ISSN 1392-3196
Zemdirbyste-Agriculture, vol. 95, No. 3 (2008), p. 158–165
UDK 633.11“32”:632.4:632.952

INFLUENCE OF FUNGICIDES AND VARIETY RESISTANCE ON FUNGAL FLORA OF BARLEY GRAIN

Pille SOOVÄLI, Mati KOPPEL

Jõgeva Plant Breeding Institute,
Aamisepa 1, Jõgeva alevik, Estonia
Email: pille.soovali@jpbi.ee, mati.koppel@jpbi.ee

Abstract

The effects on disease incidence, grain yield and quality are commonly studied in fungicide trials. Less attention is paid to the effect of fungicides on fungal contamination of harvested grain. The aim of the current studies was to identify the effects of used fungicide treatment and the resistance of variety on seed contamination by phytopathogenic and saprophytic fungi. The harvested grain of three spring barley varieties was examined for the fungal contamination at Jõgeva Plant Breeding Institute in 2004 to 2006. Eight fungal genera were identified; the most common pathogens were *Cochliobolus sativus* and *Fusarium* spp. and saprophytes *Alternaria* spp., *Cladosporium* spp. and *Phoma* spp. It was found that the fungicide and genotype factors contributed to the variance seen in fungal contamination. The time of fungicide application had clear effect on the incidence of phytopathogenic fungal species. Fungal contamination was also highly influenced by environmental conditions of the experimental year. The results illustrate the possibility of use of fungicide and variety resistance based disease control strategy for reduction of seed contamination by fungal spores.

Key words: spring barley, fungicides, phytopathogenic fungi, saprophytes.

Introduction

Chemical control strategies are general tools to keep plants free of diseases and to avoid yield losses. Fungicide application can be beneficial by decrease fungi by a direct preventive and curative effect /Bertelsen et al., 2001/. Barley grain carries commonly a numerous microbial fungal populations. The extent and the activity of this mycoflora are determined by the state of the grain and the environmental conditions /Noots et al., 1999; Lõiveke et al., 2004/. All fungi require organic nutrients for their energy source and as carbon nutrients for cellular synthesis /Deacon, 2006/. Increased water sensitivity may be the result of microorganisms living on the surface of the grain, which compete with the embryo for available oxygen /Kelly, Briggs, 1992/. Early application of fungicides against foliar diseases on spring barley is common practice among farmers in Estonia. Whether this has any effect on infection of the mature grain has been little investigated. The overall aim of the study was to test the effect of fungicide application on the presence and amount of pathogenic and non-pathogenic fungi on harvested barley grain. The side objectives were to evaluate the effect of reduced fungicide dose, timing of fungicide application and variety resistance on the fungal contamination in harvested grain.

Materials and Methods

Field trials on disease control on spring barley were arranged with three replicates in a randomized design 20m² plots at the rate of 500 viable seeds per 1m² at Jõgeva Plant Breeding Institute. The trials were organized in two series.

The effect of fungicide dose, timing of application and resistance of varieties were tested in trials of growing seasons 2004–2005. Spring barley varieties 'Anni', 'Barke' and 'Extract' and two – treatment regimes of tebuconazole fungicide were tested in the trial. The split application of dose 0.5 l ha^{-1} at growth stage BBCH 32–51 and of 0.5 l ha^{-1} at stage BBCH 57–65 was compared with single application of reduced dose 0.16 l ha^{-1} at stage BBCH 59 (2004) and 0.15 l ha^{-1} at stage BBCH 61–65 (2005). The effect of different fungicides was tested in 2006, when ten fungicides were applied in 3/4 rate of registered full dose at growing stage BBCH 37–41 in variety 'Barke'. Untreated plots were used as control. The dosages and content of active ingredients of used fungicides are given in Table 1.

Table 1. Product names, doses and active ingredients of fungicides used in the field trials

Product	Active ingredients per litre	Dose applied l ha^{-1}
2004		
Folicur EW 250	250 g tebuconazole	2 x 0.5; 0.16
2005		
Folicur EW 250	250 g tebuconazole	2 x 0.5; 0.15
2006		
Amistar Opti 480 SC	80 g azoxystrobin + 400 g chlorothalonil	2.5
Amistar Xtra 280 SC	200 g azoxystrobin + 80 g cyproconazole	0.75
Fandango 200 EC	100 g protioconazole + 100 g fluoxastrobin	0.8
Duett Ultra	310 g metyltiophanat + 187 g epoxiconazole	0.6
Tango Super	84 g epoxiconazole + 250 g fenpropimorph	1.5
Sphere 267,5 EC	80 g cyproconazole + 187.5 g trifloxystrobin	0.6
Delaro 325 SC	150 g trifloxystrobin + 175 prothioconasole	0.8
Prosaro 250 EC	125 g protioconazole + 125 g tebuconazole	0.8
Falcon 460 EC	167 g tebuconazole + 43 g triadimenol + 250 g spiroxamin	0.6
Dukes 475 EC	60 g prothioconazole + 250 g spiroxamin 165 g tebuconazole	0.8

Disease occurrence in the field was scored as per cent of infected leaf area by *P. teres* and *C. sativus* and expressed as an average of the infection score on second leaf (L-2; first leaf under the flag leaf). Samples of harvested grain were analyzed for presence of fungal spores separately for each single fungal species. Seed samples were examined under a microscope Olympus CX 31 (40x enlargement) after the incubation by the method of moist chamber (10 days in Petry dishes at 20° C under 12 h dark/light

regime) according to the identifying manual /Barnett, 1956/. Analyses were done for 25 seeds in three replications per treatment. The percentage of occurrence of fungal species was evaluated for each trial variant: (number of grains with occurrence of species / total number of grains) x 100. The achieved results were analyzed by the analysis of variance, Agrobase (Agrobase^{TM} 20, 1999), p = 0.05.

Field meteorological weather station Metos Compact was used for recording the meteorological data from the beginning of the vegetation period until the harvest. The weather conditions varied quite significantly during the trial period (Table 2). The summer was cooler than average in 2004; the sum of effective temperatures exceeded the long-term average by 26 degrees in 2005 and the sum of effective temperatures remained 12 degrees below the long-term average in 2006. June 2004 was very rainy (207 mm respectively – long-term average 65 mm) as well May 2005, when the sum of precipitation was 84 mm respectively (long-term average 47 mm). In general, the vegetation periods of 2005 and 2006 were considerably drier and warmer and were not favourable for the development of high occurrence of foliar diseases.

Table 2. Decade average air temperature (^{o}C) and sum of precipitation (mm) of summers 2004–2006 and a long – term (1964–2005) average at the Jõgeva PBI

Average t° C	05. I	05. II	05. III	06. I	06. II	06. III	07. I	07. II	07. III	08. I	t° Σ
2004	15	6.5	8.7	12.7	12.2	14.5	14.8	15.9	18	19.6	1406
2005	7,4	9.4	14.6	12	15.3	14.7	17.5	19.4	16.7	17.2	1472
2006	12,5	8.9	10.2	11.7	18.1	18.3	20.4	17.8	16.2	16.7	1434
1964–2005	8,8	10.7	11.7	14.1	14.5	15.6	16.1	16.3	16.9	16.4	1446
Precipitation mm	05. I	05. II	05. III	06. I	06. II	06. III	07. I	07. II	07. III	08. I	mm Σ
2004	2	9	6	38	54	115	18	12	48	6	307
2005	17	34	33	9	20	13	3	10	40	75	253
2006	0	13	33	9	0	26	0	0	8	20	109
1922–2006	11	17	19	12	26	27	23	28	32	31	255

Results and Discussion

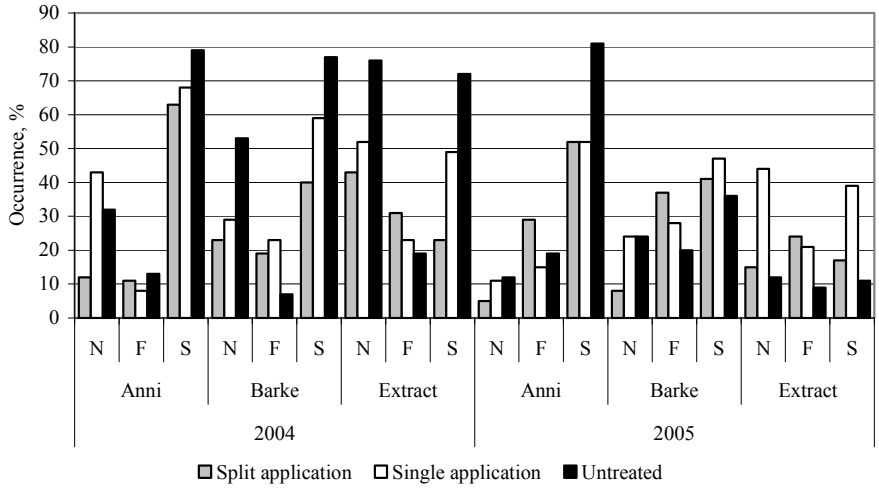
Fungicide applications and resistance of varieties caused significant differentiation in foliar infection level in trials of 2004–2005. The dominating fungi were major barley pathogens *Pyrenophora teres* causing net blotch and *Cochliobolus sativus* causing spot blotch (Table 3). Tebuconazole fungicide was effective in control of net blotch in both years in single and split applications. The significant effect in control of *C. sativus* was achieved only in 2004. Tebuconazole applications had no effect in control of low level infection of *C. sativus* in 2005. Significant effect of variety resistance on infection level of foliar diseases was detected in both diseases in both years.

Table 3. The impact of tebuconazole fungicide on foliar disease infection in different barley varieties at Jõgeva PBI 2004–2005. Disease incidence on the second leaf (%, assessed at BBCH 71–73)

Variety	Treatment	*P. teres* %		*C. sativus* %	
		2004	2005	2004	2005
Anni	Tebuconazole 2 x 0.5	6.7	0.8	10.4	0.5
	Tebuconazole 0.15	10.8	1.1	9.2	0.6
	Untreated	15.3	1.5	19.3	0.9
Barke	Tebuconazole 2 x 0.5	9.0	0.6	8.7	0.6
	Tebuconazole 0.15	15.6	2.6	10.1	1.6
	Untreated	39.3	2.7	23.0	0.8
Extract	Tebuconazole 2 x 0.5	12.7	1.1	10.3	1.1
	Tebuconazole 0.15	17.3	1.9	17.9	2.4
	Untreated	43.6	1.7	42.8	1.4
PD 0.05		2.5	0.5	2.3	0.4

Occurrence of necrotrophic fungus *C. sativus,* facultative saprophytes *Fusarium* spp. and saprophytic fungi *Alternaria* spp., *Cladosporium* spp. and *Phoma* spp. were identified on the harvested grain. Weather and growing conditions favoured occurrence of foliar diseases and contamination of harvested grain with fungal spores in 2004. The prevailing fungi on the harvested grain were *C. sativus* – 76% occurrence on seeds of variety Extract, members of genus *Alternaria* spp. and *Cladosporium* spp. – 79% occurrence in seeds of variety ʻAnni'. *Fusarium* spp. covered the range from 8% in seeds of variety ʻAnni' to 31% in variety ʻExtract' (Figure 1). The disease occurrence was medium in 2005 when weather conditions were warm and droughty at the grain formation stage. The highest occurrence of *C. sativus* was observed in variety ʻExtract' by 44%, *Fusarium* spp. in ʻBarke' by 37% and *Alternaria* spp. and *Cladosporium* spp. in ʻAnni' by 81% respectively (Figure 1).

Despite the low incidence of foliar infection of *C. sativus* and small differences between infections in different years, the grain contamination of untreated variants varies significantly between the years and varieties. Split application of tebuconazole was effective in reduction of kernel contamination with *C. sativus* in all variants except in variety ʻExtract' in 2005. This is an indication of activity of tebuconazole in control of phytopathogenic fungi in later stages of plant development Single application of reduced dose decreased kernel contamination with *C. sativus* only in more susceptible varieties ʻBarke' and ʻExtract' in more humid 2005.



N = necrotrophes, F = facultative saprophytes, S = saprophytes
LSD0.05 = 0.10 (N); 0.11 (F, S)

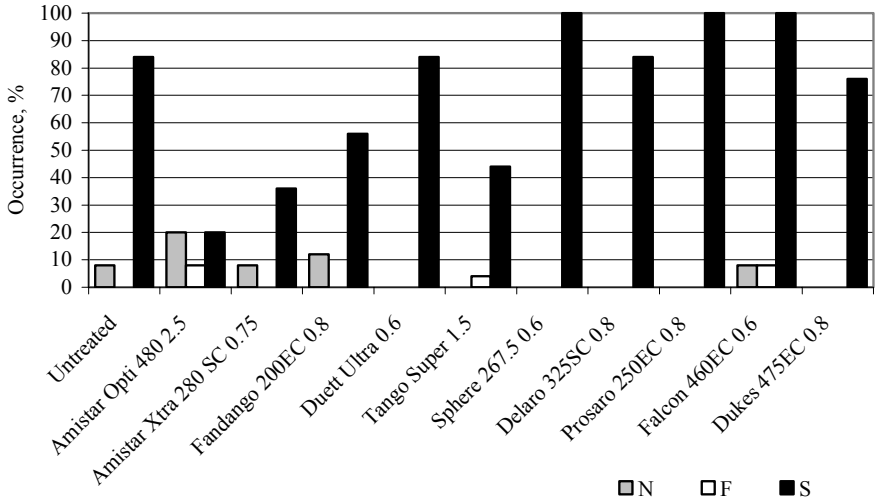
Figure 1. Occurrence (%) of phytopathogenic and saprophytic fungi on grain of spring barley varieties with different tebuconazole applications at growth stages 32–51 + 57–65 (split) and 59 (2004) or 61–65 (2005) (single)

The disease infection level of spring barley variety 'Barke' was low in 2006 (Table 4). The majority of used fungicides had very limited effect in reduction of infection of foliar diseases. Only applications of fungicides containing metyltiophanat + epoxiconazole and epoxiconazole + fenpropimorph resulted in significant decrease of net blotch. No significant differences were detected in foliar infection of *C. sativus*.

Table 4. The impact of fungicides on foliar disease infection of spring barley variety 'Barke' at Jõgeva PBI 2006. Disease incidence on the second leaf (%, assessed in BBCH 71–73)

Treatment	*P. teres* %	*C. sativus* %
Untreated	1.9	1.6
Amistar Opti 480 2.5	1.7	1.4
Amistar Xtra 280 SC 0.75	1.9	1.3
Fandango 200 EC 0.8	1.7	1.5
Duett Ultra 0.6	1.3	1.9
Tango Super 1.5	1.3	1.3
Sphere 267.5 0.6	1.8	1.0
Delaro 325SC 0.8	1.4	1.3
Prosaro 250EC 0.8	1.5	1.2
Falcon 460EC 0.6	1.8	1.3
Dukes 475EC 0.8	1.5	1.3
PD 0.05	0.6	0.8

The evaluation of the extent of mycological contamination of harvested barley grain showed, that five fungicides had 100% effect in control of *C. sativus* and *Fusarium* spp. in 2006 (Figure 2). The application of other five fungicides resulted in low occurrence of *C. sativus* (up to 20%) and *Fusarium* spp. (up to 8%). Fungicides containing azoxystrobin + chlorothalonil, azoxystrobin + cyproconazole, epoxiconazole + fenpropimorph and prothioconazole + fluoxastrobin worked effectively in reduction of contamination with saprophytes. Application of fungicides containing cyproconazole + trifloxystrobin, protioconazole + tebuconazole and tebuconazole + triadimenol + spiroxamin resulted in increase of grain contamination with *Alternaria* spp. and *Cladosporium* spp. In these treatments all kernels were contaminated with saprophytic fungi.



N = necrotrophes, F = facultative saprophytes, S = saprophytes
LSD0.05 = 0.23 (N); 0.27 (F); 0.54 (S)

Figure 2. Occurrence (%) of phytopathogenic and saprophytic fungi in grain of spring barley variety 'Barke' with different fungicide applications at growth stage 37–41

Influence of environmental conditions, fungicide treatment and variety on contamination of grain with pathogens and saprophytes is presented in Table 5. Focusing on data of mycoflora, it was confirmed that yearly conditions gave high significant effect on the occurrence of great majority of phytopathogenic fungi. The effect of year was not significant only for genus *Alternaria.*

Applied fungicides had high significant effect in control of occurrence of most saprophytes during the whole trial period. Only the occurrence of *Phoma* spp. was not influenced with fungicide applications. Significant increase of occurrence of *Fusarium* spp. was observed in varieties 'Barke' and 'Extract' in result of tebuconazole applications in 2004–2005 trials. *Fusarium* contamination was absent in untreated control, but was detected in three fungicide treated variants in 2006.

Table 5. Sums of squares of analysis of variance of contamination of harvested barley grain with pathogens and saprophytes at Jõgeva PBI in 2004–2006

Source of variation		C. sativus	*Fusarium* spp.	*Alternaria* spp.	Cladosporium spp.	Phoma
2004–2005	d.f.	SS	SS	SS	SS	SS
Year	1	2.15***	1.28***	0.04 ns	0.80***	2.46***
Treatment	2	1.76***	1.93***	0.89**	0.58**	0.13 ns
Variety	2	1.96***	0.22 ns	3.54***	0.59**	0.43 ns
Residual	1344	94.13	96.57	95.53	98.03	96.98
2006						
Treatment	10	8.27**	20.06***	14.65***	13.23***	2.86 ns
Residual	264	91.73	79.93	85.35	86.77	97.14

*** – significant at 0.01; ** – significant at 0.05; ns – non-significant

The results from the trials using eleven fungicides and three varieties showed that choice of fungicide had significant impact on the contamination and proportion of fungal species present on the harvested grain of spring barley.

Climatic conditions influenced the extent of mycological contamination more than varietal resistance. This indicates that variety in general has a lower impact on the saprophytes and conditions of vegetation period and fungicide treatment have greater impact on pathogens than saprophytes. According to Deacon (2006) more humid growing periods are related to spread of fungal species. The majority of economically damaging fungal species undergoes multiple cycles of infection in a single season and can cause serious damage to a range of cereal crops. Infection by C. sativus may occur at any stage of plant development. The disease develops faster at temperatures above 20^{o} C. The fruiting structures develop readily on moistened diseased plant tissue /Zillinsky, 1983/.

Fusarium species can survive in the soil in the form of thick-walled resting spores for years. The plants are infected at the tillering stage or into crown node. In the case of prolonged rainfall at the flowering stage rain splashes carry the conidia up to the flowering heads /Zillinsky, 1983/. The more common saprophytic genera such as Alternaria and Cladosporium invade leaves and heads of ripening plants. These organisms are aggressive spore producers. Under conditions favourable to the fungi they may invade living plant tissues or developing grain, usually during the maturation stage /Zillinsky, 1983/. Analysis of the weather conditions of our study period illustrates again that the high precipitation in vegetation period increases the need of fungicide use in spring barley. The proportion of Fusarium spp. infection was lower than that with C. sativus in all trials. Notable is presence of Fusarium contamination only in three fungicide treated variants in trial of 2006. Fusarium contamination might be influenced by long lasting fungicidal effect; the plants remain greener for longer period and serve as a good growing medium for Fusarium species /Mesterhazy, Batok, 2001/. The differences in grain infection level between years leads to the conclusion that there was strong influence of weather conditions at grain maturing time as suggest Legzdina and Belicka, 2001. Our results agree with the experience from Norway where significant

increase of Fusarium infection was detected in fungicide treated plots compared with untreated plots /Henriksen, Elen, 2005/.

Conclusions

Weather conditions of the growing period, resistance of the cultivated variety as well as choice of fungicide and its application regime influence the contamination of barley grain with necrotrophic and saprotrophic fungi. Fungicides do well in controlling the foliar infection of barley diseases but have minor influence in reduction of kernel contamination with saprotrophic fungi. Certain fungicides or fungicide application regimes can increase the grain contamination with saprophytic fungi

Received 2008-07-24
Accepted 2008-08-25

REFERENCES

1. Agrobase™ 20. The Software Solution for Plant Breeders // Addendum and Instructional Guide. – Winnipeg, Manitoba,1999. – 95 p.

2. Barnett H. L. Illustrated Genera of Imperfect Fungi // Burgess Publishing Co. – Minneapolis, 1956. – 217 p.

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

4. Deacon J. W. Fungal Biology // Fungi as plant pathogens / University of Edinburgh, UK. – 2006, 4th ed., p. 213–279

5. Henriksen B., Elen O. Natural *Fusarium* Grain Infection Level in Wheat. Barley and Oats after Early Application of Fungicides and Herbicides // Journal of Phytopathology. – 2005, vol. 153, iss. 4, p. 214–220

6. Henriksen K. E., Jorgensen L. N., Nielsen G. C. PC-Plant Protection – a Danish tool to reduce fungicide input in cereals // Brighton Crop Protection Conference. Pest & Diseases. – 2000, p. 835–840

7. Kelly L., Briggs D. E. The influence of grain microflora on the germinative physiology of barley // J. Inst. Brew. – 1992. – 98 p.

8. Legzdina L., Belicka I. Hulless barley grain infection with diverse fungal pathogens // Sustainable Systems of Cereal Crop Protection against Fungal Diseases as the Way of Reduction of Toxin Occurrence in Food Webs. – Kromeriz, Czech Republic, 2001, p. 198–203

9. Lõiveke H., Ilumäe E., Laitamm H. Microfungi in grain and grain feeds and their potential toxicity // Agronomy Research. – 2004, vol. 2, iss, 2, p. 195–205

10. Meier U. Growth stages of mono- and dicotyledonous plants. BBCH Monograph. 2. // Federal Biological Research Centre of Agriculture and Forestry. Germany. – 2001, p. 14–18. [2008 01 15]. http://old.mendelu.cz/~upsr/contents/bbcheng.pdf

11. Mesterhazy A., Batok T. Control of Fusarium head blight with fungicides // International conference Healthy Cereals 2–6 July 2001, Kroměřiž, Czech Republic. – 2001, p. 72–77

12. Noots J., Delcour J. A., Michiels W. From field barley to malt: detection and specification of microbial sensitivity for quality aspects // Critic. Rev. Microbiol. – 1999, vol. 25, p. 121–153

13. Zillinsky F. J. Common Diseases of Small Grain Cereals // A Guide to Identification. CIMMYT. – Mexico, 1983. – 141 p.