A Multi-aspect Comparative Investigation on the Use of Strobilurin and Triazole - based Fungicides for Winter Wheat Disease Control

Irena Gaurilčikienė, Bronislava Butkutė and Audronė Mankevičienė,
Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry
Lithuania

1. Introduction

Fungicides continue to be essential for the effective control of plant diseases. The azoles class of fungicides has been the leading agents for the control of fungal pathogens of plants since their introduction in the mid 1970s. This class of fungicides is also called sterol demethylation inhibitors (DMIs) because they inhibit the formation of cytochrome P450 sterol 14 alpha-demethylase (450 14DM), an enzyme required for the biosynthesis of ergosterol. Ergosterol is an important compound required for fungal membrane integrity and cell cycle progression (Dahl et al., 1987). DMIs represent one of the largest groups of systemic fungicides that have been used to control agriculturally important fungal pathogens (Zhan et al., 2006). In the mid 1980s, the first DMIs fungicide propiconazole has been investigated and introduced in the agronomic practice of Lithuania for winter wheat disease control (Šurkus et al., 1988) and currently 10 DMIs active substances alone or in mixtures are registered for cereal disease control. In spite of their long-term use, a widespread resistance to azoles in plant pathogenic fungi has not occurred. The shift to decreasing sensitivity propiconazole to *Phaeosphaeria nodorum* the causal agent of Stagonospora blotch of wheat has been reported since 1994 (Peever et al, 1994) although until 1994 only minor changes in the sensitivity of *Mycosphaerella graminicola* populations – c.a. Septoria leaf blotch, to DMIs fungicides in European countries was confirmed (Turner et al., 1996). The slightly resistant field isolates were not cross-resistant to the DMI fungicides, which act at a different stage of sterol biosynthesis (Hollomon, 1994). Sometimes the cross-resistance is only partial. Whilst cross-resistance extends to all DMIs (Gisi et al., 2004) a recent rapid decline in the efficacy of some DMIs fungicides in controlling powdery mildew and Septoria leaf blotch of wheat has been noted. Some fungicides, notably epoxyconazole and prothioconazole, are still very effective in controlling *M. graminicola* (Cools & Fraaije, 2008).
In the beginning of 90s a new class of fungicides, generated on the basis of the fungus *Strobilurus tenacellus* secondary metabolites was developed (Ammermann et al., 1992; De Vleeschauwer et al., 1996). Strobilurins, with a chemistry based on a natural product from a mushroom, are fungicide 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 also inhibit ethylene biosynthesis. This latter effect is responsible for the greening effect which results in delayed senescence with higher amount of chlorophylls (Habermeyer et al., 1998) and index of photosynthesis (Häuser-Hahn et al., 2004; Oerke et al., 2005). According to Inagaki et al. (2009), strobilurins can act on delaying root water uptake, resulting in postponement of soil dehydration, which contributes to a slight increase of grain yield in some wheat genotypes in the field under water deficit conditions.

These broad-spectrum strobilurins or Qol (quinone outside inhibitors) fungicides based on affecting the mitochondrial respiration of the fungus cell by inhibiting the electron transport at the cytochrome-bc_1 complex have been registered in Lithuania since the year 2000 and nowadays 7 active substances mostly in mixture with azoles are used in agricultural practice. It is known that the foliar strobilurin treatment in wheat had resulted in both the suppressing foliar diseases and maintenance of green leaf area as a result - period of grain filling being extended, and thus likely contributing to a yield increase (Gooding et al., 2000; Dimmock & Gooding 2002). Qol fungicides gave adequate control of the main cereal foliar diseases despite the disease pressure. Strobilurins have a single-site mode of action and have therefore been classified as at high risk of resistance (Brent & Hollomon, 2007). Consequently, for a short time the strains of powdery mildew (*Blumeria graminis*) and Septoria (*M. graminicola)* with reduced sensitivity to Qols were identified (Sierotzki et al., 2000; Gisi et al., 2002). In recent years, the efficacy of strobilurin fungicides against Septoria leaf blotch and powdery mildew has declined markedly because of widespread cross-resistance (Sierotzki & Gisi, 2006). Fungicides 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 (Kendall & Hollomon, 1994).

Septoria leaf blotch (*Mycosphaerella graminicola* (Fuckel) J. Schröt., syn. *Septoria tritici* Berk. & M.A. Curtis*)*, Stagonospora blotch (*Phaeosphaeria nodorum* (E. Müll.) Hedjar. syn. *Stagonospora nodorum* (Berk.) E. Castell. & Germano; *Septoria nodorum* (Berk.) Berk.*)* and tan spot *(Pyrenophora tritici repentis* (Died.) Drechsler, syn. *Drechslera tritici-repentis* (Died.) Shoemaker*)* are the main foliar diseases and can be a constraint to wheat yields in Lithuania and in many countries with a temperate climate. Despite the differences in biology, these diseases frequently occur together. Both Septoria and Stagonospora blotch are economically important worldwide (Cunfer & Ueng, 1999). *P. nodorum* is known to be more pathogenic at late growth stages and can cause high levels of leaf and glume blotch at higher temperatures. The cooler temperatures of spring favour development of leaf blotch caused by *M. graminicola*. The greater prevalence of *P. nodorum* later in the season may be a consequence of increasing susceptibility of wheat plants to pathogen (Shaner & Beuchley, 1995). The prevalence of *P. nodorum* later in the season also can be predicted by interaction between pathogens. As Nolan et al. (1999) reported *P. nodorum* inhibits *M. graminicola* and it was concluded that the latter has a stimulatory effect on spore production by *P. nodorum*. Tan spot has recently become recognised as one of the important and widespread diseases of wheat. Losses due to tan spot have been chronically 3–15% and as high as 50% of grain yield (Hosford et al., 1987). The pattern of diseases development varied in each year under the influence of different rainfall and temperature patterns.

In Central Europe severe epidemics of Fusarium Head Blight (FHB) occur once or twice a decade only, but it can sharply reduce yield and quality traits of cereals (Miedaner at al., 2001). Surveys carried out between 1951 and 1985 recorded 19 FHB outbreaks with a wheat grain yield reduction of 5-15% in the years when moderate epidemics of FHB were recorded and up to 40% in years when disease epidemics were severe (Pirgozliev et al., 2003).

Under suitable conditions the foliar diseases as well as FHB cause dramatic symptoms and can result in substantial losses in both yield and quality through the production of undersized grain either by affecting grain processing qualities or by producing a range of toxic metabolites. This paper provides the results of a multi-aspect comparative investigation on the use of strobilurin and triazole - based fungicides for winter wheat disease control. The aim was to study yield losses resulting from foliar and ear diseases epidemics and define the relationships between disease severity, green canopy retention, grain quality and yield.

2. Winter wheat leaf disease control with strobilurin-based and triazole

On the basis of field trials conducted during the period 2002–2004 at the Lithuanian Institute of Agriculture in Dotnuva the efficacy of fungicides against fungal leaf diseases of winter wheat cv. Zentos was explored. The impact of strobilurin based Allegro Plus, Rombus, Amistar, Opera, Acanto and triazoles Opus and Tilt (Table 1) on the epidemic progress of Septoria leaf blotch (*M. graminicola)* and Stagonospora blotch (*P. nodorum)* – both diseases were not separated, and tan spot (*P. tritici-repentis)* was surveyed. The trials were arranged in randomized blocks in the plots 10 m in length and 2.5 m in width with four replicates. The fungicides, except Folicur, were applied at the end of booting (BBCH 47), Folicur was applied at anthesis for FHB control.

Commercial name	Applied at BBCH	Active ingredients (g l^{-1})	Rate	
			fungicide (l ha^{-1})	active ingredients (g ha^{-1})
Allegro Plus	47	Krezoxim–methyl; epoxyconazole; fenpropimorf (125+125+150)	1.0	125+125+150
Rombus	47	Trifloxystrobin; propiconazole (125+125)	1.0	125+125
Amistar	47	Azoxystrobin (250)	1.0	250
Opera	47	Pyraclostrobin + epoxyconazole (133+50)	1.5	200+75
Acanto	47	Picoxystrobin (250)	1.0	250
Opus	47	Epoxyconazole (125)	1.0	125
Tilt	47	Propiconazole (250)	0.5	125
Folicur	65	Tebuconazoze (250)	1.0	250

Table 1. Characteristics of fungicides used for the winter wheat disease control

Disease assessments on leaves were conducted periodically with 7-10 days interval from the end of booting (BBCH 47) to late milk stage (BBCH 79). Plant growth stages were identified according to the BBCH scale (Phenological growth stages..., 1997). Percent of leaf area showing symptoms of leaf diseases was used to quantify disease severity. Disease severity was assessed on each plot in five randomly selected places on three adjacent tillers on three upper leaves using a percentage scale 0, 1, 5, 10, 25, 50, 75. The leaf positions on tillers were numbered relative to the uppermost leaf - the flag leaf. Thus the leaf immediately below the flag leaf (F) was designated F–1, the second leaf below the flag leaf – F–2. AUDPC (Area Under the Disease Progress Curve) was calculated by trapezoidal integration in accordance with 7-10 days interval disease severity data over the season.

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i-1}}{2}\right)(t_{i+1} - t_i) \quad (1)$$

where: y_i – disease severity %, t_i – interval of data records (days), n – number of assessments (Campbell & Madden, 1990). The plots were harvested and yields in t ha^{-1} were adjusted to 15% moisture content, thousand grain weight (TGW) was calculated. The significance of data was determined by the Fisher's criterion with a significance level of $P≤0.01$ and ≤0.05. Significant differences from untreated in Tables are marked as **($P≤0.01$) and *($P≤0.05$). Linear correlation analysis was used to examine the relationships between grain yield and AUDPC.

In 2002, fungal leaf diseases incidence and severity in winter wheat were slight due to extremely dry and unusually hot weather until harvest. In both 2003 and 2004, the pressure of fungal leaf diseases was severe. The unusually hot period during the wheat ripening stage in 2003 provoked the intense outbreak of tan spot *(P. tritici-repentis)*. At milk ripe abundant and severe tan spot infection dwarfed Septoria leaf and Stagonospora blotch on wheat leaves. However, the warm and rainy prolonged ripening season in 2004 promoted the severe infection of Septoria disease (Stagonospora blotch and Septoria leaf blotch). Both diseases were not separated, though in years 2002-2004 Stagonospora blotch *(P. nodorum)* considerably prevailed over Septoria leaf blotch *(M. graminicola)*. AUDPC allows expression of entire season's leaf diseases epidemic on different fungicide treatments. AUDPCs were plotted for each of the topmost three leaves from the data of the fungicide use.

Our experimental findings suggest that both strobilurin and triazole fungicides significantly suppressed the epidemic progress of Septoria disease (*P. nodorum* and *M. graminicola)* and tan spot *(P. tritici-repentis)* on the upper three leaves of winter wheat irrespective of dissimilar diseases pressure in particular years. In 2002 the development of both Septoria disease and tan spot was slight due to droughty weather and the AUDPC values in fungicide treated plots were low and comparable (Table 2). In both 2003 and 2004, infection of leaf diseases was vigorous. The lowest AUDPC value was recorded in the treatment with Opera (pyraclostrobin + epoxyconazole), while the highest – in the treatment applied with Tilt (propiconazole). AUDPCs of the other strobilurin fungicides and triazole Opus (epoxyconazole) were comparable. Our results suggest that the weakest control of Septoria disease ant tan spot was in Tilt treatment. As was reported by Milus and Chalkley (1997) propiconazole was the least effective treatment against *P. nodorum*. In order of precedence between DMI fungicides in 1994 epoxyconazole showed the best efficacy against Septoria both *M. graminicola* and *P. nodorum* (Kendall & Hollomon, 1994). Still recent epoxyconazole and prothioconazole are referred as very effective in controlling *M. graminicola* (Cools & Fraaije, 2008). According to sensitivity test of 42 isolates from Sweden, collected in 2003-

2005, the *P. nodorum* population is still sensitive to propiconazole, prothioconazole and cyprodinil, even though some isolates varied in sensitivity to triazoles (Blixt et al., 2009). The results of semi-field trial carried out in Denmark showed that propiconazole + fenpropimorf had only a residual preventive effect for 10 days in comparison to azoxystrobin, which showed a very effective, long preventive effect against *P. nodorum*, lasting for three weeks or more, however against *M. graminicola* both propiconazole and azoxystrobin had a similar curative effect (Jorgensen et al., 1999). After the strobilurin-based fungicides were introduced in 1994, this new class of fungicides provided a superior disease control and additional favourable effects on the physiology of the plant (Fraaije et al., 2003). In the years 2003 and 2004 the high efficacy of Qol fungicides was confirmed by our results (Table 2).
The first QoI-resistant isolates of *M. graminicola* were detected in the UK in 2001 at low frequency in QoI-treated plots (Fraaije et al., 2005), and subsequently in 2002 in five European countries (Gisi et al., 2004). The G143A mutation causing QoI resistance was first detected during 2002 in all tested populations (Torriani et al., 2009). During 2003 and 2004, the frequency of resistant isolates increased rapidly in northern Europe. The resistance is now widespread across the entire UK, whereas in France and Germany resistance levels are higher in the north than in the south (Fraaije et al., 2005). Resistance to QoI fungicides in *P. tritici-repentis* was detected in 2003 in Sweden and Denmark. The rapid increase of the frequency of the mutations F129L and G143A in pathogen population was observed and in 2005 both mutations were found in a significant proportion of the isolates from Sweden, Denmark and Germany (Sierotzki et al., 2007). A first report of variability in sensitivity of *P. nodorum* to azoxystrobin was made by Blixt et al. (2009). In the majority of the *P. nodorum* isolates, collected in 2003-2005 in Sweden, the mutation G143A, associated with loss of sensitivity to strobilurins was found. Recently Lithuanian population of *M. graminicola* has been showing medium resistance levels and *P. tritici-repentis* resistance levels are highly variable (www.frac.info). As for the increment of pathogen resistance to fungicides, the results of efficacy of the tested fungicide nowadays presumptively may be different than our presented. In commercial practice in Lithuania Qol-based fungicides are widely used and till now have given a good response to grain yield due to adequate disease control and maintenance of green leaf that act as grain filling prolongation.
Our results suggest that the highest yield and thousand grain weight (TWG) increase in experimental years 2002-2004 were recorded in Opera (pyraclostrobin + epoxyconazole) treatment, as top effective against Septoria disease and tan spot, and the lowest in Tilt (propiconazole) treatment, where it gave the lowest efficacy against these diseases (Table 8). In the case of high disease pressure in 2003 and 2004, the grain yield (X) and AUDPC (Y) of both Septoria disease and tan spot on F, F–1 and F–2 leaves showed strong negative linear correlations at a probability level of $P \le 0.01$. Regression coefficient R2y/x for Septoria *(P. nodorum* and *M. graminicola)* ranged between 73.3-91.9% and for tan spot – 83.6-92.7%. However, in the droughty year 2002, regarding slight disease severity only Septoria disease AUDPC on F-1 leaves against yield showed a significant ($P \le 0.01$) negative correlation (R2 71.4%). Our experimental findings on the correlation between the disease severity and yield agree with those obtained in Australia. As Bhathal et al. (2003) reported the different rates of progress of both tan spot and Stagonospora blotch caused similar losses in grain yield, ranging from 18% to 31%. The infection by either disease on the flag or penultimate leaf provided a good indication of yield losses.

Treatment#	AUDPC					
	Septoria disease (*P. nodorum* and *M. graminicola)*			Tan spot (*P. tritici-repentis*)		
	F §	F–1 §§	F–2 §§§	F §	F–1 §§	F–2 §§§
2002						
Untreated	8.6	9.6	4.3	18.4	14.7	1.1
Allegro Plus	0	0.2	0.3	9.0	5.8	1.3
Rombus	0.1	0.7	0.6	7.1	3.8	0.7
Amistar	0.4	2.2	0.6	3.1	3.9	0.6
Opera	0.1	0.2	0.3	2.7	2.2	1.2
Acanto	1.0	5.2	0.3	6.7	4.7	1.0
Opus	0.1	0	0.6	8.5	6.4	0.4
Tilt	0.2	1.6	0	7.0	4.7	1.4
2003						
Untreated	43.3	10.9	151.8	287.6	411.3	446.3
Allegro Plus	0.2	2.1	30.5	110.9	60.8	136.0
Rombus	0.9	1.5	42.8	109.2	62.5	128.8
Amistar	0.8	0.9	39.6	79.4	47.0	106.5
Opera	0	0.2	35.8	41.7	12.4	33.9
Acanto	4.2	1.6	36.9	107.8	62.0	103.8
Opus	0.1	0.4	38.6	89.3	30.9	96.6
Tilt	8.7	1.4	37.6	208.6	180.2	291.9
2004						
Untreated	135.2	490.1	649.7	108.6	155.5	153.8
Allegro Plus	5.5	45.0	98.8	33.4	48.9	52.2
Rombus	12.3	60.5	133.5	31.1	63.7	76.3
Amistar	8.9	53.3	100.1	28.0	40.8	49.0
Opera	1.2	19.2	40.8	9.9	14.0	28.6
Acanto	29.2	84.7	127.3	39.9	40.7	43.0
Opus	7.8	35.5	103.2	22.6	35.4	57.8
Tilt	39.1	156.2	306.7	51.7	63.6	75.1
In all treatments AUDPC value difference significant at P ≤0.01; #See Table 1 for fungicide rates; §F – flag leaf; §§F-1 – first below flag leaf; §§§F-2 – second below flag leaf.						

Table 2. The effect of strobilurin and triazole fungicides on the epidemic progress of Septoria disease (*P. nodorum* and *M. graminicola)* and tan spot (*P. tritici-repentis*) in winter wheat.

3. The incidence of Stagonospora blotch *(P. nodorum)* on winter wheat ears and grain

The epidemic incidence of Stagonospora blotch in winter wheat canopy is determined by the amount of rainfall, number of rainy days, previous crop and straw residue on soil surface, infected seeds and other factors (Hansen et al., 1994). Gradual epidemics of Stagonospora blotch were characterized by disease arising on successive leaf layers as they appeared during sustain periods of weather suitable for inoculum's transport and infection. Ordinary spores from infected plants are transported by rain splash to 0.5 m and to 40 cm above ground (Griffits & Ao, 1976). Wheat seed infection by *P. nodorum* is common, and infected seeds can be an important source of primary inoculum for foliar epidemics. According to Griffiths and Ao (1976) under the conditions prevailing to *P. nodorum* the significant distance for spreading from infected plant is about 2 m until heading. With this degree of spread less than 1000 infected plants per hectare are needed at the pre-heading stage to generate a widespread and severe attack and this situation arises if only 0.016% of seeds sown gave rise to infected plants. Transmission of the pathogen from infected seeds to coleoptiles can approach 100% over a wide soil temperature range; transmission to the first leaves is less than 50% and is most efficient at soil temperature below 17 °C. At least 44% of infected first leaves can be symptomless. (Shah & Bergstrom, 2000).

On the basis of field trials the efficacy of strobilurin-based and triazole fungicides on Stagonospora blotch on ears and laboratory analyses of grain infestation with *P. nodorum* is discussed. Percent of affected ears was used to quantify the disease incidence. Disease severity was assessed on each plot in five randomly selected places on three adjacent ears using a percentage scale 0, 1, 5, 10, 25, 50, 75. Grain infestation with *P. nodorum* in 2003 was tested using blotter-freezing method (Kietreiber, 1981) and in 2004 - both blotter-freezing and SNAW agar method (Manandhar & Sunfer, 1991).

As our experiments indicated, both strobilurin and triazole fungicides significantly suppressed the incidence and severity of Stagonospora blotch on leaves (Table 2). However, in the case of sudden outbreak of infection on ears, the incidence of glum blotch was irresistible. In 2003, abundant and severe tan spot infection prevailed and blanketed out Stagonospora blotch on wheat leaves until milk ripe. After some heavy rains at milk ripe Stagonospora blotch developed rapidly and fully occupied ears not only in untreated, but also in fungicide treated plots. In contrast, during 2004 disease developed rapidly before milk ripe and slowly later in the season. Rainy period during stem elongation and from heading to fruit development stage in that year caused a severe Stagonospora blotch infection on leaves (Table 2). Later in the season lack of precipitation and hot weather limited disease severity on glumes. Irrespective of Stagonospora blotch dissimilar incidence and severity on ears in 2003 and 2004 (Table 3), *P. nodorum* infection on wheat seeds was comparable. Similar results were obtained in Poland: correlation of level of seed infestation and subsequent disease severity was in many cases statistically insignificant (Arseniuk et al., 1998). In 2003, in spite of similar fungicide efficacy on leaves and ears only in Opera treatment significant decrease of *P. nodorum* seed infection was observed. In 2004, seed infection in parallel was tested by Blotter-freezing test and on SNAW (Septoria nodorum agar for wheat). Both Blotter-freezing and SNAW showed comparable results. In both experimental years strobilurin fungicides gave better protection of grain against *P. nodorum* infection, in comparison with triazoles.

Wheat seed infestation by *P. nodorum* is common, the extent and range depending mainly on rainfall during the production season (Shah & Bergstrom, 2000). According to Shaw et al., (2008) long term (1844-2003) archived samples analysis fluctuations in amount of *P. nodorum* in grain were related to changes in spring rainfall, summer temperature and national SO_2 emission. *P. nodorum* seed infection is influential primary infection source in wheat crop. *P. nodorum* transmission to seedling coleoptile and leaves occurred over a broad temperature range. Under the high densities at which wheat is sown, a significant number of infected seedlings per unit area may originate from relatively low initial seed infection levels and transmission efficiencies (Shah & Bergstrom, 2000). As Arseniuk et al. (1998) reported planting of highly infected seed resulted in more severe Stagonospora blotch than did the planting of healthy or only slightly infected seed; however, environmental conditions contributed considerably to disease severity. Planting in clean fields, using seeds with a low level of seed-borne inoculum in addition treated with fungicides and applying foliar fungicide application contributed toward reducing leaf infestation by *P. nodorum*, severity of leaf and glume blotch and incidence *P. nodorum* in the harvested seed (Milus & Chalkley, 1997).

Treatments[#]	Infection on ears		Infected grain (%)	
	Incidence (%)	Severity (%)	Blotter test	SNAW
2003				
Untreated	100	28.8	22.3	-
Allegro Plus	100	19.4**	17.5	-
Rombus	100	17.6**	21.5	-
Amistar	100	12.8**	19.8	-
Opera	100	12.9**	9.7*	-
Acanto	100	12.1**	18.0	-
Opus	100	18.8**	23.2	-
Tilt	100	21.65	26.2	-
2004				
Untreated	100	3.65	31.5	27.4
Allegro Plus	85.0**	1.12*	22.0	25.6
Rombus	81.6**	1.08*	15.6**	23.4
Amistar	73.3**	0.80**	18.0*	13.3**
Opera	58.4**	0.58**	19.8*	11.9**
Acanto	76.7**	1.03*	18.0*	11.9**
Opus	80.0**	1.22*	21.7	21.2
Tilt	90.0*	2.73	21.0	26.1

* Difference significant at P ≤0.05. ** Difference significant at P ≤0.01;
#See Table 1 for fungicide rates;

Table 3. The incidence and severity of Stagonospora blotch on winter wheat ears and grain infestation with *P. nodorum* in response to strobilurin-based and triazole fungicides

During the last several years in Lithuania a clear predomination of *M. graminicola* over *P. nodorum* has been observed (unpublished data). It may be determined by mild winters and agronomic factors. As Shaw et al. (2008) reported, annual variability among of *M. graminicola* or *P. nodorum* was dominated by weather factors occurring over a period longer than the growing season.

4. The impact of triazole and strobilurin-based fungicides on the incidence of Fusarium head blight toxic fungi and mycotoxins on winter wheat grain.

The *Fusarium* species predominantly found associated with FHB in wheat and other small-grain cereals all over Europe are *F. graminearum, F. avenaceum, F. culmorum, F. poae.* Among the less encountered species are *F. cerealis, F. equiseti, F. sporotrichioides, F. tricinctum* and several others witch are less pathogenic, but also toxigenic (Bottalico & Perrone, 2002; Birzele et al., 2002). In addition to true *Fusarium* species, *Monographella nivalis* may also cause head blight and can be particularly prevalent where cooler and wetter conditions prevail (Nicholson et al., 2003). The predominant *Fusarium* species on cereal grain during 1999–2002 under Lithuanian conditions were *F. culmorum, F. equiseti, F. avenaceum, M. nivalis* and *F. oxysporum.* The frequency of detection of *Fusarium* spp. micromycetes on cereal grain was 93.5 % (Lugauskas et al., 2004). Most species producing inocula, grow best, and are more pathogenic to cereal heads at warm temperatures and under humid conditions (Doohan et al., 2003). FHB is of particular concern because of the ability of the *Fusarium* species to produce mycotoxins in the grains that are harmful to human and animal consumers. The most frequently encountered mycotoxins in FHB in Europe have proved to be deoxynivalenol (DON) and zearalenone (ZEA) produced by *F. graminearum* and *F. culmorum*. Nivalenol was usually found associated with deoxynivalenol and its derivatives, formed by *F. graminearum, F. cerealis, F. culmorum* and, in northern areas, by *F. poae*. Moreover, from central to northern European countries moniliformin has been consistently reported, as a consequence of the widespread distribution of *F. avenaceum*, whereas the occurrence of T-2 has been recorded in conjunction with sporadic epidemics of *F. sporotrichioides* and *F. poae*. The beauvericin has recently been found in Finnish wheat colonized by *F. avenaceum* and *F. poae* (Bottalico & Perrone, 2002; Nicholson et al., 2003).

The control of *Fusarium* spp. and *M. nivalis* and the production of DON, including both naturally and artificially inoculated trials, were reported. Application of fungicides to reduce FHB gave a different control of these fungi. Tebuconazole selectively controlled *F. culmorum* and *F. avenaceum* and reduced levels of DON, but showed little control *M. nivalis*. Application of azoxystrobin, however, selectively controlled *M. nivalis* and allowed greater colonisation by toxigenic *Fusarium* species. This treatment also led to increased levels of DON detected. Azoxystrobin application two days post-inoculation increased the production of DON per unit of pathogen in artificially inoculated field trials. This result indicates the potential risk of increasing DON contamination of grain following treatment with azoxystrobin to control head blight in susceptible cultivars (Simpson et al., 2001). The effect of different fungicide treatments on FHB, grain yields and DON was evaluated after artificial inoculation under field conditions with a mixture of *F. graminearum* and *F. culmorum* on five different cultivars of soft and durum wheat. Treatments with cyproconazole + prochloraz and mixture tebuconazole + azoxystrobin significantly reduced the FHB disease severity (by 25 and 77 %) and DON content (by between 32 and 89 %) in the grain as compared with the inoculated control. Yields and thousand grain weight were higher in the plots subjected to fungicide treatments (Haidukowski et al., 2005).

The aim of our experiments was to investigate the efficacy of strobilurin-based and triazole fungicides on FHB in winter wheat and laboratory analyses of grain contamination with micromycetes which can occur on ripening ears of wheat and mycotoxins in relation to the application timing and environmental factors. Furthermore, control of these fungi, and in particular the prevention of increasing concentration of mycotoxins are discussed.

For investigation of mycoflora of grain at different maturity stages (BBCH 75 and BBCH 85–87) non–disinfected grain, taken directly from ears randomly from each plot (10 grains per ear, 10 ears per plot) were planted on PDA (potato dextrose agar). The plates were incubated in the dark at 25 °C for 7 days. The infection level of grain was evaluated in percent (0 – all grains healthy, 100% – all grains infected). Microscopic studies of *Fusarium* fungi were carried out after 7–8 days and were identified on the basis of their cultural and morphological characteristics according to Leslie and Summeral (2006).

The mycotoxins DON, ZEA, and T-2 were analysed by the CD-ELISA (competitive direct enzyme-linked immunosorbent assay) method. The Veratox® quantitative test kits (Neogen corporation, Food Safety Diagnostics), approved by the AOAC Research Institute (Certificate N 950702) were used for the analysis. The optical densities of samples and controls from standard curve were estimated by a photometer Neogen Stat Fax®303 Plus, using filter of 650 nm. Measured absorbances were automatically converted to the mycotoxin concentration units - μg kg^{-1}. The results were estimated taking into account the lowest calibration curve's mycotoxin concentration value (LOD-limit of detection), which is for: DON – 100.0 μg kg^{-1} (ppb); ZEA – 10.0 μg kg^{-1} (ppb); T-2 – 7.5 μg kg^{-1} (ppb).
The occurrence of *Fusarium* spp. in wheat ears depended mostly on the climatic conditions during flowering. Continuous rainfall during and after flowering with lower temperatures and longer period of open inflorescence increased grain infection level (Birzele et al., 2002). In 2003, wheat flowered from 20 to 26 of June. During the three ten–day periods before flowering warm weather conditions prevailed, except for several days with weak rain during the heading stage. Only the showery rainfall before and during the flowering occurred. Folicur was applied on 23 June. In 2004, continuous rainfall predominated before, during and after flowering, with lower temperatures. That year wheat flowered from 24 June to 2 July. Folicur was applied on 28 June.
In 2003, the appearance of FHB was late and slight. At dough stage only 12 % of ears showed slight symptoms of FHB (Table 4).

Treatment #	BBCH 75		BBCH 85	
	FHB Incidence (%)	Grain surface infection (%)	FHB Incidence (%)	Grain surface infection (%)
2003				
Untreated	0	50.5	12.0	33.3
Opera	0	45.3	1.0**	40.8
Tilt	0	45.3	4.0	12.5**
Opera and Folicur	0	35.8*	4.0	34.0
Tilt and Folicur	0	35.0*	4.0	14.3**
2004				
Untreated	55.0	5.8	38.3	23.5
Opera	31.7*	8.5	25.0*	56.0*
Tilt	53.3	10.0	41.6	50.0
Opera and Folicur	41.7	6.0	23.3*	24.0
Tilt and Folicur	61.7	9.3	31.6	15.8

* Difference significant at $P \leq 0.05$; ** Difference significant at $P \leq 0.01$;
#See Table 1 for fungicide rates and application time

Table 4. Incidence of FHB and grain infection in wheat at milk (BBCH 75) and dough (BBCH 85) ripe stages in 2003 and 2004

A significant reduction in diseased ears in Opera treated plots at booting was obtained. Slow disease incidence might have resulted from dry plant debris before flowering, because when soil moisture content is below 30 %, ascospore production is not possible. When it is greater

than 80 %, ascospore production is at its maximum. Peak ascospore release occurred 2–4 days after rainfall and its dispersal is associated with rainfall too (Xu, 2003). In 2004, due to the rainy weather, FHB appearance was early and abundant. The first symptoms of infected glumes were spotted at milk ripe. At that time half of the ears were diseased. Later in the season, at dough stage the incidence of FHB was similar to that at milk ripe. Only in Opera treated plots the incidence of FHB was significantly lower than in untreated. The consistent control of FHB, achieved through fungicides under field conditions, depends on the application timing. Studies where fungicides were applied between BBCH 32 and 50 failed to reveal any significant reduction of FHB (Hutcheon & Jordan, 1992). However, when fungicides were applied between BBCH 59 and 70 significant reductions were achieved in both the severity of FHB and concentration of mycotoxins in harvested grain. According to Mathies and Buchenauer (2000), applications of either tebuconazole or prochloraz, 2 days post inoculation (BBCH 65) reduced disease severity by 56 and 41 %, whilst application 8 days pre–inoculation and 9 days post–inoculation were less effective.
In 2003, in spite of unfavourable conditions for FHB at milk ripe in fungicide untreated plots *Fusarium* spp. was detected on 50.5 % non–disinfected grain, taken directly from ears (Table 4). Application with fungicides at the end of booting did not influence grain infection level, however, additional application with tebuconazole significantly decreased grain infection. Later, at hard dough stage grain contamination with *Fusarium* spp. was lower than at milk ripe. It might be related to glum residual surface infestation, which at milk ripe together with grain got into PDA. Significant decrease of grain contamination at that stage was recorded in the treatment with Tilt applied at booting and additionally with Folicur at flowering. In 2004, at milk ripe there were found only 5.8–10 % *Fusarium* spp. contaminated grain, nevertheless at dough ripe the infection level increased markedly, especially in the plots treated with fungicides at booting. In the plots applied with Folicur at flowering *Fusarium* spp. contaminated grain content was twice as low as in the plots treated only at booting.
In spite of different levels of FHB infection, *Fusarium* infected grain content (internal infection) after harvesting in untreated plots was found similar to that in 2004 and 2003 (Table 5). It might have been determined by severe epidemics of foliar diseases, early dried leaves and early matured plants in theses plots. In 2003, *Fusarium* spp. infected grains in Folicur treated plots decreased significantly. That year in all grain samples there was detected DON and a slight grain contamination with ZEA and T-2 was found. In all fungicide treated plots ZEA concentration in grain was significantly lower, and especially low in Folicur treatment. T-2 was detected in all grain samples, but at negligible quantities and the influence of fungicide treatment was inconsistent. In 2004, in fungicide treated plots in post–harvested samples *Fusarium* spp. infected grain made up 12.3–20.8 %, while in untreated 7.8 %. In Opera treatment the increase (20.8 %) was significant. DON concentration in grain samples taken from fungicide treated plots at booting was significantly higher than in untreated and treated with Folicur at flowering. That year relatively high concentration (65.4 μg kg^{-1}) of ZEA was found in untreated grain samples. ZEA in fungicide treated harvested grain samples was not detected.
Survey of results of two years' investigation showed that chemical control of FHB and grain contamination with *Fusarium* spp. under field conditions might be inconsistent. Field trials conducted to assay the efficacy of fungicides against FHB gave conflicting results. Effective chemical control of FHB is confounded by the fact that disease is caused by a complex of pathogens which interact with one another and with saprophytic species such as *Alternaria*

Treatment #	*Fusarium* infected grain (%)	Content of mycotoxins μg kg^{-1}		
		DON	ZEN	T-2
2003				
Untreated	7.5	160.3	27.7	<LOD
Opera	7.8	129.8*	13.8*	7.5
Tilt	4.0	125.0*	12.5*	<LOD
Opera and Folicur	2.3	122.0*	n.d.	<LOD
Tilt and Folicur	3.3	144.8	<LOD	8.1
2004				
Untreated	7.8	126.1	65.4	n.d.
Opera	20.8	135.0	n.d.	7.9
Tilt	15.0	142.0	n.d.	<LOD
Opera and Folicur	12.3	125.0	n.d.	8.0
Tilt and Folicur	15.8	n.d.	n.d.	n.d.

* Difference significant at $P \leq 0.05$; ** Difference significant at $P \leq 0.01$;
#See Table 1 for fungicide rates and application time

Table 5. Effect of different mode and action fungicides on internal *Fusarium* spp. infection and contamination with mycotoxins DON, ZEN and T-2 in harvested grain

spp. and *Cladosporium* spp. (Pirgozliev et al., 2003). According to Milus and Parsons (1994) fungicides benomyl, chlorothalonil, fenbuconazole, flusilasole, propiconazole, tebuconazole had no effect on FHB, mycotoxins levels or yield of harvested grain. Conversely, Siradinou and Buchenauer (2001) showed that applications of tebuconazole 2 days before and 2 days post-inoculation of wheat plots with *F. culmorum* reduced severity of FHB and DON content in wheat grain by 61–89 % and 50–70 %, respectively and metconazole by 69 % and 71 %, respectively. Application of fungicides to reduce FHB gave a differential control of *Fusarium* spp. and *Monographella nivalis*, which is known as non–toxigenic causal agent of FHB. Tebuconazole selectively controlled *F. culmorum* and *F. avenaceum* and reduced levels of DON, but showed little control of *M. nivalis*. Application of azoxystrobin, however, selectively controlled *M. nivalis* and allowed greater colonization by toxigenic *Fusarium* spp. This treatment also led to increased levels of DON detected (Simpson, 2001).

Due to the lack of consistently effective control measures, FHB continues to pose a significant threat to the yield and quality of wheat. Based on our experimental evidence from both field experiments and laboratory investigations, in the future we have to focus on some of the complex interactions between *Fusarium* infections, differential effects of fungicides, environmental factors and associated mycoflora which can occur on ripening ears of wheat. Experimental years were not conducive to the spread of FHB, as a result, efficacy of fungicide use against this disease did not stand out sufficiently, especially in mycotoxin tests. Discussions have been raised in scientific literature recently about the “masked” or conjugated mycotoxins (Berthiller et al., 2007; Galaverna et al., 2009). It is believed that in growing plants, especially under the effects of natural and anthropogenic factors (including fungicides) the already formed toxic metabolites can biotransform into other very similar chemical compounds that are simply not detected by the methods used by us. As a result, our future research will be focused on masked mycotoxins in naturally contaminated cereals.

5. Efficacy of strobilurin based and triazole treatments on the winter wheat senescence, grain yield and quality.

Yield losses due to diseases vary between crops and regions and are often 10–20 % (Hewitt, 2000). Research evidence on the effects of disease incidence and fungicide use on grain quality and safety are diverse and sometimes controversial (Mesterhazy et al., 2003; Covarelli et al., 2004; Wang et al., 2005; Polišenská & Tvaružek 2007; Gärtner et al., 2008; Clarck, 2003; Everts et al., 2001; Ruske et al., 2003). This part of our study was aimed to estimate the effects of fungicides containing strobilurins and triazoles on the senescence of winter wheat cv. Zentos leaves, grain yield and quality.

Measurements of the concentrations of chlorophylls, total nitrogen in flag leaves of fungicides - treated plants were started on the third day after treatment and were repeated weekly until the end of vegetation (5-6 weeks). Photosynthetic pigments and nitrogen analyses were not done on the vegetative mass of the treatments that had lost greenness. Chlorophylls a, b and carotenoids concentration was determined according to Ermakov (Ermakov et al., 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. Protein concentration in grain was calculated by multiplying the total nitrogen content, measured after Kjeldahl, by the coefficient 5.7. Wet gluten content was determined in flour (milled with Perten 3100 mill) by hand washing with excess water according to AACC method 38-10. Gluten deformation index was measured by IDK-1M instrument (standard LST:1522). The falling number and the sedimentation value according to Zeleny (Zeleny index) were determined following the ICC standard methods 107/1, 118 and 116/1.

The experimental data were compared by the analysis of variance (ANOVA), where the F-ratio was significant, the least significant difference (LSD) was calculated for $P \leq 0.05$ (symbol *) and $P \leq 0.01$(symbol **). The weather conditions after the resumption of vegetative growth of winter wheat were diverse and were discussed in the previous section of the chapter.

In the absence of fungicide, green leaf area decline was associated with drought or infection with a number of foliar pathogens (Gooding et al., 2000). Loss of chlorophyll is the classical indicator of senescence in plants. Our observation showed very low and inconsistent differences in the concentrations of chlorophylls a+b as well as the differences in nitrogen concentrations between treatments during the first weeks after fungicide spraying. As a result, the current paper will discuss the data of only the last three assessments. Lower concentrations of chlorophylls were identified in the leaves from untreated plots compared with those in the samples taken from nearly all fungicide treated ones starting with the fourth week from the spray application in 2003 and 2004 and the fifth week in 2002 (Table 6). No advantage of strobilurins over triazoles to prolong green leaf area retention was noted under the conditions of the year 2002. Inappreciably lower concentrations of chlorophylls in 2003 were identified in the 5th week after the fungicide spraying in wheat leaves of the treatments sprayed with triazole fungicide Opus (epoxyconazole), and especially with Tilt (propiconazole). In the 6th week in 2002 in all treatments, in 2003 in the untreated and Tilt treated plots, in 2004 in the control treatment only, the leaves had lost their greenness, whereas the vegetation of the rest of the treatments in 2003 and 2004 still continued. Alterations in nitrogen concentrations subject to fungicide application or harvest year have the similar trend like those of chlorophylls. Crop infection with foliar fungal diseases cause leaf drying, colour alterations and reduction of greenness, i.e. leaf pigment loss.

Year	2002		2003			2004		
Week after F treatment	4	5	4	5	6	4	5	6
Fungicide[#]	Chlorophylls a+b (mg /100 g fresh matter)							
Control	238	157	263	204	NA	261	357	NA
Allegro Plus	241	201	310	280	171	310	360	168
Rombus	271	167	340	306	239	284	375	175
Amistar	226	169	346	305	193	301	385	164
Opera	241	173	331	301	219	283	365	187
Acanto	209	199	317	273	207	302	365	169
Opus	205	205	311	266	190	297	361	172
Tilt	223	179	325	256	NA	291	370	151
	Nitrogen (g kg^{-1} dry matter)							
Control	25.2	25.6	35.6	29.8	NA	33.1	27.8	NA
Allegro Plus	26.4	28.2	37.6	32.4	24.7	30.6	30.1	24.8
Rombus	27.4	27.4	37.9	35.7	28.0	31.7	30.1	29.8
Amistar	25.3	27.1	38.7	33.4	24.7	35.4	30.1	27.6
Opera	27.9	27.0	39.4	34.5	25.0	34.6	31.9	27.8
Acanto	25.2	26.5	35.8	33.4	25.5	31.3	35.1	29.1
Opus	25.4	26.5	37.9	32.0	24.5	32.4	32.2	27.3
Tilt	27.3	26.7	38.5	29.5	NA	33.4	31.1	26.9

[#]See Table 1 for fungicide rates; ¶NA – not analysed

Table 6. Alteration of the concentrations of chlorophylls a+b and nitrogen in the flag leaves of winter wheat cv. Zentos as affected by the fungicide applied

Strobilurins inhibit respiration of fungi, while triazoles and morpholines arrest biosynthesis of fungi sterols. These fungicides exert protecting and eradicant effects. Strobilurins and triazoles fungicides 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.

In sum we can conclude, that all fungicides tested prolonged retention of green canopy of wheat plants in 2003-2004. Chlorophylls concentrations in flag leaves at the end of plant vegetation, i.e. in the fifth week after fungicide treatment, were higher in the plots treated with strobilurins compared with those treated with triazole Tilt and untreated plots. There is abundant information in literature that strobilurins have considerably stronger positive effect on plant metabolic activity than any other compounds (e.g. azoles) (Beck, 2004), with the extension of flag leaf life, related to higher chlorophyll content (Habermeyer et al., 1998; McCartney et al., 2007) and higher CO_2 consumption, i.e. photosynthetic index (Häuser-Hahn et al., 2004; Oerke et al., 2005). According to Zhang et al. (2010) fungicide, including strobilurins, treatments appeared to delay the senescence of wheat and increase the grain yield of wheat, owing to retarding the enhancement of active oxygen species and the decrease of antioxidative enzyme activity during aging of wheat. However, slow up of leaf senescence depends on a variety (Beck, 2004; Habermeyer et al., 1998; Oerke et al., 2005; Ruske et al., 2003).

Two-way analysis of variance (ANOVA) was carried out according to the following scheme: A factor fungicide applied, B factor harvest year were used to reveal the significance of factors for winter wheat grain yield and quality components tested, as well as for protein and gluten yields (Table 7). The tested factors were significant at $P \leq 0.01$ probability for wheat grain, protein and gluten yields. The interactions of these factors significantly at $P \leq 0.01$ affected grain yield and at $P \leq 0.05$ protein yield.

Factor	Fisher's test							
	GY	Pr	Glu	GluDI	ZI	FN°	PrY	GluY
Fungicides (F)	16.24**	0.1	1.29	0.59	0.69	0.63	11.67**	7.55**
Harvest year (Yr)	524.5**	90.76**	149.4**	93.9**	266.0**	57.42**	544.1**	202.1**
Interaction FxYr	2.48**	0.93	0.47	0.54	1.02	0.75	2.14*	1.22

Table 7. Significance of the effect of the fungicide applied and harvest year (2002-2004) on grain yield (GY), protein (Pr), gluten (Glu) concentrations, gluten deformation index (GluDI), Zeleny index (ZI), falling number (FN°), grain protein and gluten yield (PrY, GluY) according to Fisher's test

However, plant protection with fungicides composed of different active ingredients did not have any impact on grain quality parameters. The interaction between the use (treatments) of plant protection against fungal diseases and harvest year was also statistically insignificant. 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; Cesevičienė & Mašauskienė, 2008; Triboi et al., 2003; Souza et al., 2004). So, fungicide efficacy on grain quality depended firstly on the weather conditions being conducive to the occurrence of fungal diseases and then on fungicide applied.

Fungicide application significantly increased grain yield in all fungicide-treated plots in 2003 and 2004, and in most plots - in 2002, and especially in the conditions of 2004 (Table 8). 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 fungicides used was considerable on the yield increase: the extra yield was statistically insignificant in the plots treated with fungicide, containing only one active ingredient - strobilurin Amistar and Acanto, and triazole Tilt. Both in 2003 and 2004, in all fungicide-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 fungicide Opera, while the least increase was recorded for the plots applied with triazole Tilt. The plots sprayed with the other fungicide containing strobilurins and triazole Opus gave a similar average grain yield increase. The yield increased due to flag leaf's senescence delay, which was related to chlorophylls concentration (Table 6 and 8). Because the flag leaf makes up nearly 75% of the effective leaf area that contributes to grain fill (Kelley, 2001), keeping it free of diseases with foliar fungicide application can be beneficial for kernel development.

Fungicides help to retain vegetation of the flag leaves, allowing conditions for larger grains growth. Since in 2002 drought and heat occurred, upper leaves withered up early in all plots, including fungicide-treated ones and TGW increase in most of the treatments was minor (Table 8). However, in 2003 and 2004 the TGW increase was significant in fungicide treated plots, with the two exceptions in 2003 – plots sprayed with strobilurin based fungicide

Treatments[#]	2002		2003		2004	
	Yield increase	TGW increase	Yield increase	TGW increase	Yield increase	TGW increase
Untreated	7.52§	47.1§	6.11§	45.2§	8.50§	49.7§
Allegro Plus	0.94	0.4	0.78	1.2	1.85	3.8
Rombus	0.62	0.2	0.73	2.2	1.39	4.3
Amistar	0.28	0.6	1.02	2.4	1.84	5.5
Opera	0.88	1.0	1.19	3.0	2.16	5.9
Acanto	0.22	0.2	0.93	2.5	1.54	5.5
Opus	0.67	1.0	0.86	2.5	1.56	4.8
Tilt	0.34	0.5	0.58	1.4	0.70	2.5
LSD $_{0.05}$	0.461	0.88	0.475	1.42	0.549	1.12
LSD $_{0.01}$	0.616	1.17	0.635	1.90	0.734	1.50

[#]See Table 1 for fungicide rates; ¶TGW – thousand grain weight g; §Grain yield and TGW in untreated plots

Table 8. Effects of fungicide treatment on grain yield (t ha^{-1}) and thousand grain weight (g) increase

Allegro Plus and triazole Tilt. The largest grains grew and the highest TGW increase due to the use of fungicides was achieved in 2004, thanks to prolonged period of grain filling and maturation through cool and rainy weather.

Our findings suggest that compared with untreated plots, all fungicides applied in the experiment had only an insignificant effect on all the tested wheat grain quality parameters with rare exceptions (Table 9). Since the protein and gluten are the most important and obligatory indicators describing grain quality, we paid more attention to their variation peculiarities. The tables do not present the data of protein as well as gluten concentration and other quality parameters values for separate treatments of each year since quality variation among treatments was insignificant. In relation to the fungicides use, protein concentration in grain fluctuated within the similar range: 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, even though fungicide prolonged green leaf area retention compared with untreated plots. kg^{-1} DM in untreated plots, even though fungicide prolonged green leaf area retention compared with untreated plots. Similar regularities and conclusions are found and discussed in literature: the effects of fungicides on grain protein concentration and its relationship with green area retention of the flag leaf were inconsistent over years (Kelley, 2001; Everts et al., 2001). Protein concentration depended on the year. Protein concentration in the grain of 2003 harvest, averaged across all treatments, was 138 g kg^{-1} and ranged from

Quality parameter	2002			2003			2004		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Protein, g kg^{-1}	130	126	132	138	136	141	146	144	148
Gluten, g kg^{-1}	245	240	254	299	287	311*	274	268	278
Gluten DI, units	85.1	84.0	89.2	78.4	76.0	81.6	67.3	63.2	70.6
Zeleny Index, ml	43.5	39.8	47.2*	63.6	62.1	65.2*	57.3	54.8	58.8
Falling N°, s	417	398	426	468	461	475	373	366	383

Table 9. Variation of wheat grain quality as affected by the fungicide applied and harvest year

136 g kg^{-1} in the plots treated with Rombus (trifloxystrobin+propiconazole), Opera (pyraclostrobin+epoxyconazole) and Opus (epoxyconazole) to 141 g kg^{-1} in the plots treated with Acanto (picoxystrobin) and the fungicide, containing three active ingredients of different chemical groups, i.e. Allegro Plus (kresoxim-methyl + epoxyconazole + fenpropimorph).

In the yield of 2004 protein concentration varied from 144 g kg^{-1} (strobilurin Amistar and triazole Tilt treatments) to 148 g kg-1 in Rombus treated plots, with 146 g kg^{-1} on average. The grain from the 2002 harvest contained less protein, but the differences between treatments did not exceed 95% confidence limits. 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 (Gooding et al., 2003; Triboi & Triboi-Blondel, 2002). The differences in mean annual values of grain quality parameters show that harvest year conditions affected wheat grain quality formation more significantly, than fungicides use. The fact that fungicide-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 fungicide use (Ruske et al., 2003). While comparing the data of grain yield and protein concentration of the year 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 fungicide 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 fungicide-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 fungicide treatments.

Since gluten is some portion of proteins – it is based on a water-insoluble fraction of ones - glutenins and gliadins, there are the presumable consistent patterns of variation the same as for protein concentration. Actually, fungicides tested against fungal diseases mostly insignificantly affected wet gluten concentration in grain (Table 9). Only in 2003 in plots treated fungicide Allegro plus, composed of three active ingredients, grain contained more gluten on average than grain in other plots and significantly (at $P \leq 0.05$) more than in plots treated with only one strobilurin azoxystrobin containing fungicide Amistar and fungicide-

untreated plots. Grains matured in fungicide Allegro plus treated plots exhibited the highest gluten content in 2002 also, and in Amistar – sprayed plots grain had less gluten than the rest treatments all three years of investigation. This observation agrees with the inferences made by other researchers: none of the fungicides caused any significant changes in the wet gluten content or had only minor effect (Tanács et al., 2005; Wang et al., 2004).
Sedimentation is a protein property to swell in weak acid solutions. This is the important bread baking characteristics describing indicator. By averaged data of replications Zeleny index varied in a range 39.8 - 65.2 ml (Table 9) and met the local requirements for first class of grain quality. Due to the use of different fungicides statistically significant differences between the treatments were found in only two cases: in 2002 in the strobilurin-containing fungicide Allegro plus treated plots Zeleny index was higher than that in the other treatments and significantly (at $P \leq 0.05$) higher than in plots treated with Opus, Opera, Amistar and untreated plot. In triazole Tilt fungicide treated plots of 2003 yield matured grains exhibited the highest sedimentation (65.2 ml), which was significantly (at $P \leq 0.05$) higher than in the untreated (63.0 ml), treated with strobilurins Amistar (63.2 ml), Opera (62.1 ml) and triazole Opus (63.0 ml) plots. In Amistar - sprayed treatment matured grain Zeleny index was the lowest or one of the least every year.
For buying wheat in Lithuania the falling number must be not less than 200 seconds. This indicator of grain quality in all treatments satisfied requirements of local standard and was significantly greater than 200s: from 366-382 s in 2004 to 461-475 s in the yield of 2003 harvest (Table 9). In 2002-2003, sunny and dry weather during crop maturation and harvest time could be a factor underlying the very low alpha-amylase, i.e. a high falling number. Plant protection products use did not reveal any reliable influence in any year. Grain yield of 2004 in all treatments exhibited high quality, elastic gluten. Grains matured in 2002 had weaker gluten, i.e. gluten deformation index was higher, but satisfied the local requirements of food grains; the differences among fungicide-treated and untreated plots were statistically unreliable.
Although fungicide use had only a minor effect on the grain quality, the response of protein and wet gluten yield to the plant protection was marked each experimental year (Fig. 1). In most cases protein and gluten yields were higher for the strobilurin-treated plots (with a small exception mostly in 2002) than for those treated with triazole Tilt and untreated. Protein and gluten yields in the plots treated with Opus (3481 and 6896 kg ha^{-1} during 3 years) were similar to those produced in the plots treated with fungicides containing some strobilurins like Rombus, Amistar and Acanto (3440-3500 and 6735-6804 kg ha^{-1} respectively). The highest average protein yield, like that of gluten, was obtained when the fungicide used contained both strobilurin and epoxyconazole (Fig. 1, treatments 2 and 5). When estimating protein and gluten yield according to years, it is obvious that in 2004 protein yield was by approximately 1.43-1.57 times higher than that in 2003 and gluten yield - by 1.26-1.48 times higher than that in 2002.
Having estimated the relationship of FHB incidence and grain *Fusarium* spp. (%) contamination with grain quality, statistically significant correlations were found with many grain quality indicators (Table 10). However, the data of these correlations should be viewed critically. Having calculated respective correlations for separate years, a weak but also negative correlation was established only between FHB incidence and Gluten DI and Zeleny index both in 2003 and 2004.

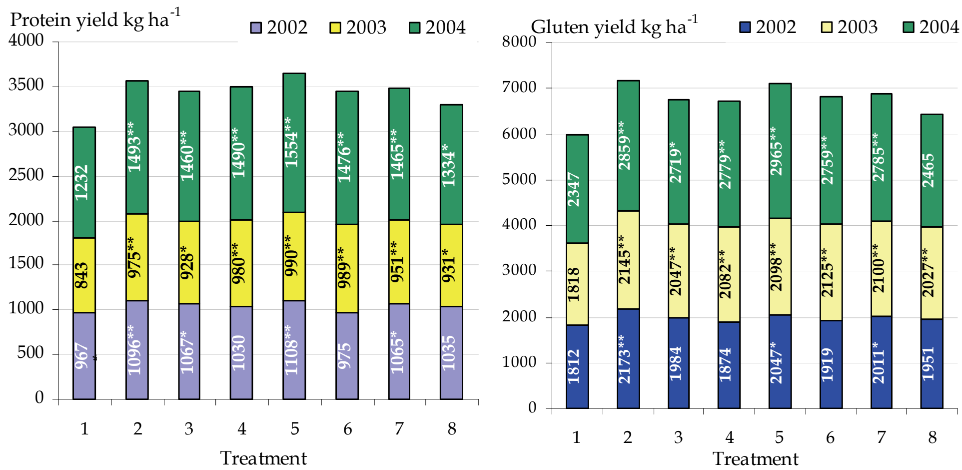


Fig. 1. Effect of fungicide treatment on grain protein and gluten yields. 1- no fungicides, 2- Allegro Plus, 3- Rombus, 4- Amistar, 5- Opera, 6- Acanto, 7- Opus, 8- Tilt; *, **difference from fungicide untreated plot significant at $P \leq 0.05$ and at $P \leq 0.01$ respectively; fungicide a.i. see in Table 1

FHB incidence (%) with:	Coefficient of linear correlation r	*Fusarium* spp. % with:	Coefficients of linear correlation r
Fusarium spp	0.76**		
Protein	0.842**	Protein	0.604**
Gluten	-0.772**	Gluten	-0.004
Gluten DI	-0.888**	Gluten DI	-0.697**
Zeleny Index	-0.867**	Zeleny Index	-0.147

Table 10. FHB incidence (2003-2004) and grain contamination with *Fusarium* spp. (2002-2004) correlation with grain quality indicators. Calculated by the data averaged over replications.

In our experiments, as well as in those reported in the scientific literature, fungal diseases, like FHB, rusts (*Puccinia* spp.) and powdery mildew (*Blumeria graminis*) incidence and fungicide use had only a minor, and harvest year considerable effect on the grain quality (Dimmock & Gooding, 2002; Gärtner et al., 2008). Gärtner et al. (2008) found that the falling number is more dependent on the prevailing climatic conditions than on FHB infection. Due to the FHB, the protein content declined, but only slightly, and the Zeleny index decreased only in some varieties. The Fusarium infection did not noticeably influence either the protein content or the water absorption ability of wheat flour (Wang et al., 2005). The fact that fungicide application significantly increased protein and gluten yields is related to the grain yield increase in fungicide treated plots, especially in the year 2004 which was conducive to the occurrence of fungal diseases. To make summarising conclusions on this subject, more detailed further research is needed, since too narrow range of quality data variation among treatments appeared each year.

6. Conclusions

The results of the multi-aspect comparative investigation on the use of strobilurin and triazole - based fungicides for winter wheat disease control suggest that the foliar application of both strobilurin-based and triazole fungicides gave an adequate control of Septoria *(P. nodorum, M. graminicola)* and tan spot *(P. tritici-repentis)*. The foliar fungicide application contributed towards reducing incidence of *P. nodorum* in the harvested seed; however, both the control of FHB and assay of mycotoxin contaminations yielded contradictory results. Under the fungicide use a substantial grain yield increase was obtained. The grain yield showed the significant negative linear correlation with AUDPC for both Septoria and tan spot.

Fungicides prolonged green leaf area retention. This effect depended on the year and fungicide origin. The application of fungicides (both strobilurins and triazoles) at the end of booting – heading stages (BBCH 47-55) significantly increased grain protein and gluten yields, but did not diminish grain quality, however, meteorological conditions of the year played a decisive role for grain quality, compared with fungicide treatments. The highest average protein yield, like that of gluten, was obtained when the fungicide used contained both strobilurin and epoxyconazole.

7. References

Ammerman, E.; Lorenz, G. & Schelberger, K. (1992). BAS 490 F – a broad-spectrum fungicide with a new mode of action, *Proceedings of Brighton crop protection conference. Pest and Disease,* pp. 403-410, ISBN 0948404655, Brighton, England, November 23-26, 1992, Farnham, Surrey

Arseniuk, E.; Goral, T.; Sowa, W.; Czembor, H. J.; Krysiak, H. & Scharen, A. L. (1998). Transmission of *Stagonospora nodorum* and *Fusarium* spp. on triticale and wheat seed and the effect of seedborne *Stagonospora nodorum* on disease severity under field conditions. *Journal of Phytopathology*, Vol., 146, Iss. 7, (August 1998) pp. 339-345, ISSN 0931-1785

Beck, Ch. (2004). Einfluss von fungiziden auf die ertragsphysiologie von weizen. *Inaugural-Dissertation*, Institute for Plant Diseases, University of Bonn, Germany. http://hss.ulb.uni-bonn.de:90/2005/0537/0537.pdf

Berthiller, F.; Sulyok, M.; Krska, R. & Schuhmacher, R. (2007). Chromatographic methods for the simultaneous determination of Mycotoxins and their conjugates in cereals. *International Journal of Food Microbiology*, Vol. 119, (October 2007) pp. 33-37, ISSN 0168-1605

Bhathal, J. S.; Loughman, R. & Speijers, J. (2003). Yield reduction in wheat in relation to leaf disease from yellow (tan) spot and Septoria nodorum blotch. *European Journal of Plant Pathology,* Vol. 109, No. 5, (June 2003) pp. 435–443, ISSN 0929-1873

Birzele, B.; Meier, A.; Hindorf, H.; Krämer, J. & Dehne, H. W. (2002). Epidemiology of *Fusarium* infection and deoxynivalenol content in winter wheat in the Rhineland, Germany. *European Journal of Plant Pathology*, Vol. 108, No. 7, (September 2002) pp. 667–673, ISSN 0929-1873

Blixt, E.; Djurle, A.; Yuen, J. & Olson A. (2009). Fungicide sensitivity in Sweden isolates of *Phaeosphaeria nodorum*. *Plant Pathology,* Vol. 58, Iss. 4, (August 2009) pp. 655-664, ISSN 0032-0862

Bottalico, A. & Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology*, Vol. 108, No. 7, (September 2002) pp. 611–624, ISSN 0929-1873

Brent, J. K. & Hollomon D. W. (2007). *Fungicide resistance: the assessment of risk*. *FRAC Monograph No. 2*, pp. 1–28. Fungicide Resistance Action Committee. www.frac.info

Campbell, C. L. & Madden, L. V. (1990). Temporal analysis of epidemics. I: Description and comparison of diseases progress curves. In: *Introduction to Plant Disease Epidemiology*, Campbell, C. L. & Madden, L. V. (Eds.), pp. 161–202. Wiley-Interscience (January 1990), ISBN-10: 0471832367, New York

Cesevičienė, J. & Mašauskienė, A. (2008). The effect of harvest time on winter wheat grain protein content and sedimentation index. *Zemdirbyste - Agriculture*, Vol. 95, No. 1, pp. 58–72, ISSN 1392-0227

Clark, D. C. (2003). Agronomic implications of some morphological and biochemical effects of trifloxystrobin in wheat growing. *Pflanzenschutz-Nachrichten Bayer*, Vol. 56, Iss. 2, pp. 281-296, ISSN 03401723

Cools, H. J. & Fraaije, B. A. (2008). Are azole fungicides losing ground against Septoria wheat disease? Resistance mechanisms in *Mycosphaerella graminicola*. *Pesticide Management Science,* Vol. 64, Iss. 7, (July 2008) pp. 681-684, ISSN 1526-4998

Covarelli, L.; Turner, A. S. & Nicholson, P. (2004). Repression of deoxynivalenol accumulation and expression of *Tri* genes in *Fusarium culmorum* by fungicides in vitro. *Plant Pathology*, Vol. 53, Iss. 1, (February 2004) pp. 22-28, ISSN 1365-3059

Cunfer, B. M. & Ueng, P. P. (1999). Taxonomy and identification of *Septoria* and *Stagonospora* species on small–grain cereals. *Annual Review of Phytopathology*, Vol. 37, (September 1999) pp. 267–284, ISSN 0066-4286

Dahl, C.; Biemann, H. P. & Dahl, J. (1987) A protein kinase antigenically related to pp60v-src possibly involved in yeast cell cycle control: positive in vivo regulation by sterol. *Proceedings of the National Academy of science of the United States of America,* Vol. 84, N 12, pp. 4012– 4016, ISSN 0027-8424

De Vleeschauwer, J.; Schelberger, K.; Saur, R. & Defloor K. (1996). BAS 494 02 F, a new broad-spectrum fungicide for disease control in cereals. Proceedings of the 48th International Symposium on Crop Protection, Gent (Belgium), 7 May 1996. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen Universiteit Gent*, Vol. 61/2a, pp. 367-376, ISSN 1379-1176

Dimmock, J. P. R. E. & Gooding, M. J. (2002). The influence of foliar diseases, and their control by fungicides, on the protein concentration in wheat grain: a review. *The Journal of Agricultural Science,* Vol. 138, No. 4, pp. 349-366, ISSN 0021-8596

Doohan, F. M.; Brenan, J. & Cooke, B. M. (2003). Influence of climatic factors on *Fusarium* species pathogenic to cereals. *European Journal of Plant Pathology*, Vol. 109, No. 7, (September 2003) pp. 755–768, ISSN 0929-1873

Ermakov, A. I.; Arasimovich, V. V.; Jarosh, N. P.; Peruanskij, J. V.; Lukovnikova, G. A. & Ikonnikova, M. I. (1987). *Methods of Biochemical Tests of Plants*, A. I. Ermakov (Ed.), 3rd edition, pp. 107– 109. Agropromizdat, Leningrad, Russia

Everts, K. L.; Leath, S. & Finney, P. L. (2001). Impact of powdery mildew and leaf rust on milling and baking quality of soft red winter wheat. *Plant Disease*, Vol. 85, No. 4, (April 2001) pp. 423-429, ISSN 0191-2917

Fraaije, B. A; Lucas, J. A.; Clark, W. S. & Burnett, F. J. (2003). QoI resistance development in populations of cereal pathogens in the UK. *Proceedings BCPC International Congress. Crop Science and Technology*, pp. 689–694, ISBN 1 901396 63 0, Glasgow, 10-12 November 2003, British Crop Protection Council, Alton, Hants, UK

Fraaije, B. A; Burnett, F. J.; Clark, W. S.; Motteram, J. & Lucas, J. A. (2005). Resistance development to QoI inhibitors in populations of *Mycosphaerella graminicola* in the UK, In: *Modern Fungicides and Antifungal Compounds IV*, Dehne, H. W.; Gisi, U.; Kuck, K. H.; Russell, P. E. & Lyr, H. (Eds), pp. 63–71, British Crop Protection Council, Alton, Hants, ISBN 978-3-941261-02-0, UK

Gärtner, B. H.; Munich, M.; Kleijer, G. & Mascher, F. (2008). Characterisation of kernel resistance against *Fusarium* infection in spring wheat by baking quality and mycotoxin assessments. *European Journal of Plant Pathology*, Vol. 120, No. 1, (January 2008) pp. 61–68, ISSN 0929-1873

Galaverna, G.; Dall'Asta, C.; Mangia, A.; Dossena, A. & Marchelli, R. (2009). Masked Mycotoxins: an Emerging Issue for Food Safety. *Czech Journal of Food Sciences,* Vol. 27, Special Iss. 1, 2009, pp. 89-92, ISSN 1212-1800

Gaurilčikienė, I. & Ronis, A. (2006). The effect of strobilurin fungicides on the development of foliar diseases of winter wheat. *Agronomy Research,* Vol. 4, Special Iss., 177-180, ISSN 1406-894X

Gisi, U.; Sierotzki, H.; Cook A. & McCaffery, A. (2002). Mechanisms influencing the evoliution of resistance to Qo inhibitor fungicides. *Pest Management Science*, Vol. 58, Iss. 9, (September 2002) pp. 859-867, ISSN 1526-4998

Gisi, U.; Pavic, L.; Stanger, C.; Hugelshofer, U. & Sierotzki, H. (2004). Dynamics of *Mycosphaerella graminicola* populations in response to selection by different fungicides. *Proceedings of 14th International Reinhardsbrunn Symposium Modern fungicides and antifungal compounds IV*, ISBN-10: 1901396398, ISBN-13: 978-1901396393, Friedrichroda, Thuringia, Germany, April 25-29, 2004,: Dehne, H. W.; Gisi, U.; Kuck, K. H.; Russell, P. E. & Lyr, H. (Eds.), British Crop Protection Council, Amazon, UK

Griffiths, E. & Ao, H. C. (1976). Dispersal of *Septoria nodorum* spores and spread of glume blotch of wheat in the field. *Transaction of British Mycological Society,* Vol. 67, No. 3, pp. 413-418, ISSN 0007-1536

Gooding, M. J.; Dimmock, J. P. R. E.; France, J. & Jones, S. A. (2000). Green leaf area decline of wheat flag leaves: the influence of fungicides and relationships with mean grain weight and grain yield. *Annals of Applied Biology*, Vol. 136, pp. 77–87, ISSN 0003-4746

Gooding, M. J.; Ellis, R. H.; Shewry, P. R. & Schofield, J. D. (2003). Effects of restricted water availability and increased temperatures on the grain filling, drying and quality of winter wheat. *Journal of Cereal Science*, Vol. 37, Iss. 3, (May 2003) pp. 295–309, ISSN 0733-5210

Guttieri, M. J.; Stark, J. C.; O'Brien, K. M. & Souza, E. (2001). Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. *Crop Science*, Vol. 41, No. 2, (March 2001) pp. 327–335, ISSN 0011-183X

Habermeyer, J.; Gerhard, M. & Zinkernagel, V. (1998). The impact of strobilurins on the plant physiology of wheat. *Proceedings of 7th International Congress of Plant*

Pathology, http://www.bspp.org.uk/icpp98/5.6/3.html, Edinburgh, Scotland, 9-16 August, 1998, British Society for Plant Pathology, Birmingham, B3 2PP, UK

Haidukowski, M.; Pascale, M.; Perrone, G.; Pancaldi, D.; Campagna, C. & Visconti, A. (2005). Effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated under field condition with *Fusarium graminearum* and *Fusarium culmorum*. *Journal of the Science of Food and Agriculture*, Vol. 85, No. 2, (May 2005) pp. 191–198, ISSN 1097-1210

Hansen, J. H.; Secher, B. J. M. & Jorgensen, L. N. (1994). Thresholds for control of *Septoria* spp. in winter wheat based on precipitation and grows stage. *Plant Pathology*, Vol. 3, Iss. 1, (February 1994) pp. 183-189, ISSN 0032-0862

Häuser-Hahn, I.; Baur, P. & Schmitt, W. (2004). Fluoxastrobin (HEC 5725) – biochemistry and chemodynamic behaviour of a new leaf systemic strobilurin fungicide. *Pflanzenschutz-Nachrichten Bayer*, Vol. 57, Iss. 3, pp. 437-450, ISSN 03401723

Hewitt, G. (2000). New modes of action of fungicides. *Pesticide Outlook*, Vol. 11, Iss. 1, pp. 28-32, ISSN 0956-1250, http://pubs.rsc.org/ej/PO/2000/B006311H.pdf

Hollomon, D. W. (1994) Do morpholine fungicides select for resistance? In: *Fungicide Resistance*, Heaney, S.; Slawson, D.; Hollomon D. W.; Smith, M; Russell, P. E. & Parry, D. W. (Eds.), pp. 281-289, British Crop Protection Council, Farnham, ISBN 90-72398-07-6, Surrey

Hosford, R. M.; Larez, J. C. R. & Hammond, J. J. (1987). Interaction of wet period and temperature on *Pyrenophora tritici-repentis* infection and development in wheat's of differing resistance. *Phytopathology*, Vol. 77, pp. 1021–1027, ISSN 0031-949X

Hutcheon, J. A. & Jordan, V. W. L. (1992). Fungicide timing and performance for Fusarium control in wheat. In: *Proceedings of the Brighton Crop Protection Conference. Pest and Disease,* Vol. 2, pp. 633–638, ISBN 0948404655, Brighton, England, November 23-26, 1992, Farnham, Surrey

Jorgensen, L. N.; Henriksen, K. E. & Nielsen, G. C. (1999). Adjusting thresholds for Septoria control in winter wheat using strobilurins. *Proceedings of Fifth International Septoria Workshop Septoria and Stagonospora diseases of Cereals: A Compilation of Global Research,* pp. 173-175, ISBN 970-648-035-8, CIMMYT, Mexico, (Septebmer 20-24) 1999, van Ginkel, M.; Mc Nab, A. & Krupinskij, J. (Eds.), D. F.: CIMMYT, Mexico

Inagaki, M. N.; Mori, M. & Nachit, M. M. (2009) Effect of a strobilurin-class fungicide on water use in synthetic bread wheat genotypes grown under increasing water deficit conditions. *Cereal Research Communications*, Vol. 37, No. 4, (December 2009) pp. 513–519, ISSN 0133-3720

Kelley, K. (2001). Planting date and foliar fungicide effects on yield components and grain traits of winter wheat. *Agronomy Journal*, Vol. 93, No. 2, (March 2001) pp. 380–389, ISSN 0002-1962

Kendall, S. & Hollomon, D. W. (1994). Towards the rational use triazole mixtures for cereal disease control. In: *Proceedings of the Brighton Crop Protection Conference. Pest and Disease*, pp. 549-556, ISBN 10 0948404809, Brighton, England, 21-24 November, 1994, British Crop Protection Council, Farnham

Kietreiber, M. (1981) Filterpapier-fluoreszenztest für die festellung von *Septoria nodorum* in *Triticum aestivum* unter berücksichtiung des keimruhe befindlichen saatgutes. *seed Science & Technology*, Vol. 9, pp. 717-723

Leslie, J. F. & Summerell, B. A. (2006). *The Fusarium Laboratory Manual*. Blackwell Publ., ISBN-13 978-0-8138-1919-8, ISBN 10 0-8138-1919-9, Iowa, USA

Lugauskas, A.; Krasauskas, A. & Repečkienė, J. (2004) Ekologiniai veiksniai, lemiantys mikromicetų paplitimą ant javų grūdų ir sojų sėklų. *Ekologija*, No. 2, pp. 21–32, ISSN 0235-7224

McCartney, C.; Mercer, P. C.; Cooke, L. R. & Fraaije, B. A. (2007). Effects of a strobilurin-based spray programme on disease control, green leaf area, yield and development of fungicide-resistance in *Mycosphaerella graminicola* in Northern Ireland. *Crop Protection,* Vol. 26, Iss. 8, (August 2007) pp. 1272-1280, ISSN 0261-2194

Manandhar, J. B. & Cunfer, B. M. (1991). An improved selective medium fort he assay of *Septoria nodorum* from wheat seed. *Phytopathology*, Vol. 81, pp. 771-773, ISSN 0031-949X

Mesterhazy, A.; Bartok, J. & Lamper, C. (2003). Influence of wheat cultivar, species of *Fusarium* and isolate aggressiveness on the efficacy of fungicides for control of FHB. *Plant Disease*. Vol. 87, No. 9, (September 2003) pp. 110-115, ISSN 0191-2917

Mathies, A. & Buchenauer, H. (2000). Effect of tebuconazole (Folicur) and prochloraz (Sportak) treatments on Fusarium head scab development, yield and deoxynivalenol (DON) content in grains of wheat following artificial inoculation with *Fusarium culmorum*. *Journal of Plant Disease and Protection*, Vol. 107, pp. 33–52, ISSN 18613829, 18613837

Miedaner, T.; Reinbrecht, C.; Lauber, U.; Schollenberger, M. & Geiger, H. H. (2001) Effects of genotype and genotype-environment interaction on deoxynivalenol accumulation and resistance to *Fusarium* head blight in rye, triticale, and wheat. *Plant Breeding*, Vol. 120, pp. 97-105, ISSN 179-9541

Milus, E. A. & Parsons, C. E. (1994). Evaluation of foliar fungicides for controlling Fusarium head blight of wheat. *Plant Disease,* Vol. 78, (July 1994) pp. 697–699, ISSN 0191-2917

Milus, E. A. & Chalkley, D. B. (1997). Effect of previous crop, seedborne inoculum and fungicides on development of Stagonospora blotch. *Plant Disease,* Vol. 81, November 1997, pp. 1279–1283, ISSN 0191-2917

Nicholson, P.; Chandler, E.; Draeger, R. C.; Gosman, N. E.; Simpson, D. R.; Thomsett, E. & Wilson, A. H. (2003). Molecular tools to study epidemiology and toxicology of Fusarium head blight of cereals. *European Journal of Plant Pathology*, Vol. 109, (September 2003) pp. 691–703, ISSN 0929-1873

Nolan, S.; Cooke, B. M. & Monaham, F. J. (1999). Studies on the interaction between *Septoria tritici* and *Stagonospora nodorum* in wheat. *European Journal of Plant Pathology*, Vol. 105, (December 1999) pp. 917-925, ISSN 0929-1873

Oerke, E.-C.; Steiner, U.; Beck, C. & Dehne, H.-W. (2005). Influence of strobilurines on host plant physiology. In: *Proceedings of the 14th International Reinhardsbrunn Symposium: Modern Fungicides and Antifungal compounds IV*, Dehne, H.-W.; Gisi, U; Kuck, K. H.; Russell, P. E; Horst Lyr, H. (Eds.), Friedrichroda, Thuringia, 25-29 April, 2004, British Crop Production Council, ISBN 1901396398, Alton, UK

Peever, T. L.; Brants, A.; Bergstrom, G. S. & Milgrooin, M. G. (1994). Selection for decreased sensitivity to propiconazole in experimental field populations of *Stagonospora nodorum* (syn. *Septoria nodorum*). *Canadian Journal of Plant Pathology*, Vol. 16, (June 1994) pp. 109-117, ISSN 0706-0661

Phenological growth stages and BBCH-identification keys of cereals (1997). In: *Growth stages of Mono– and Dicotyledonous Plants. BBCH-Monograph*. Meier, U. (Ed.), pp. 12–16, Blackwell Wissenschafts–Verlag, ISBN 10 3826331524, Berlin, Wien

Pirgozliev, S. R.; Edwards, S. G.; Hare, M. C.; Jenkinson, P. (2003). Strategies for the control of *Fusarium* head blight in cereals. *European Journal of Plant Pathology*, Vol. 109, (September 2003) pp. 731–742, ISSN 0929-1873

Polišenská, I. & Tvaružek, L. (2007). Relationships between deoxynivalenol content, presence of kernels infected by *Fusarium* spp. pathogens and visually scabby kernels in Czech wheat in 2003–2005. *Cereal Research Communications*, Vol. 35, No. 3, (September 2007) pp. 1437–1448, ISSN 0133-3720

Ruske, R. E.; Gooding, M. J. & Jones, S. A. (2003). 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*, Vol. 140, Iss. 4, (October 2003) pp. 395-407, ISSN 0021-8596

Shah, D. A.; Bergstrom, G. C. (2000). Temperature dependent seed transmission of *Stagonospora nodorum* in wheat, *European Journal of Plant Pathology*, Vol. 106, (November 2000) pp. 837-842, ISSN 0929-1873

Shaw, M. W.; Bearchell, S. J.; Fitt, B. D. L. & Fraaije, B. A. (2008). Long term relationships between environment and abundance in wheat of *Phaoshaeria nodorum* and *Mycosphaerella graminicola*. *New Phytologist,* Vol. 177, pp. 229-238, ISSN 0028-646X

Shaner, G. & Beuchley, G. (1995). Epidemiology of leaf blotch of soft red winter wheat caused by *Septoria tritici* and *Stagonospora nodorum*. *Plant Disease,* Vol. 79, (September 1995) pp. 928–938, ISSN 0191-2917

Sierotzki, H.; Wullschleger, J. & Gisi, U. (2000). Point mutation in cytochrome *b* gene conferring resistance to strobilurin fungicides in *Erysiphe graminis* f. sp. *tritici* field isolates. *Pesticide Biochemistry and Physiology,* Vol. 68, (October 2000) pp. 107–112, ISSN 0048-3575

Sierotzki, H. & Gisi, U. (2006) Mechanisms and diagnostics of QoI resistance in cereal pathogens. *Aspects of Applied Biology*. *Fungicide resistance: Are we winning the battle but losing the war?*, Vol. 78, (September 7-8) 2006, Edinburgh, pp. 17–20, ISSN 0265-1491

Sierotzki, H.; Frey, R.; Wullschleger, J.; Palermo, S.; Karli, S.; Godwin, J. & Gisi, U. (2007). Cytochrome b gene sequence and structure of *Pyrenophora teres* and *P. tritici-repentis* and implications for QoI resistance. *Pest Management Science*, Vol. 63, (November 2007) pp. 225–233, ISSN 1526-4998

Simpson, D. R.; Weston, G. E.; Turner, J. A.; Jennings, P. & Nicholson, P. (2001). Differential control of head blight pathogens of wheat by fungicides and consequences for mycotoxin contamination of grain. *European Journal of Plant Pathology*, Vol. 107, No. 4, (May 2001) pp. 421–431, ISSN 0929-1873

Siradinou, E. & Buchenauer, H. (2001). Chemical control of Fusarium head blight on wheat. *Journal of Plant Disease and Protection*, Vol. 108, pp. 231–243, ISSN 1861-3829

Souza, E. J.; Martin, J. M.; Guttieri, M. J.; O'Brien, K. M.; Habernicht, D. K.; Lanning, S. P.; McLean, R.; Carlson, G. R. & Talbert, L. (2004). Influence of genotype, environment, and nitrogen management on spring wheat quality. *Crop Science*, Vol. 44, No. 2, (March 2004) pp. 425–432, ISSN 0011-183X

Šurkus, J.; Dabkevičius, Z. & Brazauskas, R. (1988). Fungicidas tiltas javams apsaugoti nuo ligų. LŽMTI mokslinių straipsnių rinkinys, No. 62, pp. 93-100

Tanács, L.; Matuz, J.; Gero, L. & Petróczi, I. M. (2005). Effects of NPK fertilizers and fungicides on the quality of bread wheat in different years. *Cereal Research Communications,* Vol. 32, No. 2-3, (June 2005) pp. 627-634, ISSN 0133-3720

Torriani, S. F. F.; Brunner, P. C.; McDonald, B. A. & Sierotzki, H. (2009). QoI resistance emerged independently at least 4 times in European populations of *Mycosphaerella graminicola. Pest Management Science*, Vol. 65, (February 2009) pp. 155–162, ISSN 1526-4998

Triboi, E.; Martre, P. & Triboi-Blondel, A.-M. (2003). Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *Journal of Experimental Botany*, Vol. 54, Iss. 388, (July 2003) pp. 1731–1742, ISSN 0022-0957

Triboi, E. & Triboi-Blondel, A.-M. (2002). Productivity and grain or seed composition: a new approach to an old problem – invited paper. *European Journal of Agronomy*, Vol. 16, No. 3, (April 2002) pp. 163–186, ISSN 1161-0301

Turner, J. A.; Elcock, S. J. & Hims, M. J. (1996). Monitoring triazole fungicide sensitivity in population of *Septoria tritici*. *Proceedings of Brighton crop protection conference. Pest and Diseases*, pp. 695 - 700, ISBN 0948404655, Brighton, England, November 18-21, 1996, Farnham, Surrey

Wang, J.; Pawelzik, E.; Weinert, J.; Zhao, Q. & Wolf, G. A. (2004). Effect of fungicide treatment on the quality of wheat flour and breadmaking. *Journal of Agricultural and Food Chemistry*, Vol. 52, No. 25, (November 2004) pp. 7593-600, ISSN 0021-8561

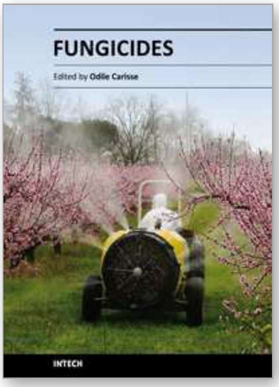
Wang, J.; Wieser, H.; Pawelzik, E.; Weinert, J.; Keutgen, A. J. & Wolf, G. A. (2005). Impact of the fungal protease produced by *Fusarium culmorum* on the protein quality and bread making properties of winter wheat. *European Food Research and Technology.* Vol. 220, No. 5-6, (May 2005) pp. 552-559, ISSN 1438-2377

Zhan, J.; Stefanato, F. L. & Mcdonald, B. A. (2006). Selection for increased cyproconazole tolerance in *Mycosphaerella graminicola* through local adaptation and in response to host resistance. *Molecular Plant Pathology*, Vol. 7, No. 4, (July 2006) pp. 259–268, ISSN 1464-6722

Zhang, Y. J.; Zhang, X.; Chen, C. J.; Zhou, M. G. & Wang, H. C. (2010). Effects of fungicides JS399-19, azoxystrobin, tebuconazloe, and carbendazim on the physiological and biochemical indices and grain yield of winter wheat. *Pesticide Biochemistry and Physiology*, Vol. 98, Iss. 2, (October 2010) pp. 151-157, ISSN 0048-3575

Xu, X. (2003). Effects of environmental conditions on development of Fusarium ear blight. *European Journal of Plant Pathology*, Vol. 109, No. 7, (September 2003) pp. 683–689, ISSN 0929-1873

www.frac.info/frac/work/FRAC_QoI_Minutes2009Final.pdf



Fungicides

Edited by Odile Carisse

ISBN 978-953-307-266-1

Hard cover, 538 pages

Publisher InTech

Published online 14, December, 2010

Published in print edition December, 2010

Plant and plant products are affected by a large number of plant pathogens among which fungal pathogens. These diseases play a major role in the current deficit of food supply worldwide. Various control strategies were developed to reduce the negative effects of diseases on food, fiber, and forest crops products. For the past fifty years fungicides have played a major role in the increased productivity of several crops in most parts of the world. Although fungicide treatments are a key component of disease management, the emergence of resistance, their introduction into the environment and their toxic effect on human, animal, non-target microorganisms and beneficial organisms has become an important factor in limiting the durability of fungicide effectiveness and usefulness. This book contains 25 chapters on various aspects of fungicide science from efficacy to resistance, toxicology and development of new fungicides that provides a comprehensive and authoritative account for the role of fungicides in modern agriculture.