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Effects of foliar fungicides on the mycoflora of glumes of *Triticum aestivum*

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In the years 1983-1984, the effect of three foliar fungicides, i.e., Bayleton 25 WP, Dithane M-45 and Funaben K on the mycoflora associated with glumes of spring *Triticum aestivum* cv. Kolibri cultivated in the field was investigated. During each vegetative period, glumes were collected in the milky ripe of seeds. Fungi species associated with glumes were determined based of colonies isolated from glumes incubated in Petri dishes with potato glucose agar. The fungicide which reduced the most the overall number of fungal isolates was Bayleton 25 WP. The number of species was most reduced following Dithane M-45 application. The mycoflora of glumes which had been untreated and treated with fungicides was compared with fungicides-treated and fungicide-untreated seeds and leaves. The highest similarity in the mycoflora of fungicide-untreated plant parts was found when glumes and seeds were compared. The mycoflora of fungicide-treated glumes, leaves, and seeds varied, depending on the year and fungicide applied.

Key words: Fungicides, mycoflora of glumes.

INTRODUCTION

Plant surfaces are colonized by many fungi (Dickinson, 1967; Jenkyn, Prew, 1973; Last, Deighton, 1965). The fungi most frequently colonizing leaves, glumes, and seeds of *Triticum aestivum* L. are *Alternaria alternata* (Fr.) Keissler, *Cladosporium* spp., *Epicoccum purpurascens* Link, *Fusarium* and *Helminthosporium* spp., *Septoria nodorum* Berk., yeast-like fungi, and non-sporulating fungi (Bashi, Fokkema, 1977; Błaszkowski, 1994 a, b, c; Dickinson, Skidmore, 1976; Dickinson, Wallace, 1976; Flanningan, 1971). In general the species composition of fungi communities of glumes is similar to that of leaves and seeds, altough climatic conditions (Bashi, Fokkema, 1977), agrochemical treatments (Hill, Lacey, 1983), the age of leaves (Dickinson, 1967; Last, 1955), and position of glumes towards other leaves or ears (Hesseltine, Bothast, 1977; Hudson, Webster, 1958; Last, Deighton, 1965) may have an influence on these communities.

Fungicides play an important role in the control of plant diseases. However, treatments usually affect both pathogenic and saprophytic mycoflora (D e C a l, M e l g a r e j o, 1992). The latter may include important antagonists (F o k k e m a, 1973; F o k k e m a, V a n D e r M e u l e n, 1976). Moreover, it was demonstrated that the changes in fungi communities of flag leaves, glumes, and seeds of wheat nad barely highly depended on, e.g., the species composition of the mycoflora (H i l l, L a c e y, 1983; L u k e, B a r n e t t, M o r e y, 1977), the fungicides used (D i c k i n s o n, 1973), and their time of application (D i c k i n s o n, W a l l a c e, 1976).

Studies devoted to determining the effect of fungicides on epiphytic mycoflora of *T. aestivum* and other cereal plants mainly concerned fungal communities associated with leaves and seeds (e.g., D i c k i n s o n, W a l l a c e, 1976; H i l l, L a c e y, 1983; M a g a n, L a c e y, 1986; M i l l s, W a l l a c e, 1968). Only D i c k i n s o n (1973) indicated changes in glume mycoflora following the application of enthirimol and zineb on plants. However, no studies were published which contain some information about the fungi communities of leaves, glumes, and seeds after fungicide treatments.

The aims of this study were to determine the effect of three fungicides on the mycoflora of *T. aestivum* glumes and to compare fungi communities of leaves, glumes, and seeds of *T. aestivum* after these chemicals had been applied.

MATERIALS AND METHODS

In the years 1982-1984, a field experiment was conducted at the Agricultural Experiment Station Lipki near Stargard Szczeciński (Poland). The following conditions were set up:

— forecrop (1982-1984) – *Solanum tuberosum* L.

— experimental design – randomized complete block design with four replicates,

— plant – spring wheat (*Triticum aestivum* L.), cv. Kolibri,

— fertilization (kg/ha) – N - 80; P_2O_5 - 110; K_2O - 120,

— fungicides – (1) Bayleton 25 WP, containing 25 % of triadimefon, at a rate of 0.5 kg/ha; (2) Dithane M-45, containing 80 % of mancozep, at a rate of 1.8 kg/ha; and Funaben K, containing 40 % of carbendazim and 40 % of captafol, at a rate of 1.5 kg/ha.

Seeds of *T. aestivum* were sown on 23, 21, and 20 April 1982, 1983, and 1984, respectively. Each replicate (a plot of dimensions of 1.8 x 1.8 m) was separated from the neighbouring ones by protective strips 1.8 m wide seeded with *Secale cereale* L. The fungicide sprays were applied with the knapsack sprayer Armitsu. Plants were treated with fungicides twice during each vegetative period, i.e., at the time of shooting (stage 6-7 after Feekes) (L a r g e, 1954) and at the beginning of heading (stage 10.1). Control plants received water-spray applications.

At the milky ripe stage of seeds (stage 11.2-3), 10 randomly selected ears were separately collected from each plot. The ears were subsequently transferred to plastic bags, transported to the laboratory, and refrigerated at 4°C until the next day. In the laboratory, glumes separated from the central part of the ears were placed into a bulb with 100 ml of sterile distilled water and shaked vigorously for 120 seconds. After drying between two pats of sterile blotting-paper, fourteen glumes randomly selected from each treatment were placed in 10-cm Petri dishes (5 glumes per dish) containing potato glucose agar (PGA). The outter glume surface contacted the agar medium surface. The Petri dishes were incubated at room conditions for 10-14 days. At the end of this period, fungi colonies growing out of each seed were transferred individually to PGA slants and identified.

Fungi species were identified according to A r x (1970), B a r n e t t (1960), B o o t h (1971), D e V r i e s (1959), D o m s c h and G a m s (1970), D r e c h s l e r (1923), E l l i s (1971), G a m s (1971), G i l m a n (1945), R a p e r and T h o m (1949), R a p e r and F e n n e l (1965), and Z y c h a, S i e p m a n n and L i n n e-m a n n (1969). Except for *S. nodorum*, representatives of each of the other species were grown from single conidia in Petri dishes of PGA at room temperature with a 12-h photoperiod under cool white fluorescent lamps located 40 cm above cultures. Cultures were grown for 10-14 days. *Septoria nodorum* was cultured on oatmeal agar, as the medium produces distinctive colonies with abundantly sporulating pycnidia.

The mycoflora of glumes were compared to that of seeds and leaves (B ł a s z-k o w s k i, 1994 b, c) using Sorensen's similarity coefficient C. This is obtained by means of the formula: 2c/(a+b), where c = number of species in common in both floras; a = number of species in one flora; b = number of species in the other flora.

RESULTS

During the two-year study, a total of 714 fungi colonies were isolated from fungicide-treated and untreated glumes (Tab. 1). They represented 21 species in 12 genera. More isolates were obtained in 1983 (401) than in 1984 (313). The fungi communities determined in 1983 included more species (5-13, depending on the type of fungicide treatment) than those in 1984 (6-9).

The fungi which most frequently occurred over the two years of study were *Alternaria* spp., *Mucor hiemalis*, a pink yeast-like fungus, and non-sporulating fungi. In 1983, *Ulocladium botrytis* was isolated frequently. The fungi dominating in the recovered populations were: *Alternaria* spp., a pink yeast-like fungus, and non-sporulating fungi.

The fungi potentially pathogenic to *Triticum aestivum* were represented by *F. culmorum*, *Fusarium graminearum*, *F. poae*, *Helmithosporium sativum* and *Septoria nodorum*. Except for *S. nodorum*, they occurred, however, rarely and comparised a small proportion. In the overall number of fungi recovered the proportion of *S. nodorum* in the overal number fungi isolated was over 4-fold higher in 1984 than in 1983.

Table 1

The effects of fugnicides on the occurrence of fungi associated with glumes of *Tricticum aestivum*

Fungus	Bayleton 25 WP		Dithane M-45		Funaben K		Control	
	1983	1984	1983	1984	1983	1984	1983	1984
Alternaria alternata (Fr.) Keissler	30	18	37	19	37	27	37	38
Aureobasidium pullulans (de Bary) Arn.	26	–	24	12	13	–	24	1
Bipolaris sorokiniana (Sacc. Shoemaker) = *Helminthosporium sativum* Pammel, King, Bakke	–	1	–	–	1	4	–	1
Botrytis cinerea Pers.: Fr.	–	–	–	2	–	–	–	–
Cladosporium cladosporioides (Fres.) de Vries	–	–	–	–	–	–	1	–
C. herbarum Link. Fr.	1	1	–	–	–	–	6	–
Epicoccum purpurascens Link	1	1	–	–	1	–	1	23
Fusarium avenaceum (Corda Fr.) Sacc.	1	–	–	–	–	–	1	–
F. culmorum (W. G. Smith) Sacc.	–	–	–	–	–	–	3	1
F. graminearum Schwabe	1	–	–	–	–	–	–	–
F. lateritium Nees	1	–	–	–	–	–	2	–
F. semitectum Berk. et Rav.	1	–	6	–	4	–	3	–
F. sporotrichoides Sherb.	1	–	–	–	–	–	–	–
F. poae (Peck) Wollenw.	–	–	–	–	–	1	–	1
Fusarium sp. 1	–	–	–	–	–	1	–	–
Fusarium sp. 2	–	–	–	–	–	1	–	–
Fusidium sp.	–	–	–	–	5	–	–	–
Mucor hiemalis Wehmer	3	1	3	1	1	–	2	5
Rhizopus nigricans Ehrenb.	–	3	–	–	–	–	–	–
Septoria nodorum Berk.	–	–	–	2	–	–	4	16
Ulocladium botrytis Preuss	2	–	1	–	5	–	6	–
Yeast-like pink	11	30	–	5	–	23	1	1
Non-sporulating	19	10	24	34	34	25	16	4
Total	98	65	95	75	101	82	107	91
No. of species	12	7	5	6	8	6	13	9

On average of two years, the fungicide which reduced the most the overall number of fungi isolates obtained from fungicide-treated glumes compared with that from control glumes was Bayleton 25 WP (by 18.5 %), followed by Dithane M-45 (14.4 %) and Funaben K (7.8 %). All fungicides reduced more the overall number of colonies in 1984 than in 1983. The rate of reduction caused by Bayleton 25 WP in 1984 was over 3-fold higher than in 1983.

The fungicide which reduced the most number of species in fungi communities was Dithane M-45 (by 47.4 % on average of two years), followed by Funaben K (35.9 %) and Bayleton 25 WP (29.9 %). However, the rare of reduction of the number of species associated with glumes treated with fungicides relative to the number of species of control glumes highly differed depending on both the fungicides compared and the year of the study. For example, the rate of reduction after plant treatments with Bayleton 25 WP in 1984 was 3-fold higher than that in 1983. In contrast, glumes of plants treated with Dithane M-45 yielded almost 2-fold less species in 1983 than in 1984. The effect of Funaben K on the occurrence of species was similar in the two years of studies.

On average of two years, the mycoflora of plant parts collected from fungicide-untreated plots (Tab. 2) was most similar in the glume seed comparison (C = 0.61), followed by those leaves at 11.2-3/glumes (0.58), leaves at 10.5.4/glumes (0. 52), and leaves at 10.5.1/glumes (0.41).

Table 2

Similarity coefficients of fungi populations of *Triticum aestivum* isolated in 1983 and 1984 from (A) glumes and leaves, at three stages of plant development, (B) seeds and leaves, (C) glume and seeds

Fungicide	Year	(A) glumes/leaves at:			(B) seeds/leaves at:			(C) glumes/seeds
		10.5.1	10.5.4	11.2-3	10.5.1	10.5.4	11.2-3	
Bayleton 25 WP	1983	0.50	0.52	0.64	0.61	0.73	0.38	0.48
	1984	0.63	0.50	0.71	0.63	0.74	0.80	0.53
Dithane M-45	1983	0.50	0.46	0.67	0.71	0.44	0.40	0.35
	1984	0.62	0.46	0.50	0.70	0.40	0.32	0.48
Funaben K	1983	0.43	0.40	0.71	0.57	0.53	0.47	0.56
	1984	0.25	0.36	0.50	0.22	0.33	0.46	0.53
Control	1983	0.40	0.60	0.62	0.32	0.53	0.56	0.67
	1984	0.42	0.44	0.53	0.50	0.37	0.43	0.55

When comparing fungi communities of glumes and leaves following fungicide treatments, the lowest similarity coefficient was obtained in the leaves at 10.5.1/ /glumes/Funaben K comparison. The leaves at 10.5.1/glumes/Funaben K, 1984 coefficient was much lower than that of leaves at 10.5.1/glumes/Funaben K, 1983. In adolition, the species composition of the leaf mycoflora of the Funaben K – treated plants collected at 10.5.4 also least resembled that of glumes. The mycoflora of glumes and leaves of plants at 11.2-3 was more or less similar in all the experimental combinations considered.

Except for the leaves at 10.5.1/seeds/Dithane M-45, 1983 and 1984 comparisions, fungi communities of leaves and seeds were least similar after Dithane M-45 and Funaben K treatments. The similarity values were always lower in 1984.

The mycoflora of glumes and seeds differed the most following plant treatments with Dithane M-45. Most of the species in common were found in the fungi communities from Funaben K-treated plants.

DISCUSSION

The growing season of 1984 was much more rainy and cool than that of 1983. Rain may decrease the occurrence of epiphytic fungi due to washing spores from plants (H i l l, L a c e y, 1983; L a c e y, 1975) or to leaching nutrietens from the phylloplane (F o k k e m a, 1971). The optimal temperature for the growth and sporulation of most fungi isolated from glumes in this study is higher than that of 1984 (D o m s c h, G a m s, A n d e r s o n, 1980). This may explain both the lower overall number of fungi and the number of species found in 1984 than in 1983.

The frequent occurrence of *Alternaria* spp., *Mucor hiemalis, Ulocladium botrytis*, a pink yeast-like fungus, and non-sporulating fungi in the mycoflora of glumes examined by the author supports the findings of many investigators indicating that these fungi are commonly associated with *Triticum aestivum*, including leaves (B a s h i, F o k k e m a, 1977; B ł a s z k o w s k i, 1994 c; D i c k i n s o n, S k i d m o r e, 1976; M a g a n, L a c e y, 1986), glumes (F l a n n i g a n, C a m p b e l l, 1977), and seeds (B ł a s z k o w s k i, in press a; F l a n n i g a n, 1971; L u k e, B a r n e t t, M o r e y, 1977; Ł a c i c o w a, 1964).

The 4-fold higher number of *S. nodorum* present in the fungi communities of glumes collected in 1984 as compared to that in the mycoflora of 1983 probably resulted from favourable temperature and humidity conditions for the establishment of infection by this pathogen (B r ö n n i m a n n, 1968; L e i t e r i t z, F o c k e, 1977; T y d e s l e y, T h o m p s o n, 1980; W e b s t e r, C o o k, 1979).

The strongest inhibitory effect of Bayleton 25 WP on the overall numbers of fungi associated with glumes contradicts earlier results of investigations conducted by the author (B ł a s z k o w s k i, 1994 b, c), who indicated that Bayleton 25 WP was the least and Funaben K the most toxic to the mycoflora of leaves and seeds of *Triticum aestivum*. Highly variable effects of triadimefon have also been recognized in investigations with ectomycorrhizal fungi (see M a r x, C e r d e l l, F r a n c e, 1986). However, the reasons of these discrepancies can not be explained at this time.

The rank of coefficients of similarity of seed, glume, and leaf fungi communities found in this study clearly reveals the origination of the mycoflora of the plant parts compared, being closest in the nearest positioned plant parts. The age and distance between plant parts strongly influence the quantitative and qualitative composition of associated mycoflora (D i c k i n s o n, 1967; L a s t, 1955; L a s t, D e i g h t o n, 1965).

The results of comparison of seed, glume, and leaf mycofloras following fungicide application are in agreement with those of B ł a s z k o w s k i (1994 b, c) and the findings of W e b s t e r and C o o k (1979) and E d g i n g t o n, K n e w, B a r r o n (1971) indicating that carbendazim derivatives have a broad spectrum of fungitoxicity.

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