Reaction of Winter Wheat Varieties to *Fusarium graminearum* and *F. culmorum* in Field Infection Trials and the Efficacy of Fungicides

MARIE VÁŇOVÁ, LUDVÍK TVARŮŽEK and HANA HRABALOVÁ

Agricultural Research Institute Kroměříž, Ltd., Kroměříž, Czech Republic

Abstract

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Fifteen winter wheat varieties were tested for their reaction to *Fusarium culmorum* and *F. graminearum* in the ear in 1996, 1998 and 1999. To ensure a sufficient level of infection in the field the varieties were artificially inoculated with spores of the two species at a ratio of 1:1. The infection was evaluated using a 10-score scale at three times: first at the milk stage (BBCH 71–75), i.e. about 14 days after inoculation; two further readings were taken at 7-day intervals. The data were used to calculate the average infection for each variety at each reading, and were statistically evaluated. The parameter "area under disease progress curve" (AUDPC) was also calculated. Significant differences between varieties were found in all investigated years. On the average of the 3 years, the variety Ebi showed the lowest infection. Other varieties with significantly low infection were Estica and Atlet. The variety with the highest infection was Bruneta, followed by Samanta, Saskia and Samara. The degree of infection was considerably influenced by weather conditions in the year. A lower infection was observed in 1996 when rainfall in the period from 1st to 20th June was below normal, while rainfalls in 1998 and 1999 were higher in this period. The efficacy of fungicides was assessed in the highly susceptible variety Bruneta. All tested preparations significantly decreased the degree of infection in comparison with the untreated control. In 1998 the efficacy of fungicides was rather low (from 15.51 to 37.55%), while in 1999 it was higher (from 36.74 to 58.20%). In both years the preparation Folicur BT was most efficacious.

Keywords: winter wheat; *Fusarium graminearum*; *Fusarium culmorum*; resistance of cultivars; efficacy of fungicides

Fusarium graminearum and *F. culmorum* cause serious damage to grains in winter wheat ears. *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein.] Petch) is typical of areas with warmer climatic conditions where corn and rice are produced, while *F. culmorum* (W.G. Smith) Sacc. (unknown teleomorph) is characteristic of colder areas where several small-grain cereals are produced (HOMDORK *et al.* 2000a).

The level of ear infection is influenced by various factors that determine the progress of the disease; if these factors coincide, the weight of an infected ear can be reduced by as much as 50% in extreme cases (SUTY 1996). As reported by DILL-MACKY and JONES (1997), the yield reductions in 1993–1996 were 33, 18 and 5%, respectively, in the conditions of Minnesota. HOMDORK *et al.* (2000a) gave yield reductions by 24.2–45% for conditions in Europe and in experiments.

Besides yield losses, the value of the grain is diminished by mycotoxins produced by some fungi of the genus *Fusarium*. In the phytosanitary-toxicological aspect greatest attention has been paid to deoxynivalenol (DON) (OBST *et al.* 1997; SUNDHEIM *et al.* 1997); other trichothecenes have also been studied.

Their incidence is usually higher in more humid areas or in years with rainy weather from the beginning of anthesis to harvest. OBST *et al.* (1997) consider the time between 50% of ears started heading (GS 55) and end of anthesis (GS 69) as most suitable for successful infection, provided that the average temperature is higher than 18°C for 24 h and precipitation exceeds 5 mm.

The cropping system is another risk factor: an important roles are played by the forecrop, soil tillage and varietal resistance (DILL-MACKY & JONES 1997). GROTH *et al.* (1999) stated that besides high resistance of the host variety, the only effective protection was a tillage method that would bury organic matter residues and thus potential sources of inoculum. The highest incidence of fusaria, and consequently the highest content of deoxynivalenol, were reported by OBST *et al.* (1997) after corn as a forecrop.

SUTY and MAULER-MACHNIK (1996) presented results of a study on perithecium formation on corn straw. Perithecia were produced on 50% of corn residues on the ground surface. The proportion of ripe perithecia was 41% in May, increasing to 86% in June. Higher temperatures and humid weather favoured perithecium formation and the release of ascospores from ripe perithecia. KHONGA and SUTTON (1988) did not find any perithecium formation on plowed-in corn straw, and the level of infected ears on winter wheat decreased from 27% (without plowing) to 7% (after plowing-in).

Disease incidence is influenced by the amount of released ascospores, weather conditions at heading time and varietal resistance (SUTTON 1982).

Treatments with fungicides are considered as partly effective measures even if applied at the optimal time.

Under conditions in the Czech Republic, the incidence of Fusarium head blight on winter wheat has been related to weather conditions during anthesis and to the level of variety susceptibility.

The risk of a higher incidence of the disease will consequently increase with an increasing proportion of cereals (mainly winter wheat and corn) in a crop rotation, and by reduced soil tillage.

In risky areas it is advisable to benefit from different varietal resistance and partial fungicidal efficacy.

This paper presents results of 3-year field trials that were conducted to study the reaction of 15 winter wheat varieties after artificial inoculation, and the efficacy of fungicide applications on susceptible varieties.

MATERIAL AND METHODS

Testing the reaction of varieties

Field trials were performed on plots of the Kroměříž Agricultural Research Institute, Ltd., in 1996, 1998 and 1999 (235 m above sea level, average annual temperature 8.7°C, average annual precipitation 599 mm). Pea for grain was the forecrop in all experimental years, and the fertilizer schedule was: 300 kg NPK/ha (15:15:15) in the fall before sowing and 50 kg N pure nutrients per ha in the growing season in form of ammonium-calcium saltpeter.

Each variety was sown in three replicates into plots 1 m^2 in size using a microplot seeder.

To achieve sufficient disease severity every year, the trials were artificially inoculated with spores of *Fusarium graminearum* and *F. culmorum*. Their ratio in the inoculum was 1:1 (MESTERHÁZY 1978). After the pathogenicity of fungal isolates had been checked, they were cultured on solid nutrient media (sterile wheat grain) and kept under NUV light for 3–4 following sporulation stimulation. Then the substrate was dried and stored for 3 months.

Conidial concentration of each species in the inoculum was adjusted under a microscope to 1 million conidia per ml suspension of each species and put into plot at 30 ml on 1 m^2 by spraying.

Inoculation with spore suspension was carried out at the beginning of anthesis in a variety when about 50% of its spikelets had extruded anthers.

Disease grade was assessed visually on a six-score scale:

Grade	% of ear diseased
0	0
1	10
2	25
3	50
4	75
5	100

The assessment was carried out three times: the first at the milk stage (BBCH 71–75), i.e. about 14 d after inoculation; two more scorings followed at 7-day intervals.

Ten ears of each variety were assessed in three replications. The values were used to calculate an average disease grade for each variety at each date of assessment; these data were used to determine the "area under disease progress curve – AUDPC" (SHANER & FINNEY 1997) by the formula:

$$AUDPC = \text{sum}\,((x_i + x_{i+1})/2) \times T_i$$

where: x_i – % disease at *i*-th assessment
x_{i+1} – % disease at *i*+1-th assessment
T_i – number of days between assessments

For individual years the varieties were arranged in ascending order by increasing total AUDPC.

Statistical data processing was based on analysis of variance; differences were compared by Tukey's test ($P = 0.05$).

Evaluation of fungicidal efficacy

The variety Bruneta was used for these trials in 1998 and 1999; it had been selected in trials of the preceding year as a variety susceptible to Fusarium head blight. After a cereal (winter wheat) as forecrop the trial was sown on 2^{nd} October and 14^{th} October 1998. NPK fertilizer was applied in the fall (15:15:15, at a dose of 300 kg/ha), followed by additional fertilizing with ammonium-calcium saltpeter (30 kg N pure nutrients per ha) and DAM (50 kg N pure nutrients per ha) during the growing season. To prevent infection with other pathogens all plots were treated with a single dose of the mixture Bavistin + Atlas at a dose of 300 g + 0.2 l/ha (carbendazim 500 g/kg + quinoxyfen 500 g/l) to control *Tapesia yallundae* (syn. *Pseudo-*

cercosporella herpotrichoides) and powdery mildew (*Blumeria graminis* syn. *Erysiphe graminis*). Amistar (azoxystrobin 250 g/l) at a dose of 0.5 l/ha was applied to control leaf rust of wheat (*Puccinia recondita*) and *Septoria* sp.

Fungicides were applied at the beginning of anthesis (BBCH 61–64); artificial inoculation with a suspension of conidia of *Fusarium* was carried out 24 h later in the same way as in the variety trials. An identical method was used for disease rating and for statistical evaluation.

Fungicides used to control Fusarium head blight:

Fungicide	Active ingredient	Content of a.i.	Dose applied per ha
Falcon	spiroxamine	250 g/	0.6 l
	tebuconazole	167 g/l	
	triadimenol	43 g/l	
Folicur BT	tebuconazole	125 g/l	0.75 l; 1.0 l
	triadimefon	100 g/l	
Tilt 250EC	propiconazole	250 g/l	0.5 l
Caramba	metconazole	60 g/l	0.8–1.0 l

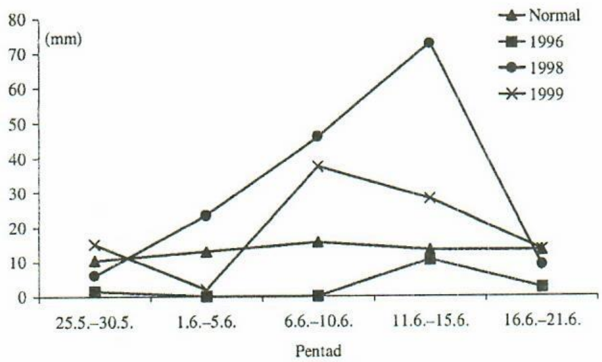

Fig. 1. Mean temperatures from 26th May to 20th June in 1996, 1998 and 1999

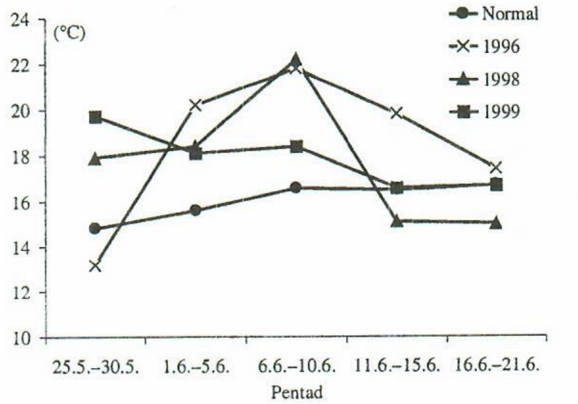

Fig. 2. Precipitation means from 26th May to 20th June in 1996, 1998 and 1999

Table 1. Assessment of the reaction of winter wheat varieties *to Fusarium culmorum* and *F. graminearum* infection of ears – year 1996 (date of inoculation: 1st– 5th June1996; DC: 61)

Variety	Percent disease			AUDPC	Significance (0.1)
	1st assessment	2nd assessment	3rd assessment		
Brea	0	0	10	35	A
Ebi	0	10	25	157.5	B
Athlet	10	10	25	192.5	C
Estica	10	10	25	192.5	C
Siria	10	10	25	192.5	C
Versailles	10	10	25	192.5	C
Šárka	0	25	25	262.5	D
Ritmo	10	10	50	280	E
Ilona	10	25	50	385	F
Alka	10	50	50	560	G
Astella	10	50	75	647.5	H
Bruneta	10	50	75	647.5	H
Samanta	10	50	75	647.5	H
Saskia	10	50	75	647.5	H
Samara	10	75	100	910	I

1st assessment: day 14 after inoculation; 2nd assessment: day 21 after inoculation; 3rd assessment: day 28 after inoculation

RESULTS

The reaction of 15 winter wheat varieties was studied in the years 1996, 1998 and 1999. The tests were also planted in 1997, but were destroyed by floods and heavy rains and, therefore, could not be included in the overall assessment.

Table 2. Assessment of the reaction of winter wheat varieties *to Fusarium culmorum* and *F. graminearum* infection of ears – year 1998 (date of inoculation: 5th June1998; DC: 61)

Variety	Percent disease			AUDPC	Significance (0.1)
	1st assessment	2nd assessment	3rd assessment		
Ebi	10	25	25	322.5	A
Estica	0	25	50	387.5	B
Athlet	10	50	50	610	C
Alka	10	50	75	710	D
Astella	10	50	75	710	D
Brea	10	50	75	710	D
Ilona	10	50	75	710	D
Bruneta	25	50	75	762.5	E
Siria	25	50	75	762.5	E
Šárka	25	50	75	762.5	E
Ritmo	10	50	100	810	F
Saskia	25	75	75	950	G
Samara	50	75	75	1037.5	H
Versailles	50	75	75	1037.5	H
Samanta	25	75	100	1050	I

1st assessment: day 14 after inoculation; 2nd assessment: day; 21 after inoculation; 3rd assessment: day 28 after inoculation

Table 3. Assessment of the reaction of winter wheat varieties *to Fusarium culmorum* and *F. graminearum* infection of ears – year 1999 (date of inoculation: 3rd June1999; DC: 61)

Variety	Percent disease				AUDPC	Significance (0.1)
	1st assessment	2nd assessment	3rd assessment	4th assessment		
Ebi	0	5	10	10	137.5	A
Athlet	0	5	17.5	17.5	208.75	B
Estica	0	10	17.5	25	273.75	C
Ritmo	0	17.5	25	50	461.25	D
Alka	10	17.5	17.5	50	462.5	E
Samara	10	17.5	25	37.5	473.75	F
Siria	0	10	37.5	50	478.75	G
Ilona	17.5	17.5	25	50	548.75	H
Brea	10	25	37.5	62.5	693.75	I
Versailles	10	25	50	62.5	775	J
Šárka	10	25	50	75	812.5	K
Astella	25	37.5	37.5	62.5	875	L
Samanta	10	37.5	50	62.5	881.25	M
Saskia	10	37.5	50	62.5	881.25	M
Bruneta	25	62.5	75	87.5	1406.25	N

1st assessment: day 12 after inoculation; 2nd assessment: day 22 after inoculation; 3rd assessment: day 29 after inoculation; 4th assessment: day 35 after inoculation

Table 4. Statistical evaluation of the reaction of winter wheat varieties, 1996–1999 and means

Variety	AUDPC value			Mean	Significance (0.1)
	1996	1998	1999		
Brea	0	0	10	35	A
Ebi	157.5	322.5	137.5	205.83	A
Estica	192.5	387.5	273.75	284.58	A B
Athlet	192.5	610	208.75	337.08	A B C
Siria	192.5	762.5	478.75	477.92	A B C D
Brea	35	710	693.75	479.58	A B C D
Ritmo	280	810	461.25	517.08	A B C D E
Ilona	385	710	548.75	547.92	A B C D E
Alka	560	710	462.5	577.5	A B C D E
Šárka	262.5	762.5	812.5	612.5	A B C D E
Versailles	192.5	1037.5	775	668.33	B C D E
Astella	647.5	710	875	744.17	C D E
Samara	910	1037.5	473.75	807.08	D E
Saskia	647.5	950	881.25	826.25	D E
Samanta	647.5	1050	881.25	859.58	D E
Bruneta	647.5	762.5	1406.25	938.75	E

Table 5. Significance of differences in AUDPC for the factor: years 1996, 1998, 1999

Year	Mean square	Significance (0.1)
1 – 1996	396.66	A
3 – 1999	624.66	B
2 – 1998	755.50	B

Table 6. Analysis of variance for the relationship of *Fusarium* progress AUDPC in winter wheat to the year and variety

Variability source	Degrees of freedom	Mean square	*F*	Significance
Year	2	494 656.81	12.844	**
Varieties	14	142 371.79	3.697	*
Residual	28	38 513.874		
Total	44			

**significance at 0.01; *significance at 0.05

Table 7. Efficacy of fungicide applications to control Fusarium head blight in the variety Bruneta in 1998 (fungicide application: 4rd June 1998; date of inoculation: 5th June 1998; DC : 61)

Treatment	Dose per ha	Fusarium head blight scoring	Percent efficacy	Significance (0.1)
Control		2.45		A
Falcon	0.6	2.07	15.51	B
Folicur BT	0.75	1.83	25.31	C
Tilt	0.5	1.77	27.76	D
Caramba	1.2	1.66	32.24	E
Caramba	0.8	1.53	37.55	F
Folicur BT	1	1.53	37.55	F

Table 8. Efficacy of fungicide applications to control Fusarium head blight in the variety Bruneta in 1999 (fungicide application: 2[st] June 1998; date of inoculation: 3[rd] June 1998; DC: 61–65)

Treatment	Dose per ha	Fusarium head blight scoring	Percent efficacy	Significance (0.1)
Control		2.23		A
Control		2.08		A
Caramba	1.2	1.36	36.74	B
Caramba	0.8	1.25	41.86	C
Falcon 460 EC	0.6	1.02	52.5	D
Tilt 250 EC	0.5	1	53.48	E
Folicur BT 225 EC	1	0.98	54.4	F
Folicur BT 225 EC	0.75	0.9	58.2	G

Disease severity was lowest in 1996, while in 1998 and 1999 it was statistically significant higher. The difference between 1998 and 1999 was not statistically significant. As identical isolates of both fusaria and identical spore concentrations for inoculation were used in these trials, differences in severity could be caused by different temperature and moisture conditions after inoculation.

Figs. 1 and 2 show precipitation and temperatures from 1[st] to 20[th] June; the year 1996 had below normal precipitation in this period, while in 1998 and 1999 it was higher. The temperature sum for 1996 was higher than those for 1998 and 1999. The data demonstrate obvious effects of weather conditions and the fact that the excessive precipitation in 1998 did not further increase disease severity statistically significant.

Differences between the varieties were statistically significant in all experimental years (Tables 1–3). The 3-year mean shows that disease grade was lowest in the variety Ebi (Table 4). The varieties Estica and Athlet also had significantly lower disease grades. These three varieties are later ones, their anthesis is delayed by 3–5 days. All other varieties showed statistically significant higher disease grades. The highest disease grade was observed in the variety Bruneta, preceded by Samanta and Saskia. These three are part of a collection of earlier varieties.

Fungicidal efficacy was evaluated in the highly susceptible variety Bruneta by a scale described in the Method section. Using this method, all fungicides statistically significant reduced the disease severity in comparison with the untreated control. Differences between the fungicides were also statistically significant except for Caramba (0.8 l) and Folicur BT (1.0 l) in 1998. Total percent efficacy was low in 1998, ranging from 15.51 to 37.55%; in 1999 it was higher, between 36.74 and 58.2%. Folicur BT had the highest efficacy in both experimental years: by a dose of 1 l/ha in 1998, by one of 0.75 l/ha in 1999.

DISCUSSION

To assess the reaction of winter wheat varieties to Fusarium head blight, the method of artificially induced infection under field conditions has routinely been used until now (GROTH *et al.* 1999). If this is accompanied by sprinkler irrigation, reproducibility of the results may be enhanced. But disease intensity is variable without irrigation, so that in some years the reaction cannot be assessed at all due to a low disease grade which blurs the differences between the genotypes. The authors cited above state that neither is it possible to assess lodged stands where conditions for infection of ears near the ground surface are more propitious and the ears are thus exposed to higher infection pressure than those in stands without lodging.

The year 1997 was excluded from our experimental results because heavy rains caused severe lodging. There was a significant difference between the year 1996 on the one hand and the years 1998 and 1999 on the other, and disease severity was sufficiently high in all 3 years to allow evaluation of differences between the varieties.

The source of infection can be another reason for variability. Natural presence of fusaria in the ear is a result of asexual conidia or sexually produced ascospores on the surface of plant debris (DILL-MACKY & JONES 2000). An inoculum in form of a mixture of mycelial and conidial fragments cultivated under laboratory conditions is used for most field tests. The spore concentration is so high and spraying so homogeneous which makes it very unlikely that such natural inoculum will have an influence on the level of infection.

Differences in level of infection on varieties were significant when data on the particular years were statistically processed while taking into account the potential effects of a year. Yet more important is the statistical processing of 3-year means which was possible due to sufficiently high disease severity in all 3 years. This method of data processing and result evaluation demonstrated the explicitly lowest disease grade in the variety Ebi and the highest in Bruneta. But the differences between Ebi and the next eight varieties were not statistically significant. Neither was the difference between Bruneta and the nine varieties before it statistically significant. Consequently, the bulk of the tested varieties do not differ in reaction from each other very much; the only variety with a lower disease grade is Ebi for all experimental years. In world collections, Chinese materials are considered as important sources of resistance (GROTH *et al.* 1999).

Fungicide applications are another important protective measure. Attention is currently focused on this measure because the chances of using resistant varieties are small and ascospores produced on plant debris are an important infection source (SUTY & MAULER-MACHNIK 1996). Corn for grain and silage is a source of infection for subsequent winter wheat (DILL-MACKY & JONES 2000) as well as spring barley.

MATTHIES and BUCHENAUER (2000) evaluated the efficacy of the active ingredients tebuconazole (150 g/l in Folicur) and prochloraz (320 g/l in Sportak) in separate applications or in a mixture of both fungicides to control artificial infection of winter wheat at the GS 65 stage. Infection intensity was reduced by both fungicides in comparison with the untreated control. Fungicide applications within a short time after inoculation (2 days) were most efficient, Folicur reduced infection intensity by 56% and Sportak 41%. Treatments 8 days before infection and 9 days after infection showed lower efficiency.

In the present trials the fungicides were applied 1 day before inoculation, their efficacy was higher in 1999 than in 1998. Efficacies of 58.2% and 54.4% were achieved in 1999 by applications of Folicur BT at doses of 0.75 and 1 l/ha, but it was only 37.55% in 1998 with a dose of 1 l/ha. These variations can likely be explained by the lower content of tebuconazole in Folicur BT than in the Folicur used by MATTHIES and BUCHENAUER (2000).

HOMDORK *et al.* (2000a) reported fungicidal efficiency to be related to time, frequency of applications and fungicide dose while tebuconazole is considered as a highly efficient ingredient (PARRY *et al.* 1995). Nevertheless, yields increased after tebuconazole applied to control fusaria very differently (in a range of 31 to 80%). MIELKE and MEYER (1990) also found tebuconazole to be the most efficient fungicide, even though they failed to achieve complete suppression of infection.

Fusarium head blight in winter wheat largely decreases crop yield and quality. Lower quality is ascribed mainly to mycotoxin production. Toxins can be produced on infected grains during storage under favourable conditions, so their content is not only determined by field conditions (HOMDORK *et al.* 2000b).

The results of these trials indicate that the susceptibility of varieties grown in this country, favourable conditions for infection under field conditions, the high percentage of cereals in crop rotations and the increasing use of minimized soil tillage make it necessary to be concerned with Fusarium head blight. Consequently, not only yield losses but also mycotoxin content should be monitored.

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Souhrn

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V letech 1996, 1998 a 1999 byl hodnocen soubor 15 odrůd pšenice ozimé na odolnost k *Fusarium culmorum* a *Fusarium graminearum* v klase. Pro zajištění každoročního dostatečného stupně napadení v polních pokusech byly uměle infikovány sporami *Fusarium graminearum* a *F. culmorum* (poměr v inokulu 1 : 1). Hodnocení napadení bylo prováděno modifikovanou desetibodovou stupnicí. Hodnocení bylo prováděno třikrát – poprvé v mléčné zralosti (BBCH 71–75) tj. asi 14 dnů po inokulaci, další dvě hodnocení byla provedena v sedmidenních intervalech na 10 klasech ve třech opakováních. Ze získaných údajů bylo vypočteno průměrné napadení každé odrůdy v každém termínu. Tato data sloužila k výpočtu parametru plochy pod křivkou vývoje choroby AUDPC. Statistické zpracování bylo provedeno analýzou variance. Pro porovnání rozdílů byl použit Tukey test. Byly zjištěny průkazné rozdíly mezi odrůdami ve všech sledovaných letech. V průměru tří sledovaných let byla nejméně napadenou odrůdou Ebi, následovaly průkazně méně napadené odrůdy Estica a Athlet. Naopak nejvíce napadenou odrůdou byla Bruneta a před ní se umístily odrůdy Samanta, Saskia a Samara. Výrazný vliv na celkový stupeň napadení měl průběh počasí v daném roce. Menší napadení bylo v roce 1996, kdy období od 1. do 20. června bylo srážkově podnormální, zatímco roky 1998 a 1999 byly v tomto období srážkově bohatší. Účinnost aplikace fungicidů byla hodnocena u vysoce náchylné odrůdy Bruneta. Všechny zkoušené přípravky vykázaly průkazné snížení stupně napadení ve srovnání s neošetřenou kontrolou. V obou zkoušených letech vykazoval nejvyšší účinnost přípravek Folicur BT. V roce 1998 bylo procento účinnosti nižší (od 15,51 do 37,55 %). V roce 1999 byla účinnost aplikovaných fungicidů vyšší (od 36,74 do 58,2 %).

Klíčová slova: pšenice ozimá; *Fusarium graminearum*; *Fusarium culmorum*; odolnost odrůd; účinnost fungicidů

Corresponding author:

Ing. MARIE VÁŇOVÁ, CSc., Zemědělský výzkumný ústav Kroměříž, s.r.o., Havlíčkova 2787, 467 41 Kroměříž, Česká republika, tel.: + 420 634 30 71 30, fax: + 420 634 227 25, e-mail: vanovam@vukrom.cz