Influence of Wheat Cultivar, Species of *Fusarium*, and Isolate Aggressiveness on the Efficacy of Fungicides for Control of Fusarium Head Blight

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ABSTRACT

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Attempts to control Fusarium head blight (FHB) with fungicides have been highly variable. Variability is caused by cultivar resistance, fungicide efficacy, fungicide coverage, timing, and pathogen aggressiveness. In this research, fungicides were tested on winter wheat cultivars with different levels of resistance to FHB using different isolates of *Fusarium graminearum* and *F. culmorum* to evaluate the role of host resistance and isolate aggressiveness on severity of FHB. Fungicides were applied to groups of wheat heads to provide full coverage. Incidence and severity of FHB was measured by the severity of head symptoms, percentage of *Fusarium*-damaged kernels (FDK), yield loss, and deoxynivalenol (DON) contamination. Development of FHB was affected by fungicides, cultivars, fungal isolates, and most of the two-way interactions of these variables. Among the fungicides tested, those containing tebuconazole tended to be more effective in reducing FHB. Reduction of disease in susceptible cultivars may not be adequate to produce marketable yields under conditions of high disease pressure. In most cases, if a fungicide reduced FHB visual symptoms, similar decreases were detected in yield loss, DON concentration, and FDK reaction. In 1998, an increase in DON contamination compared with the Fusarium check was observed with azoxystrobin and carbendazim on the more susceptible cultivar. This increase in DON with some fungicide requires additional research. Research to develop more resistant cultivars, better spraying technology, and more effective fungicides is also needed.

Additional keywords: *Fusarium culmorum*, *Gibberella zeae*, mycotoxin, scab, vomitoxin

Hungary produces 1.1 to 1.3 million ha (about 3 million acres) of wheat (*Triticum aestivum* L.). The average national yield is 3.5 to 5.4 t/ha, but 6 to 9 t/ha has been reported on some farms. Fusarium head blight (FHB) of wheat, a devastating disease throughout the world, frequently occurs in Hungary. National surveys have been conducted in Hungary for 32 years to monitor the incidence of FHB. In dry years, only 5 to 6% of the grain is infected, but in years in which environmental conditions are favorable for the development of FHB, the national mean of disease incidence often exceeds 20%. Incidence in some fields reaches 100%. The first major epidemic of FHB was observed in 1970, and subsequent major epidemics were reported in 1979, 1985, 1997, 1998, and 1999. The highest incidence of grain infection across the country was 24.9% in

1999. In 1998, losses were estimated at \$100 million, including direct and indirect losses in yield, lower prices, and consequences of deoxynivalenol (DON) contamination (31).

Fungicides have been applied in an attempt to manage the disease, but efficacy has been variable and often unsatisfactory (1,4,5,8,21–24,33,34,39,41,43,54). Most researchers reported less than 50 to 60% control. In Hungary, Füzi (10) reported efficacy of fungicides at about 30%. Milus and Parsons (37) tested several fungicides against FHB, but did not observe any reduction in symptoms or DON contamination. Generally, the fungicides with triazole chemistry (tebuconazole, metconazole, and bromuconazole) are the most effective compounds (3,5,6,9,18,31,36); however, it was not always possible to reduce the ratio of visibly scabby grain to less than 5%, which is considered necessary for practical control (54).

In addition to reducing yield and grain weight, FHB results in DON contamination of grain. Several reports indicate that products containing tebuconazole reduce the concentration of DON by about 50% (2,17,33,47,48). We found (33,35) that concentration of DON decreased in proportion to FHB symptoms. For *Fusarium sporotrichioides* Shreb., Moss and

Frank (38) reported a reduction in T-2 toxin and diacetoxyscirpenol following application of tridemorph at a low rate (0.35 liters/ha, 0.24.5 kg a.i./ha), but DON concentration increased at application rates up to 5 times higher (3.75 liters/ha, 2.62 kg a.i./ha), despite reduced severity of symptoms. Gareis and Ceynowa (11) reported an increased nivalenol contamination following application of tebuconazole. Concentration of DON has been reported to increase following strobilurin application (41). D'Mello et al. (7) concluded that "sublethal doses" of fungicides (rates lower than recommended by the manufacturer), such as carbendazim, tridemorph, difenoconazole, and tebuconazole plus triadimenol, might increase mycotoxin contamination. Ueda and Yoshizawa (51) reported the occurrence of increased DON concentration at a low rate of carbendazim, but reduced concentrations at higher fungicide rates.

These contradictory results of fungicide efficacy in reducing symptoms and on mycotoxin production need clarification. One source of variability in these tests is the species and isolate of *Fusarium* involved in the complex, which vary in virulence (30,35). Annually, 500 to 600 samples of grain from commercial fields are planted on pentachloronitrobenzene agar (42) to identify the grain infection level of FHB in Hungary. The primary pathogens associated with FHB in Hungary are *F. graminearum* Schwabe, *F. culmorum* (Wm. G. Smith) Sacc., and *F. avenaceum* (Fr.:Fr.) Sacc. (27). Fourteen species of *Fusarium* were recovered from isolations from over 4,000 diseased kernels (27). *F. graminearum* (68%) and *F. culmorum* (12%) were the most frequently isolated species. *F. poae*, *F. avenaceum*, and *F. sporotrichioides* were isolated from less than 2% of the grains.

The primary objective of this study was to evaluate the efficacy of fungicides on FHB and their influence on DON concentration. In addition, different cultivars and species of *Fusarium* were included in the test to elucidate their role in FHB severity and fungicide efficacy, and to provide possible explanations for the contradictory results observed in previous research.

MATERIALS AND METHODS

Experimental design. Winter wheat plots were planted following oil rape (*Brassica napus* L.) to minimize inoculum

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of *Fusarium* spp. from debris. The plot size was 5 m² and wheat was sown at 550 seed/m². The plots were planted on 15, 18, and 22 October in 1995, 1996, and 1997, respectively, using a Wintersteiger Øyord planter (Wintersteiger GmbH, Ried, Austria). Two winter wheat cultivars with different levels of resistance to FHB were planted in the first two years and three cultivars the third year. Zombor (moderately susceptible [MS]) and Zugoly (susceptible [S]) were sown in 1996, Táltos (MS) and Zugoly (S) in 1997, and Bence (moderately resistant [MR]), Táltos (MS), and Zugoly (S) in 1998.

The fungicide treatments were replicated four times for each cultivar in a randomized complete block design. Within each fungicide plot, four isolates of *Fusarium* spp. and an uninoculated control served as subplots (also called microplots). Another treatment was inoculated but was not treated with fungicide, which is referred to as the no-fungicide treatment. There were three replicates of about 20 heads for each subplot in a factorial design.

Fungicide applications. Fungicides were applied at Feekes growth stage 10.5.1 (full flowering) with 0.5-liter hand sprayers with graduated flasks. Each fungicide was applied in 250 ml of water per 5-m² plot, with one-half applied from each side of the plot so that heads were thoroughly covered. This volume is equivalent with 203 liters/acre or 53.7 gal/acre. The rates of active ingredients are given in Table 1 and agree with the official recommendations for commercial field use in Hungary and European Union.

Inoculum production. Cultures of *F. graminearum* and *F. culmorum* were used in this study. Isolates 12377 (from maize seed; Veszto, Hungary, 1978), 40, and 44 (from wheat grains; Tulln, Austria, 1992) of *F. graminearum*; and isolates 12375 (from wheat root; Szeged, Hungary, 1978) and 12551 (from wheat stalk; Szeged, Hungary, 1978) of *F. culmorum* were used in the studies. Four isolates were used each year, but they differed each year (Table 2). Virulence of the four isolates was confirmed prior to testing using a bioassay in petri dishes (26,28,30). Isolates were

stored in test tubes on potato dextrose agar under light mineral oil (Soltrol 160) at room temperature. Isolates stored under these conditions remain viable for 3 years and do not lose pathogenicity (29).

The inocula were sprayed the day after fungicide treatment. Inoculum was produced in 10-liter flasks containing liquid Czapek-Dox medium (30,31). The medium was aerated at about 1 liter/min through a glass tube. A cotton plug was used to filter air in order to avoid contamination of the medium by other microorganisms. After 7 days at room temperature (21 to 24°C), the suspension was homogenized for 30 s in a blender.

The concentration of conidia was measured with a Buerker cell counting chamber (MOM; Hungarian Optical Works, Budapest, Hungary). The suspension contained conidia and mycelium fragments that both are capable of forming colonies and infecting plants (50). Mycelial fragments also were present (especially for isolates of *F. graminearum* that sporulate poorly); therefore, the amount of fungal inoculum in the suspension was higher than the actual

Table 1. Fungicides, active ingredients, and application rates of fungicides used in fungicide trials, 1996 to 1998

Fungicide	Active ingredient (g/liter)	Application rate (liters/ha) ^a		
		1996	1997	1998
Alert S ^b	Flusilazole 125 + carbendazim 250	1.0	1.0	NT
Alto Combi 420 ^c	Cyproconazole 120 + carbendazim 300	1.0	NT	NT
Amistar ^d	Azoxystrobin 250	NT	NT	1.0
Archer 425 Ec ^e	Propiconazole 125 + fenpropimorph 375	NT	1.0	1.0
Caramba SL ^f	Metconazole 60	NT	1.0	NT
Duett ^g	Epoxyconazole 125 + carbendazim 125	NT	NT	1.0
Falcon 460 EC ^h	Tebuconazole 167 + spiroxamine 250 + triadimenol 43	0.6, 1.2	0.6	0.6, 0.8, 1.0
Folicur BT = F. Top ^h	Tebuconazole 125 + triadimephon 100	1.0	1.0	1.0
Folicur Plus = Matador ^h	Tebuconazole 250 + triadimenole 125	1.0	NT	NT
Folicur Solo = Folicur 250 Ec ^h	Tebuconazole 250	1.0	1.0	1.0
Granit Sc ⁱ	Bromuconazole 200	1.0	NT	NT
Juwel ^g	Kresoxim-methyl 125 + epoxyconazole 125	NT	NT	1.0
Kolfugo Super ^j	Carbendazim 200	1.5	1.5	1.5
Tango ^g	Epoxyconazole 125 + tridemorph 375	NT	1.0	NT
Folicur BT + Kolfugo Super	Tebuconazole 125 and triadimephon 100 + carbendazim 200	NT	1.0 + 1.5	1.0 + 1.0

^a NT = not tested.

^b DuPont, Newark DE USA/BASF Germany.

^c Novartis, Basel, Switzerland.

^d Zeneca Agrochemicals, Fernhurst, Haslemere, Surrey, United Kingdom.

^e Syngenta, Basel, Switzerland.

^f Cyanamid, France, now BASF, Ludwigshaven, Germany.

^g BASF, Ludwigshaven, Germany.

^h Bayer, Leverkusen, Germany.

ⁱ Aventis, Crop Science Kft, Budapest, Hungary.

^j Chinoin RT, Budapest, Hungary.

Table 2. Concentration and aggressiveness of isolates of *Fusarium culmorum* and *F. graminearum* used in fungicide trials, 1996 to 1998

Isolate	1996		1997		1998	
	CFU/ml ^a	Aggress. (%) ^b	CFU/ml	Aggress. (%)	CFU/ml	Aggress. (%)
12551 <i>F. culmorum</i>	0.7 × 10 ⁵	100	...	...	4.5 × 10 ⁵	100
12375 <i>F. culmorum</i>	...	...	...	...	1.8 × 10 ⁵	100
44 <i>F. graminearum</i>	5.2 × 10 ⁵	95	2.1 × 10 ⁵	91	...	...
44 <i>F. graminearum</i>	2.6 × 10 ⁵	85	...	...	...	...
40 <i>F. graminearum</i>	4.3 × 10 ⁵	96	1.3 × 10 ⁵	22	5.0 × 10 ⁵	86
12377 <i>F. graminearum</i>	...	...	3.8 × 10 ⁵	100	2.5 × 10 ⁵	92
12377 <i>F. graminearum</i>	...	...	1.9 × 10 ⁵	100	...	...

^a Concentration of conidia in suspension. Mycelial fragments that were not counted also were present in the suspensions.

^b Aggressiveness determined by the percentage of seedling killed after 6 days using the petri dish assay (29).

concentration of conidia. The inocula were stored at 4°C until use.

Inoculation. Arbitrarily chosen groups of approximately 20 flowering heads (Feekes growth stage 10.5.1) were sprayed from each side with the inoculum (about 20 ml for each) using a 1-liter hand sprayer. The heads were covered with a polyethylene bag for 24 h, if temperature was above 20°C, and for 48 h, if the temperature was less than 20°C. The uninoculated spikes were sprayed by distilled water and bagged in the same way, and served as controls. The uninoculated groups of heads were sprayed first to avoid possible contamination. No additional moisture was applied to the heads.

As indicated previously, each of the four fungal isolates was applied in triplicate within each main plot (5 m²). The first isolate was inoculated onto heads approximately 0.5 m from the front of the plot. Subplots within a plot were separated by approximately 0.5 m. Each group of heads was labeled and loosely bound until harvest.

Disease evaluation and yield. Head symptoms were evaluated 10, 14, 18, 22,

and 26 days after inoculation on a scale of 0 to 4, where 0.1 = 2.5, 0.5 = 12.5, 1 = 25, 1.5 = 37.5, 2 = 50, 2.5 = 62.5, 3 = 75, 3.5 = 87.5, and 4 = 100% of the spikelets with FHB symptoms (29). For statistical analyses, the ratings were converted to percentages by multiplying them by 25, which is in agreement with other FHB rating schemes (45).

At harvest, each group of heads was cut by hand and placed in a separate bag. Ten heads were randomly separated and threshed at a low fan speed so that small, infected grains would not be blown away with the chaff. Moisture of grain was 13 to 16% at harvest and 12% at threshing (2 to 3 months after harvest). The grain was weighed and the percentage of *Fusarium*-damaged kernels (FDK) was estimated. Only those kernels with characteristic white or rose discoloration were considered damaged by *Fusarium* spp. This method is in agreement with the color picture series from Minnesota (19), except the scale was enlarged to 100% with 10% intervals. The three samples of grain of an isolate from each plot were pooled for DON analysis (34). Of 6 g of seed milled,

5 g were used for DON analysis. The method for DON analysis was described earlier (35).

Efficacy of each fungicide treatment for FHB, FDK, DON, and yield loss was calculated as a percentage of the inoculated control, where percent = $100 - (x/y) \times 100$, where x = value of fungicide treatment and y = value of unsprayed inoculated control. The mean of the percentage of the four variables was used to provide the percentage of overall efficacy.

Statistics. Both a randomized block and nested-factorial design were used for analysis. The severity of FHB, ratio of FDK, and DON values of the noninoculated control groups of heads were zero in all tests; therefore, these data were not included in the analyses. In all tests, the plots that were inoculated with *Fusarium* spp. without fungicide treatment served as controls to evaluate fungicide effect. The mean fungal isolate from the fungicide-treated subplot was compared with the same fungus in the unsprayed inoculated control. The data for FHB, FDK, and yield loss were averaged for the three groups of heads for an isolate within a 5-m² plot. The

Table 3. Significance of mean square values for yield loss, *Fusarium* head blight rating (FHB), percentage of damaged kernels (FDK), and concentration of deoxynivalenol (DON) for each source of variation in 1996

Source of variance	df	Mean square values ^a			
		Yield loss	FHB	FDK	DON
Fungicide	9	2,720.3***	2,420.3***	2,783.1***	657.6***
Isolate	3	27,186.3***	22,370.0***	22,869.6***	2,661.6***
Cultivar	1	31,335.0***	18,483.0***	10,948.0***	1,317.2***
Fungicide × isolate	27	76.3 ns	107.1**	358.4**	51.4**
Fungicide × cultivar	9	168.4*	124.7**	461.5**	138.5***
Isolate × cultivar	3	960.3***	955.6***	1,390.0***	79.8**
Fungicide × isolate × cultivar	27	87.7 ns	43.9 ns	158.6 ns	38.9 ns
Within	240	86.3	56.2	116.8	26.2
Total	319	...	...	...	...

^a Yield loss relative to the uninoculated check; ***, **, and * significant at $P \leq 0.001$, 0.01, and 0.05, respectively; ns = not significant.

Table 4. Effect of fungicides on mean *Fusarium* head blight (FHB) rating, percentage of damaged kernels (FDK), yield loss, and concentration of deoxynivalenol (DON) across both cultivars and four fungal isolates in 1996

Fungicide (liters/ha)	Disease parameter ^a				
	FHB (%)	FDK (%)	Yield loss (%)	DON (ppm)	Overall efficacy (%) ^b
Falcon 1.2	23.8	14.6	28.8	7.0	59.9
Folicur Plus 1.0	23.9	15.9	30.5	7.5	57.9
Folicur Solo 1.0	24.8	19.6	30.7	11.0	51.4
Fol. BT 1.0	28.5	18.4	33.5	8.3	52.1
Falcon 0.6	30.8	19.3	35.5	9.8	47.9
Alert 1.0	32.4	27.1	36.6	12.3	39.3
Alto Combi 1.0	36.5	29.7	44.3	11.1	34.1
Granit 1.0	38.2	33.1	45.0	15.7	25.8
Kolfugo Super 1.0	38.2	32.0	43.3	12.7	30.5
No fungicide ^c	51.8	43.7	58.7	22.2	0.00
Mean					
All treatments	32.9	25.3	38.7	11.8	39.9
All treatments, no fungicide	30.8	23.3	36.5	10.6	44.4
LSD ($P \leq 0.05$) ^d	3.7	5.3	4.6	2.5	5.7
Mean fungicide efficacy (%)	40.6	46.6	37.8	52.2	44.3

^a Yield loss relative to the uninoculated check; *Fusarium* head blight rating based on a 0-to-4 scale, where 0 = no head blight and 4 = 100% of the spikelets with head blight.

^b The efficacy of each fungicide for each parameter was calculated as a percentage of the inoculated control. The mean percentage of the four parameters is the overall efficacy.

^c Inoculated with fungus, but not sprayed with fungicide.

^d LSD = least significant difference.

mean for the three replicates was used to calculate the percentage difference, which was used in the analysis. For DON, which was from the pooled grains of the three replicates of an isolate within a plot, the concentration was used in the analysis.

The three-way and the single four-way analyses of variance and correlation analyses were conducted by Microsoft Excel (Microsoft Corporation, Redmond, WA). The three-way and four-way analyses were conducted with the functions given by Sváb (49) and Weber (53). Data were analyzed separately by year because cultivars, fungicides, and isolates changed.

RESULTS

In 1996, fungicides, fungal isolates, and cultivars all had a significant effect on yield loss, FHB, FDK, and DON (Table 3). The two-way interactions among these

variables, except for the interaction between fungicide and fungal isolate for yield loss, also were significant. The three-way interaction was not significant for any parameter.

All of the fungicide treatments reduced FHB, FDK, yield loss, and DON accumulation compared with the control, and there were significant differences among the fungicides (Table 4). The mean overall efficacy calculated using all four parameters was also different among fungicides. Fungicide treatments that included the active ingredient tebuconazole tended to be more effective in reducing head blight than the other fungicides. Most of the fungicides were more effective than the current standard carbendazim (Kolfugo Super).

There also were significant differences between cultivars for all four parameters.

The percentage of diseased kernels (Table 5) and the severity of FHB (Table 6) across all fungicides and fungal isolates were lower for Zombor, the more resistant cultivar, than for the more susceptible Zugoly. Similar results were observed for yield losses and DON accumulation (*data not shown*). Mean efficacy of the nine fungicides compared with the untreated control for FHB ratings was lower for the susceptible cultivar Zugoly (32%) than for the more resistant cultivar Zombor (51%). Mean efficacy of the nine fungicides compared with the untreated control for FDK was 43% for the susceptible cultivar Zugoly and 52% for the resistant cultivar Zombor. The results were similar for the other disease evaluation parameters (*data not shown*).

The fungal isolates also differed in virulence based on results for FHB, FDK,

Table 5. Effect of fungicide, fungal isolate, and wheat cultivar on percentage of *Fusarium*-damaged kernels in 1996^a

Fungicide (liters/ha)	Zombor					Zugoly					Overall
	Fungal isolate				Cv.	Fungal Isolate				Cv.	
	Fc12552	Fg44	Fg44	Fg40		Fc12551	Fg44	Fg44	Fg40		
Falcon 1.2	0.1	17.5	11.4	19.5	12.1	2.5	16.0	20.6	29.4	17.1	14.6
Folicur Plus 1.0	0.1	18.8	9.5	32.5	15.2	0.3	21.9	19.5	25.0	16.7	15.9
Folicur Top 1.0	0.1	22.5	11.3	12.3	11.5	2.1	26.3	32.5	40.6	25.4	18.4
Falcon 0.6	0.8	20.0	16.3	25.6	15.7	0.2	29.4	27.5	35.0	23.0	19.3
Folicur Solo 1.0	0.3	26.3	7.5	27.5	15.4	0.7	30.0	30.6	33.8	23.8	19.6
Alert 1.0	0.0	21.9	8.9	33.8	16.1	1.3	62.5	46.3	42.5	38.1	27.1
Alto Combi 1.0	0.2	33.1	21.3	21.3	18.9	0.2	60.6	45.6	55.0	40.4	29.7
Kolfugo S 1.5	0.1	30.6	21.3	36.9	22.2	0.9	52.5	52.5	61.3	41.8	32.0
Granit 1.0	0.2	55.0	23.8	46.3	31.3	0.8	48.8	45.6	44.4	34.9	33.1
No fungicide ^b	2.4	51.3	38.8	53.8	36.5	3.4	67.5	60.0	72.5	50.9	43.7
Mean											
All treatments	0.4	29.7	17.0	30.9	19.5	1.2	41.5	38.1	43.9	31.2	25.3
All treatments, no fungicide	0.2	27.3	14.6	28.4	17.6	1.0	38.7	35.6	40.8	29.0	23.3
LSD (<i>P</i> ≤ 0.05) ^c	...	...	...	...	7.5	...	...	...	...	7.5	5.3
Mean fungicide efficacy (%)	91.9	46.8	62.5	47.2	51.8	70.8	42.7	40.6	43.8	43.0	46.7

^a Fc = *Fusarium culmorum* and Fg = *F. graminearum*; Cv. = cultivar mean and Overall = overall mean.

^b Inoculated with fungus, but not sprayed with fungicide.

^c LSD = least significant difference.

Table 6. Effect of fungicide, fungal isolate, and wheat cultivar on *Fusarium* head blight (FHB) severity rating in 1996^a

Fungicide (liters/ha)	Zombor					Zugoly					Overall
	Fungal isolate				Cv.	Fungal isolate				Cv.	
	Fc12551	Fg44	Fg44	Fg40		Fc12551	Fg44	Fg44	Fg40		
Falcon 1.2	6.8	22.4	13.9	26.8	17.5	7.1	39.1	27.4	47.2	30.2	23.8
Folicur Plus 1.0	3.3	25.8	12.8	19.3	15.3	8.6	39.3	34.1	47.7	32.4	23.9
Folicur Solo 1.0	6.6	28.8	9.7	34.3	19.8	6.6	35.2	29.4	47.4	29.7	24.8
Folicur Top 1.0	6.7	33.6	19.3	26.1	21.4	8.8	46.6	32.0	54.7	35.5	28.5
Falcon 0.6	7.6	28.1	15.8	35.4	21.7	10.4	50.0	38.4	60.6	39.9	30.8
Alert 1.0	8.7	33.3	22.1	40.5	26.2	12.8	44.4	39.1	58.3	38.6	32.4
Alto Combi 1.0	6.1	38.0	25.6	39.4	27.3	14.2	60.6	45.8	62.3	45.7	36.5
Granit 1.0	7.3	40.6	29.3	43.3	30.1	14.4	57.1	48.4	65.0	46.2	38.2
Kolfugo 1.5	6.6	36.8	23.8	40.8	27.0	17.8	61.0	49.9	68.9	49.4	38.2
No fungicide ^b	13.4	57.3	51.3	64.0	46.5	26.9	70.9	56.8	73.9	57.1	51.8
Mean											
All treatments	7.3	34.5	22.4	37.0	25.3	12.8	50.4	40.1	58.6	40.5	32.9
All treatments, no fungicide	6.6	31.9	19.1	34.0	22.9	11.2	48.1	38.3	56.9	38.6	30.8
LSD (<i>P</i> ≤ 0.05) ^c	...	...	...	...	5.2	...	...	...	...	5.2	3.7
Mean fungicide efficacy (%)	50.7	44.3	62.7	46.9	50.7	58.4	32.1	32.6	23.0	32.4	40.6

^a FHB rating based on a 0-to-4 scale, where 0 = no head blight and 4 = 100% of the spikelets with head blight. Fc = *Fusarium culmorum* and Fg = *F. graminearum*; Cv. = cultivar mean and Overall = overall mean.

^b Inoculated with fungus, but not sprayed with fungicide.

^c LSD = least significant difference.

DON, and yield loss (results for FHB and FDK are shown in Tables 5 and 6). Isolate 12551 of *F. culmorum* was less virulent than the other three isolates. Although fungicide efficacy was affected by the fungal isolate, there was no difference between the two species of *Fusarium* in fungicide efficacy based on the percentage improvement in control of FHB of the fungicides compared with the untreated control. The correlations for FHB ratings among the fungicides between the isolates of *F. graminearum* and *F. culmorum* across cultivars were between $r = 0.9287$ and 0.9816 ($P \leq 0.001$).

Correlations between FHB ratings, percentage FDK, yield loss, and DON accumulation in response to fungicides were highly significant ($r > 0.93$; $P \leq 0.001$), which indicates that the fungicides had a similar effect on all four parameters. Although the responses were correlated, fungicide efficacy based on the percentage improvement over the unsprayed control was variable for the four disease parameters. Maximum fungicide efficacy was 38, 41, 47, and 52% for yield loss, FHB rat-

ings, percentage of FDK, and DON accumulation, respectively. This variation in efficacy demonstrates the need to consider all four parameters in describing the influence of fungicides on the development of head blight.

Results in 1997 were similar to 1996 (Table 7). Fungicides, fungal isolates, and cultivars had a significant effect on yield loss, FHB, and FDK. The two-way interactions among these variables, except for the interaction between fungicide and fungal isolate for yield loss, also were significant. The three-way interaction was significant only for the percentage of diseased kernels. Results for DON accumulation were different than in 1996. Accumulation of DON was not significant for cultivar or the cultivar–fungal isolate interaction.

The percentage of FDK in 1997 was higher than in 1996, but results for the two years were similar (Table 8). All fungicide treatments reduced FHB, FDK, yield loss, and DON accumulation compared with the unsprayed control, and there were significant differences among the fungicides. There also were differences among fungi-

cides in overall mean efficacy based on all four parameters. Among the fungicides, the mixture of tebuconazole and triadimephon plus carbendazim (Folicur Top plus Kolfugo) had the highest overall efficacy (56%). As in 1996, fungicide treatments that contained tebuconazole tended to be the most effective.

There also were significant differences between cultivars and fungal isolates for all four parameters. Severity of FHB (Table 9) across all fungicides and fungal isolates was lower for Táltos, the more resistant cultivar, than for the more susceptible cultivar Zugoly. Similar results were observed for reduction in yield loss, FDK, and DON accumulation (*data not shown*). Isolate 40 of *F. graminearum* was less virulent than the other isolates used in 1997.

As in 1996, correlations between fungicide efficacy for four parameters were significant ($r \geq 0.90$, $P \leq 0.001$) in 1997. Maximum efficacy for the most effective fungicide was between 61 and 51% for the FHB rating, percentage of FDK, relative yield loss, and DON accu-

Table 7. Significance of mean square values for Fusarium head blight (FHB) rating, percentage of damaged kernels (FDK), yield loss, and concentration of deoxynivalenol (DON) for each source of variation in 1997

Source of variation	df	Mean square values ^a			
		FHB	FDK	Yield loss	DON
Fungicide	9	1,494.88***	2,210.11***	2,185.33***	241.28***
Isolate	3	20,697.33***	70,910.66***	38,708.66***	1,854.29***
Genotype	1	15,443.00***	452.00***	2,833.00***	6.09 ns
Fungicide × isolate	27	154.11***	284.92***	78.25 ns	35.61***
Fungicide × genotype	9	176.88***	621.55***	341.88***	70.71***
Isolate × genotype	3	1,269.00***	464.83***	914.33***	6.07 ns
Fungicide × isolate × genotype	27	31.85 ns	121.53***	66.55 ns	12.15 ns
Error	240	23.60	88.6	89.8	7.80
Total	319	...	...	...	...

^a Yield loss relative to the uninoculated check; ***, **, and * significant at $P \leq 0.001$, 0.01, and 0.05, respectively; ns = not significant.

Table 8. Effect of fungicides on mean Fusarium head blight rating (FHB), percentage of damaged kernels (FDK), yield loss, and concentration of deoxynivalenol (DON) across both cultivars and four fungal isolates in 1997

Fungicide (liters/ha)	Disease parameter ^a				Overall efficacy (%) ^b
	FHB (%)	FDK (%)	Yield loss (%)	DON (ppm)	
Folicur Top 1.0 + Kolfugo 1.5	16.3	28.6	29.2	6.6	56.3
Folicur Solo 1.0	18.2	39.4	32.9	7.7	47.5
Folicur Top 1.0	22.8	43.9	37.9	9.9	37.3
Falcon 0.6	25.7	47.3	39.9	9.9	33.4
Archer 1.0	26.5	49.3	39.6	9.4	44.7
Metconazole 1.0	26.5	46.3	40.5	10.2	40.0
Alert 1.0	27.0	48.9	45.9	8.7	31.2
Kolfugo 1.5	28.5	46.0	44.6	9.6	30.7
Tango 1.0	30.5	52.1	47.3	11.7	22.6
No fungicide ^c	41.1	60.6	59.1	17.0	0.0
Mean					
All treatments	26.3	45.9	42.1	10.1	34.4
All treatments, no fungicide	24.7	44.6	39.8	9.3	...
LSD ($P \leq 0.05$) ^d	2.4	4.6	4.6	1.6	6.51
Mean fungicide efficacy (%)	40.0	26.3	32.7	45.3	36.1

^a Yield loss relative to the uninoculated check; Fusarium head blight rating based on a 0-to-4 scale, where 0 = no head blight and 4 = 100% of the spikelets with head blight.

^b Efficacy of each fungicide for each parameter was calculated as a percentage of the inoculated control. The mean percentage of the four parameters is the overall efficacy.

^c Inoculated with fungus, but not sprayed with fungicide.

^d LSD = least significant difference.

mulation, indicating that the fungicides had a similar effect on all four parameters measured.

In 1998, all main effects and two-way interactions were significant for FHB rating, percentage of FDK, yield loss, and concentration of DON (Table 10). The three-way interaction between fungicide, fungal isolate, and cultivar was significant for FHB rating and yield loss.

All of the fungicide treatments reduced FHB, FDK, and yield loss, and there were significant differences among the fungicides. Except for carbendazim (Kolfugo Super) and azoxystrobin (Amistar), all of the fungicides reduced the accumulation of DON (Table 11). Mean overall efficacy based on all four parameters also was different among fungicides. Fungicide treatments that included the active ingredient tebuconazole tended to be more effective in reducing head blight than the other fungicides. The combination of tebuconazole and triadimephon plus carbendazim (Folicur Top plus Kolfugo) had the highest overall efficacy.

Across all fungal isolates, the mean fungicide efficacy for FHB ratings was lower on Zugoly (59%) than on Bence (70%) and

Táltos (72%). Folicur Top + Kolfugo (82.92%) and Folicur Solo (83.7%) were the most effective in reducing FHB ratings. Similar results were observed for the percentage of FDK. Across all fungal isolates, the mean fungicide efficacy for percentage of FDK was 37.6% for the susceptible Zugoly, 53.7% for the intermediately susceptible Táltos, and 74.7% for the resistant Bence.

There also were significant differences between cultivars and fungal isolates for all four parameters. The results for DON accumulation are presented in Table 12. Accumulation of DON across all fungicides and fungal isolates was lowest for Bence, intermediate for Táltos, and greatest for Zugoly. Similar results were observed for reduction in yield loss, FDK, and DON accumulation (*data not shown*).

As in 1996 and 1997, correlations between fungicide efficacy of the four parameters measured (FHB ratings, percentage FDK, DON accumulation, and relative yield loss) were significant ($r = 0.92$ and 0.97 , $P \leq 0.001$). This indicates that fungicide efficacy was similar for the four parameters measured.

DISCUSSION

The microplot method used in this study originally was developed for evaluating host plant resistance (29). Many cultivars and fungal isolates could be evaluated at the same time in a relatively small area. This method was easily adapted for the evaluation of fungicides on several cultivars using different isolates of the head scab pathogen.

Efficacy of fungicides in controlling FHB is highly variable and often unsatisfactory (1,4,5,21–24,33,34,39,41,54). Some of the variability in these studies is related to the timing of the fungicide application and coverage (12). Other sources of variability are the timing and severity of infection, virulence of the isolates, and level of resistance in the cultivars planted. Using the microplot method, these sources of variability are eliminated or reduced, which provides an accurate evaluation of fungicide efficacy. Although the microplot technique eliminates or reduces problems with fungicide coverage and timing, results of fungicide efficacy observed in this study should not be expected under field conditions experienced by producers. Producers do not have the benefits of ideal

Table 9. Effect of fungicide, fungal isolate, and wheat cultivar on *Fusarium* head blight (FHB) ratings in 1997^a

Fungicide (liters/ha)	Taltos					Zugoly					Overall
	Fungal isolate					Fungal isolate					
	Fg44	Fg40	Fg12377	Fg12377-2	Cv.	Fg44	Fg40	Fg12377	Fg12377-2	Cv.	
Folicur Top 1.0 + Kolf. 1.5	14.4	0.9	17.9	10.3	10.9	24.7	2.4	38.8	20.8	21.7	16.3
Folicur Solo 1.0	16.4	1.7	17.6	13.3	12.2	28.3	4.3	39.7	23.7	24.1	18.2
Folicur Top 1.0	20.3	1.3	26.7	18.1	16.6	39.0	3.0	43.0	30.8	28.9	22.8
Falcon 0.6	19.0	2.3	30.5	23.7	18.9	41.5	4.4	49.1	35.3	32.6	25.7
Archer 1.0	20.6	0.2	30.1	7.0	14.5	48.8	4.8	59.8	41.0	38.6	26.5
Metconazole 1.0	23.5	3.2	31.9	25.0	20.9	41.4	5.3	46.1	35.9	32.2	26.5
Alert 1.0	27.7	2.6	35.7	25.3	22.8	41.3	5.6	51.0	27.2	31.3	27.0
Kolfugo 1.5	17.6	2.1	31.7	21.9	18.3	45.4	8.0	60.5	40.9	38.6	28.5
Tango 1.0	25.3	2.5	36.8	30.8	23.9	42.4	6.0	58.7	41.7	37.2	30.5
No fungicide ^b	44.1	4.3	48.4	42.1	34.7	58.5	7.0	70.9	53.5	47.4	41.1
Mean											
All treatments	22.9	2.1	30.7	21.7	19.4	41.2	5.1	54.7	35.0	33.3	26.3
All treatments, no fungicide	20.5	1.9	28.8	19.5	17.7	39.2	4.8	49.6	33.0	31.7	24.7
LSD (<i>P</i> ≤ 0.05) ^c	...	...	...	...	2.2	...	...	...	...	2.2	1.6
Mean fungicide efficacy (%)	60.4	56.8	40.6	53.7	49.1	33.0	30.4	30.0	38.3	33.2	39.9

^a FHB rating based on a 0-to-4 scale, where 0 = no head blight and 4 = 100% of the spikelets with head blight. Fc = *Fusarium culmorum* and Fg = *Fusarium graminearum*; Cv. = cultivar mean and Overall = overall mean; Kolf. = Kolfugo.

^b Inoculated with fungus, but not sprayed with fungicide.

^c LSD = least significant difference.

Table 10. Significance of mean square values for *Fusarium* head blight rating (FHB), percentage of damaged kernels (FDK), yield loss, and concentration of deoxynivalenol (DON) within each source of variation in 1998

Source of variance	df	Mean square values ^a				df for DON (ppm)
		FHB (%)	FDK (%)	Yield loss (%)	DON (ppm)	
Fungicide	10	4,601.25***	5,535.33***	3,968.07***	345.16***	9
Cultivar	2	19,243.41***	71,748.72***	18,837.52***	3,838.32***	2
Isolate	3	13,853.55***	23,769.84***	12,488.33***	953.36***	3
Fungicide × cultivar	20	450.05***	750.13***	305.06***	92.49***	18
Fungicide × isolate	30	464.98***	431.98***	234.64***	50.42**	27
Cultivar × isolate	6	1,034.48***	2,597.43***	736.66***	212.13***	6
Fungicide × cultivar × isolate	60	65.28***	225.4	91.17*	20.12 ns	54
Error	396	19.6	108.65	69.1	19.99	240
Total	527	...	...	...	...	359

^a Yield loss relative to the uninoculated check; ***, **, and * significant at $P \leq 0.001$, 0.01, and 0.05, respectively; ns = not significant.

coverage and timing of fungicide applications that were achieved with the microplots in this study. These results do, however, demonstrate the potential of these fungicides to control FHB.

In this study, there were differences among fungicides in their ability to reduce FHB. Fungicides that contain tebuconazole as one of the active ingredients tended to be the most effective, but their efficacy was reduced at lower rates (Falcon at 0.6 liter/ha). Other researchers (14,23,52) have reported satisfactory control using tebuconazole, but Hershman et al. (16) reported poor performance. Although bromuconazole (Granit) and metconazole (Caramba)

have been reported to have efficacy against FHB (3,5,6,20), they were only moderately effective in this study. The failure to perform in Hungary may be because the application rate was 33% less than the application rate in Western European countries. Results with metconazole at 1.2 and 1.5 liters/ha were similar to tebuconazole (Á. Mesterházy, *unpublished data*).

Carbendazim (Kolfugo Super), which has been the standard fungicide for control of FHB in Hungary over the past 30 years, was one of the least effective fungicides. It

has been reported to increase the efficacy of triazole fungicides against FHB (32,44). In this study, although triazoles combined with carbendazim may have been more effective than carbendazim alone, neither was better than fungicides containing tebuconazole. The mixture of tebuconazole + triadimephon (Folicur Top) and carbendazim (Kolfugo Super) was the most effective treatment against FHB in 1997 and 1998. We have observed a similar response previously with tebuconazole + spiroxamine + triadimephon (Falcon at 0.8 liter/ha) + carbendazim (Kolfugo Super at 1 liter/ha) and tebuconazole (Folicur at 1 li-

Table 11. Effect of fungicides on mean Fusarium head blight rating (FHB) in wheat rated for FHB, yield loss, percentage of damaged kernels (FDK), and concentration of deoxynivalenol (DON) across three cultivars and four fungal isolates in 1998

Fungicide (liters/ha)	Disease parameters ^a				Overall efficacy (%) ^b
	FHB (%)	Yield loss (%)	FDK (%)	DON (ppm)	
Folicur Top 1.0 + Kolfugo 1.0	7.7	16.0	15.5	4.2	70.3
Folicur Solo 1.0	8.4	20.9	19.7	3.8	68.5
Falcon 0.8	9.6	20.6	28.1	6.2	59.0
Falcon 1.0	11.5	18.4	25.9	5.7	62.0
Folicur Top 1.0 + Kolfugo 1.0	12.9	21.3	30.8	5.9	55.8
Juwel 1.0	13.6	30.4	28.8	5.9	53.4
Duett 1.0	14.1	25.4	25.9	...	...
Falcon 0.6	16.1	28.2	35.7	...	...
Kolfugo 1.5	23.3	38.4	41.9	10.4	34.0
Amistar 1.0	24.7	37.6	46.4	11.0	26.8
No fungicide ^c	41.0	50.4	58.6	11.8	0.0
Mean					
All treatments	16.5	28.0	32.5	7.2	39.1
All treatments, no fungicide	14.2	25.7	29.9	6.6	...
LSD ($P \leq 0.05$) ^d	0.7	2.8	3.4	2.0	7.74
Mean fungicide efficacy (%)	65.4	48.9	49.0	43.7	51.7

^a Yield loss relative to the uninoculated check; FHB rating based on a 0-to-4 scale, where 0 = no head blight and 4 = 100% of the spikelets with head blight.

^b Efficacy of each fungicide for each parameter was calculated as a percentage of the inoculated control. The mean percentage of the four parameters is the overall efficacy.

^c Inoculated with fungus, but not sprayed with fungicide.

^d LSD = least significant difference.

Table 12. Effect of fungicide, fungal isolate, and wheat cultivar on deoxynivalenol contamination (ppm) following fungicide application in 1998^a

Fungicide (liters/ha) ^b	Zugoly					Táltos					Bence					Over
	Fungal isolates					Fungal isolates					Fungal isolates					
	12377 Fg	40 Fg	12375 Fc	12551 Fc	Cv.	12377 Fg	40 Fg	12375 Fc	12551 Fc	Cv.	12377 Fg	40 Fg	12375 Fc	12551 Fc	Cv.	
F. Solo 1.0	7.6	1.2	10.3	16.6	8.9	3.3	1.3	0.0	3.1	1.9	0.0	0.0	0.0	2.0	0.5	3.8
F. Top 1.0 + Kolf. 1.0	10.0	4.5	5.7	13.3	8.4	4.1	0.8	0.8	9.2	3.7	0.0	0.0	0.2	1.6	0.5	4.2
Falcon 1.0	12.9	5.8	10.6	17.7	11.7	4.9	1.0	6.2	8.9	5.3	0.0	0.0	0.2	0.3	0.1	5.7
Juwel 1.0	12.5	3.8	14.1	17.6	12.0	12.3	2.0	4.8	0.0	4.8	0.0	0.0	0.9	2.3	0.8	5.9
F. Top 1.0	9.0	3.8	9.2	14.4	9.1	13.2	4.6	5.2	9.6	8.2	0.0	0.0	0.2	2.0	0.6	5.9
Falcon 0.8	12.3	6.7	17.8	19.6	14.1	6.2	5.5	1.2	2.5	3.8	0.0	0.0	1.1	2.0	0.8	6.2
Kolfugo 1.5	14.7	5.5	26.7	37.6	21.1	9.4	2.8	4.0	11.1	6.9	0.0	0.8	3.9	8.5	3.3	10.4
Amistar 1.0	20.1	12.3	25.1	26.3	21.0	14.8	2.3	11.6	9.0	9.4	0.0	0.3	2.2	7.8	2.6	11.0
No fung. ^c	14.9	8.9	21.8	22.7	17.1	9.5	6.6	18.0	20.7	13.7	0.0	0.0	4.5	13.8	4.6	11.8
Mean																
All	11.4	5.5	14.0	19.5	12.6	7.7	2.7	5.4	8.5	6.1	0.0	0.1	1.2	3.8	1.3	6.5
All, no fung.	12.4	5.5	11.6	20.4	13.3	8.5	2.5	4.2	6.7	5.5	0.0	0.1	1.1	3.3	1.1	6.6
LSD (<i>P</i> ≤ 0.05) ^d	7.2	7.2	7.2	7.2	3.6	7.2	7.2	7.2	7.2	3.6	ns	ns	ns	7.2	3.6	2.1
Mean fung. efficacy (%)	17.1	38.9	31.5	10.1	22.2	10.4	61.6	76.5	67.6	59.9	0	0	75.8	76.1	75.3	43.7

^a Fc = *Fusarium culmorum* and Fg = *F. graminearum*; Cv. = cultivar mean and Over = overall mean; ns = not significant.

^b Abbreviations: F. = Folicur, Kolf. = Kolfugo, fung. = fungicide, All = all treatments.

^c Inoculated with fungus, but not sprayed with fungicide.

^d LSD = least significant difference.

ter/ha) + carbendazim (Kolfugo Super at 1.5 liters/ha) (Á. Mesterházy, *unpublished data*). These data indicate that efficacy of existing fungicides can be improved by combining fungicides.

Although the fungicides reduced the incidence of FHB in wheat, the data also demonstrate that the most effective fungicides may not provide satisfactory control. Hart et al. (14) and McMullen et al. (23) made the same observations. Additional research is needed to develop more effective fungicides, and new formulations or combinations of existing fungicides to improve plant protection.

In this study, cultivars were a significant source of variability, and there was a significant cultivar–fungicide interaction. Infection was higher on the more susceptible cultivars. In this experiment, Zugoly was the most susceptible cultivar; however, in Hungary, there are some durum wheat and *T. aestivum* accessions that are more susceptible. Fungicide efficacy was higher on the more resistant cultivars than the more susceptible cultivars. It is important that the most susceptible cultivars be identified and withdrawn from commercial production. The knowledge of resistance level of wheat cultivars has contributed to the development of cultivar-specific fungicide treatments to control FHB in Hungary (35).

Another source of variability of some fungicide studies is the inability to control the timing and level of inoculation, as well as the variation in the virulence of different isolates of the pathogen. Reliance on natural infection often fails to produce sufficient infection pressure, and it is not possible to evaluate specific isolates of the fungus. One approach has been to inoculate plots. In North America and China, corn is inoculated with *F. graminearum* and spread in the plot area (14,25,46). Perithecia of *Gibberella zeae*, the perfect state of the fungus, develop on the inoculated corn shortly after the stem elongation and release ascospores that produce infection (47). Ascospore release and infection can be enhanced by mist irrigation. By inoculating the plots, the quantity of inoculum is greater and specific isolates can be evaluated. Although the timing of inoculation may be improved compared with natural infection, inoculation may still occur several times during and after the flowering and early grain-filling period. In addition, inoculation cannot be used for *F. culmorum* and other species that do not produce perithecia. It is possible to spray whole plots with a spore suspension to control the timing of inoculation, but to evaluate different inocula requires much more space. The microplot technique used in this study eliminates or reduces most of these problems.

Different isolates of *Fusarium* were used to evaluate their effect on disease development and to evaluate the efficacy

of fungicides on different isolates. Fungicide efficacy varied with aggressiveness of the isolate. The correlations between isolates of *F. graminearum* and *F. culmorum* in fungicide efficacy were high in this test and in other experiments (35); therefore, the fungicides tested provided protection against the two main species of *Fusarium* associated with FHB.

The results in yield, percentage of damaged grains, and concentration of DON obtained in these microplot studies cannot be related directly to commercial production. Under commercial production, about one-third (*unpublished data*) of the damaged and shriveled grains are blown out of the combine, resulting in lower yield, fewer damaged grains, and lower DON concentration (13,54). However, these results provide an indication on the efficacy of these fungicides in controlling FHB under ideal conditions. Research is being conducted to examine how the microplot technique can be used to better estimate the effect of FHB on yield, FDK, and DON concentration in producer fields.

Results on yield in this study also require careful evaluation. In this study, plots sprayed with a fungicide but not inoculated were used as one of the controls for comparisons. Fungicides that control FHB also will control leaf diseases. However, the microplot system allows us to measure yield response independently of leaf diseases. An evaluation of all diseases is needed.

In this study, the concentration of DON, FHB visual ratings, FDK severity, and yield loss were significantly correlated. Similar correlations between DON and FDK have been observed (32,46). For fungicides that are moderately effective against FHB, a decrease in DON was not always detected on susceptible cultivars. Data from Germany showed that strobilurine fungicides can increase DON concentration (40), and lower than normal rates of fungicides increased DON in other studies (7). In this study, the application of azoxystrobin (Amistar) resulted in an increase in DON only in 1998. The role of fungicides in increasing DON levels in infected wheat requires additional research.

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