layers: the fairly dry year of 2002 and the period during the winter months severely reduced soil water recharge. Temperately rainy weather coincided with wheat flowering stage, which ended late in June. An especially hot and dry period which started at the end of July–beginning of August was unfavourable for the formation, development and ripening of grain. The weather conditions in the spring of 2004 were similar to those of the year 2003, and although it was not rainy until the middle of June, the soil moisture did not drop below 15%. Because of cool weather, grain filling stage lasted longer than in the other experimental years.

Results and Discussion

Loss of chlorophyll (Chl) is the classical indicator of senescence in plants. The differences in the concentrations of Chl a + b as well as the differences in nitrogen concentrations between treatments during the first weeks after F spraying were low and inconsistent. As a result, this article will discuss the data of only the last three assessments. Lower concentrations of Chl were identified in the leaves from untreated plots compared with those in the samples taken from nearly all F treated ones starting with the fourth week from the spray application in 2003 and 2004 and the fifth week in 2002 (Table 2). No advantage of strobilurines over triazoles to prolong green leaf area retention was noted under the conditions of the year 2002. Inappreciably lower concentration of Chl in 2003 were identified in the 5th week after the F spraying in wheat leaves of the treatments sprayed with triazole F epoxiconazole (Opus), and especially with propiconazole (Tilt). In the 6th week in 2002 in all treatments, in 2003 in the untreated and propiconazole treated plots, in 2004 in the control treatment only the leaves had lost their greenness, whereas the vegetation of the rest of the treatments still continued. Alterations in nitrogen concentrations subject to F application or harvest year have the similar trend like those of Chl. Crop infection with foliar fungal diseases causes leaf drying, colour alterations and reduction of greenness, i. e. leaf pigment loss. Strobilurins inhibit respiration of fungi, while triazoles and morpholines arrest biosynthesis of fungi sterols. These F exert protectant and eradicant effects. Strobilurins and triazoles F application significantly suppressed the epidemic progress of Stagonospora leaf blotch and tan spot on the upper three leaves, including flag leaf both in 2003 and 2004 /Gaurilčikienė, Ronis, 2006/. Besides, the weakest control of leaf diseases was in propiconazole treatment.

Table 2. Alteration of the concentrations of chlorophylls a + b (mg/100 g fresh matter) in the flag leaves of winter wheat 'Zentos' as affected by the F applied

Year	Week after F treatment	Control	Allegro Plus	Rombus	Amistar	Opera	Acanto	Opus	Tilt
2002	4	238	241	271	226	241	209	205	223
	5	157	201	167	169	173	199	205	179
2003	4	263	310	340	346	331	317	311	325
	5	204	280	306	305	301	273	266	256
	6	N A	171	239	193	219	207	190	N A
2004	4	261	310	284	301	283	302	297	291
	5	357	360	375	385	365	365	361	370
	6	N A	168	175	164	187	169	172	151

NA – not analysed

Two-way analysis of variance (ANOVA) was carried out according to the following scheme: A factor F applied, B factor harvest year were used to reveal the significance of factors for winter wheat grain protein concentration and yield, and grain yield (Table 3). The tested factors were significant at $P<0.01$ probability for wheat grain and protein yields. The interactions of these factors significantly at $P<0.01$ affected grain yield and at $P<0.05$ protein yield. However, plant protection with F composed of different active ingredients did not have any impact on grain protein concentration, which is a very important grain quality indicator. The interaction between the use (treatments) of plant protection against fungal diseases and harvest year was also statistically insignificant.

Table 3. Significance of the effect of F applied and harvest year on wheat grain protein concentration and yield, and grain yield according to Fisher's criterion

Factor	Degree of freedom	Fisher's criterion		
		Protein concentration	Grain yield	Protein yield
Fungicides (F)	7	0.1	16.24**	11.67**
Harvest year (Y)	2	90.76**	524.48**	544.13**
Interaction FxY	14	0.93	2.48**	2.14*

This observation does not contradict the inferences about the significance of the air temperature and amount of precipitation at different plant development periods for the wheat grain yield and quality, including protein concentration, made by other researchers /Guttieri et al., 2001; Triboi et al., 2003; Mašauskienė, Cesevičienė, 2004; Souza et al., 2004/.

F efficacy depended firstly on the weather conditions being conducive to the occurrence of fungal diseases and then on F applied (Table 4).

Table 4. The effect of F on winter wheat productivity

Fungicide	Extra yield t ha^{-1}			
	2002	2003	2004	Mean
No fungicides	0 (7.52)	0 (6.11)	0 (8.50)	0 (7.38)
Kresoxim-methyl + epoxiconazole + fenpropimorph	0.94**	0.78**	1.85**	1.19**
Trifloxystrobin + propiconazole	0.62**	0.73**	1.39**	0.91**
Azoxystrobin	0.28	1.02**	1.84**	1.05**
Pyraclostrobin + epoxiconazole	0.88**	1.19**	2.16**	1.41**
Picoxystrobin	0.22	0.93**	1.54**	0.9**
Epoxiconazole	0.67**	0.86**	1.56**	1.03**
Propiconazole	0.34	0.58*	0.70*	0.54**
$LSD_{0.05}$	0.467	0.483	0.532	0.305
$LSD_{0.01}$	0.636	0.657	0.724	0.406

F application significantly increased grain yield, especially in the conditions of 2004. The last year was conducive to the occurrence of fungal diseases /Gaurilčikienė, Ronis, 2006/. The extra yield amounted to 0.70 and 2.16 t ha^{-1}. In 2002, the effect of not all the F used was considerable on the yield increase: the extra yield was statistically insignificant in the plots treated with F, containing only one active ingredient – strobilurin azoxystrobin and picoxystrobin, and triazole propiconazole. Both in 2003 and 2004 in all F-treated plots the yield significantly increased at 99% probability level, except for propiconazole treatment where yield increase was of 95% probability level. The data from 2003–2004 as well as the data averaged over all experimental years suggest that the highest winter wheat yield increase was obtained from the plots treated with the F pyraclostrobin + epoxiconazole, while the least increase was recorded for the plots applied with triazole propiconazole. The plots sprayed with the other F containing strobilurines and triazole epoxiconazole gave a similar average grain yield increase. The yield increased due to flag leaf's senescence delay, which was related to Chl concentration (Tables 2 and 4). Because the flag leaf makes up nearly 75% of the effective leaf area that contributes to grain fill /Kelly, 2001/, keeping it free of diseases with foliar F application can be beneficial for kernel development.

The plant protection products against fungal diseases only insignificantly affected protein concentration in grain, which amounted to 126–148 g kg^{-1} DM in the plots treated with strobilurins, 130–146 g kg^{-1} DM – with triazoles and 128–145 g kg^{-1}

DM in untreated plots (Table 5), even though F prolonged green leaf area retention compared with untreated plots. Similar regularities and conclusions are found and discussed in literature: the effects of F on grain protein concentration and its relationship with green area duration of the flag leaf were inconsistent over years /Kelly, 2001; Everts et al., 2001/. Protein concentration depended on the year. According to protein concentration, the grain grown in 2003–2004 met the requirements of the first class. Protein concentration in the grain of 2003 harvest, averaged across all treatments, was 138 g kg^{-1} and ranged from 136 g kg^{-1} in the plots treated with trifloxystrobin + propiconazole, pyraclostrobin + epoxiconazole and epoxiconazole to 141 g kg^{-1} in the plots treated with picoxystrobin and the F, containing three active ingredients of different chemical groups, i. e. kresoxim-methyl + epoxiconazole + fenpropimorph.

Table 5. Grain protein concentration and yield in relation to F application and harvest year

Treatment	Grain protein concentration g kg^{-1} DM				Grain protein yield kg ha^{-1}			
	2002	2003	2004	Average	2002	2003	2004	Average
Control	128	138	145	137	967	843	1232	1014
Allegro Plus	128	141	144	138	1096**	975**	1493**	1188**
Rombus	131	136	148	138	1067*	928*	1460**	1152**
Amistar	132	137	144	138	1030	980**	1490**	1167**
Opera	132	136	146	138	1108**	990**	1554**	1217**
Acanto	126	141	147	138	975	989**	1476**	1147**
Opus	130	136	146	137	1065*	951**	1465**	1160**
Tilts	131	139	144	138	1035	931*	1334*	1100**
Average	129.8	138.0	145.5	137.8	1043	948	1438	1143
$LSD_{0.05}$	7.21	6.99	4.04	3.79	78.56	66.54	93.16	51.30
$LSD_{0.01}$	–	–	–	–	107.96	90.60	126.84	68.12

In the yield of 2004, protein concentration varied from 144 g kg^{-1} (strobilurin azoxystrobin and triazole propiconazole treatments) to 148 g kg^{-1} in trifloxystrobin + propiconazole treated plots, with 145.5 g kg^{-1} on average. The grain from the 2002 harvest contained less protein, the differences between treatments did not exceed the least significant difference LSD_{05} between the treatments. Some literature sources suggest that if wheat does not receive nitrogen compounds during grain ripening period, less nitrogen will accumulate in grain /Triboi et al., 2003/. In 2002, during grain ripening period the weather was hot and sunny, however, shortage of rainfall and the drought interrupted plant vegetation, markedly accelerated grain ripening and at the same time nitrogen flow into grain. It is known that factors and measures aimed to maximize the yield exert an opposite effect on grain quality, especially protein concentration. For example, environmental factors that lead to high yields can also lead to a reduction in protein concentration, partly because it appears that nitrogen quantity per grain is relatively conserved when grain weight is modified by increased temperatures and restricted water availability / Triboi, Triboi-Blondel, 2002; Gooding et al., 2003/. The fact that F-treated wheat plots produced higher grain yield with undiminished protein concentration, indicates that plant protection against fungal diseases exerts a positive effect not only on dry matter but also on nitrogen accumulation in grain. This agrees with the data in literature: there were several instances where grain protein concentration was unaffected despite large (1.5 t ha^{-1}) increases in grain yield following F use /Ruske et al., 2003/.

Due to the F use, the response of protein yield was marked each experimental year (Table 5). Protein yield was higher for the strobilurin-treated plots (with a small exception) than for those treated with triazole propiconazole (Tilt) and control. Protein yield in the plots treated with triazole epoxiconazole was close to that produced in the plots treated with F containing some strobilurins like trifloxystrobin + propiconazole and picoxystrobin. The highest protein yield on average was obtained when F used contained both strobilurin and epoxiconazole: Opera and Allegro Plus. When estimating protein yield according to years, it is obvious that in 2004 when grain yield and protein concentration were the highest, protein yield was by approximately 1.5 times higher than that in 2003. Since F effect on protein yield was more obvious in 2004 than in 2002 and 2003, the difference between the highest and lowest protein yield in 2004 was 322 kg ha^{-1}, in 2002 – 141 kg ha^{-1}, and in 2003 – 147 kg ha^{-1}.

While comparing the data of grain yield and protein concentration of the years 2002 and 2003, the consequences of “dilution” effect, i.e. higher yield has a lower protein concentration, could be discerned. However, this phenomenon cannot explain such relationship between the yield and protein concentration of the grain grown in 2004. Dilution of grain protein concentration following F use, when it did occur, was small compared with what would be predicted by adoption of other yield increasing techniques such as the selection of high yielding cultivars (based on currently available cultivars) or by growing wheat in favourable climates /Ruske et al., 2003/. The year 2004 was favourable not only for dry matter accumulation but also for effective utilisation of mineral nitrogen for protein biosynthesis. Compared with the control treatment, in the F-sprayed crops photosynthesis was more intensive, during which carbohydrate accumulation and protein synthesis occurred uniformly, which prevented the consequences of “dilution” effect in significantly higher grain yield produced in the plots protected from fungal diseases by F treatments.

Conclusions

1. F prolonged green leaf area retention. This effect depended on the year: wheat leaves lost their greenness in the 6th week after F treatment in the year 2002 in all treatments, in 2003 greenness persisted in the plots treated with strobilurins and epoxiconazole, and in 2004 in all F-treated plots.

2. F application significantly increased grain yield in all F-treated plots in 2003 and 2004, and in most plots in 2002.

3. The plant protection against fungal diseases only insignificantly affected protein concentration in grain, which amounted to 126–148 g kg^{-1} DM in the plots treated with strobilurins, 130–146 g kg^{-1} DM – with triazoles and 128–145 g kg^{-1} DM in untreated plots. Protein concentration depended on the year.

4. Due to the F use, the response of protein yield was marked each experimental year. Protein yield was higher for the strobilurin-treated plots (with a small exception) than for those treated with triazole propiconazole (Tilt).

Received 2008-07-08
Accepted 2008-08-22

REFERENCES

1. Bertelsen J. R., de Neergaard E., Smedegaard-Petersen V. Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat // Plant Pathology. – 2001, vol., iss. 50, p. 190–205

2. Clark D. C. Agronomic implications of some morphological and biochemical effects of trifloxystrobin in wheat growing // Pflanzenschutz-Nachrichten Bayer. – 2003, vol. 56, iss. 2, p. 281–296

3. Everts K. L., Leath S., Finney P. L. Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat // Plant Disease. – 2001, vol. 85, p. 423–429

4. FRAC Code List 2007©*: Fungicides sorted by mode of action (including FRAC Code numbering). [2008 02 12], www.frac.info/frac/index.

5. Gaurilčikienė I., Ronis A. The effect of strobilurin fungicides on the development of foliar diseases of winter wheat // Agronomy research. – 2006, vol. 4, p. 177–180

6. Gooding M. J., Ellis R. H., Shewry P. R. et al. Effects of restricted water availability and increased temperatures on the grain filling, drying and quality of winter wheat // Journal of Cereal Science. – 2003, vol. 37, p. 295–309

7. Grossmann K., Retzlaff G. Bioregulatory effects of the fungicidal strobilurin kresoxim-methyl in wheat *(Triticum aestivum)* // Pesticide Science. – 1997, vol. 50, p. 11–20

8. Guttieri M. J., Stark J. C., O'Brien K. M. et al. Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit // Crop Science. – 2001, vol. 41, p. 327–335

9. Habermeyer J., Gerhard M., Zinkernagel V. The impact of strobilurins on the plant physiology of wheat // 7th International Congress of Plant Pathology, 9–16 August, 1998, Edinburgh, Scotland, [2008 03 19], http://www.bspp.org.uk/icpp98/5.6/3.html

10. Häuser-Hahn I., Baur P., Schmitt W. Fluoxastrobin (HEC5725) – biochemistry and hemodynamic behaviour of a new leaf systemic strobilurin fungicide // Pflanzenschutz-Nachrichten Bayer. – 2004, vol. 57, iss. 3, p. 437–450

11. Hewitt G. New modes of action of fungicides // Pesticide Outlook –2000, vol. 11, iss. 1, p. 28–32, [2008 03 19], http://pubs.rsc.org/ej/PO/2000/B006311H.pdf

12. Kelley K. Planting date and foliar fungicide effects on yield components and grain traits of winter wheat // Agronomy Journal. – 2001, vol. 93, p. 380–389

13. Kendall S., Hollomon D. W. Towards the rational use of triazole mixtures for cereal disease control // Pest and Disease / Brighton crop protection conference. – 1994, p. 549–556

14. Landau S., Mitchell R. A. C., Barnett V. et al. A parsimonious, multiple-regression model of wheat yield response to environment // Agricultural and Forest Meteorology. – 2000, vol. 101, iss. 2–3, p. 151–166

15. Mašauskienė A., Cesevičienė J. Žieminių kviečių technologinių savybių kitimas priklausomai nuo tręšimo azoto trąšomis ir grūdų laikymo trukmės // Maisto chemija ir technologija. – 2004, t. 38, Nr. 1, p. 49–60

16. Oerke E.-C., Steiner U., Beck C. et al. Influence of strobilurines on host plant physiology. In Modern Fungicides and Antifungal Compounds IV // Proceedings of the 14th International Reinhardsbrunn Symposium, 25–29 April, 2004, Friedrichroda, Thuringia / eds H.-W. Dehne, U. Gisi, K.-H. Kuck. et al., [2008 04 14], http://www.pk.uni-bonn.de/reinhardsbrunn/-abstracts/dehne-oerke.html

17. Ruske R. E., Gooding M. J., Jones S. A. The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning, remobilization and grain N accumulation in winter wheat cultivars // The Journal of Agricultural Science. – 2003, vol. 140, p. 395–407

18. Ruske R. E., Gooding M. J., Pepler S. The effect of adding azoxystrobin to a triazole programme on nitrogen uptake and partitioning by wheat // Aspects of Applied Biology. – 2001, vol. 64, p. 227–234

19. Souza E. J., Martin J. M., Guttieri M. J. et al. Influence of genotype, environment, and nitrogen management on spring wheat quality // Crop Science. – 2004, vol. 44, p. 425–432

20. Tarakanovas P., Raudonius S. Agronominių tyrimų duomenų statistinė analizė taikant kompiuterines programas ANOVA, STAT, SPLIT-PLOT iš paketo SELEKCIJA ir IRRISTAT. – Akademija (Kėdainių raj.), 2003. – 56 p.

21. Triboi E., Triboi-Blondel A.-M. Productivity and grain or seed composition: a new approach to an old problem – invited paper // European Journal of Agronomy. – 2002, vol. 16, p. 163–186

22. Triboi E., Martre P., Triboi Blondel A.-M. Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content // Journal of Experimental Botany. – 2003, vol. 54, iss. 388, p. 1731–1742

23. Yamaguchi I., Fujimura M. Recent topics on action mechanisms of fungicides // Journal of Pesticides Science. – 2005, vol. 30, iss. 2, p. 67–74

24. Ермаков А., Арасимович В., Ярош Н. и др. Спектрофотометрическое определение хлорофиллов *а* и *б* и каротиноидов // Методы биохимического исследования растений / ред. А. И. Ермаков, 3-е изд. – Ленинград: Агропромиздат, 1987, с. 107–109