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The effect of fungicides on the mycoflora of leaves of *Triticum aestivum* L.

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The effect of three foliar fungicides on the mycoflora associated with leaves of *Triticum aestivum* cv. Kolibri cultivated in the field was investigated. The total number of fungi associated with leaves increased as the season progressed. The fungicide which reduced considerably the number of both fungal colonies and species was Funaben K. The decline in the total number of fungi species of *T. aestivum* leaves was largely due to the reduction in the number of *Alternaria alternata* species and non-sporulating fungi. None of the fungicides reduced the proportion of leaves with *Cladosporium* spp. At some stages, the increase in the occurrence of some fungi species was accompanied by a decrease in the number of other fungi.

INTRODUCTION

The surface of leaves of *Triticum aestivum* L. is colonized by many fungi whose number and composition depend on, e.g., the stage of plant development, weather conditions, and conducted chemical treatments (Bashi, Fokkema, 1977; Dickinson, Wallace, 1976).

Fungicides play an important role in the control of *T. aestivum* diseases. However, their effects are rarely limited to plant diseases specific organisms they are primarily intended to control (Dickinson, Wallace, 1976). Thus, they could change diseaese severity by altering the interactions among microorganisms (De Cal, Melgarejo, 1992).

The fungi most frequently associated with *T. aestivum* leaves are *Alternaria alternata* (Fr.) Keissler, *Cladosporium* spp., *Epicoccum purpurascens* Link., *Fusarium* and *Helminthosporium* spp., *Septoria nodorum* Berk., and yeast-like fungi (Bashi, Fokkema, 1977; Dickinson, Skidmore, 1976; Dickinson, Wallace, 1976). Literature reports of the sensivity of these fungi to fungicides differ frequently. The contradictions regard the responses of both a single species to

different fungicide groups and an individual species to the same chemical. For example, Mills and Wallace (1968) found that the survival of *H. sativum* after treatment with different mercurialis varied from 0.0 to 89.2 %. According to De Cal and Melgarajo (1992), *Alternaria* spp. from peach twigs showed high sensivity to benomyl, but were tolerant to benomyl applications in Hill and Lacey's (1983) studies on ripening barley.

Fluctuations in the occurrence of individual fungal species have also been suggested to be caused by changes in the number of accompanying fungi (Mills, Wallace, 1968).

Several members of epiphytic mycoflora can be antagonistic to pathogenic fungi, including. *A. alternata*, *Cladosporium* spp. and yeast-like fungi (Dickinson, Skidmore, 1976; Dickinson, Wallace, 1976; Fokkema, 1971, 1973; Fokkema, Van Der Meulen, 1976) found to be potential biocontrol agents of *H. sativum* and *S. nodorum*. The effect of fungicides on such antagonistic fungi and other components of the mycoflora of *T. aestivum* leaves is, therefore, of great importance for the development of an integrated approach to disease control.

The aim of this study was to determine the quantitative effects of three fungicides on the composition of *T. aestivum* leaf mycoflora.

MATERIALS AND METHODS

In 1982-1984, a field experiment was conducted at the Agricultural Experiment Sation Lipki near Stargard Szczeciński. 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 plus 40 % of captafol, at a rate of 1.5 kg/ha.

Seeds of *T. aestivum* were sown on 23, 21, and 20 April in 1982, 1983, and 1984, respectively. Plots (1.8 x 1.8 m) were separated from one another 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, Large, 1954) and the begening of heading (stage 10.1). Control plants received water-spray applications.

Fourteen days after each treatment of plants with fungicides, i.e., at the stages of plant development of 10.5.1 and 10.5.4 (flowering – Large, 1954) and in the milky rape of seeds (stage 11.2-3), ten flag leaves from each plot were collected.

The leaves were subsequently placed in plastic bags, transferred to the laboratory, and stored in a refrigerator a 4°C until the next day. In the laboratory, fragments 50 mm long were cut from the middle of the leaves, placed in a bulb with 100 ml of sterile distilled water, and shaked vigorously for 120 seconds. After drying between two pats of sterile blotting-paper, these fragments were cut into 2 x 5 mm pieces. Fourteen leaf pieces randomly selected from each treatment were placed in 10 cm Petri dishes (5 pieces per dish) containing oatmeal agar. The bottom leaf surface contacted the surface of agar medium. The Petri dishes were incubated under room conditions for 10-14 days. At the end of this period, fungal colonies growing out of each leaf fragment were transferred individually to potato glucose agar (PGA) slants and identified.

Fungal species were identified according to Arx (1970), Barnett (1960), Booth (1971), de Vries (1959), Domsch and Gams (1970), Drechsler (1923), Ellis (1971), Gams (1971), Gilman (1945), Raper and Thom (1949), Raper and Fennel (1965), and Zycha and Siepmann (1969). Except for *S. nodorum*, representatives of each 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 this medium produces distinctive colonies with abundantly sporulating pycnidia.

Data were processed by analysis of variance. The statistical significance of differences between means was determined using the least significant difference (LSD) at $P = 0.05$ calculated from the Tukey test.

RESULTS

Weather conditions. A summary of the weather records from the Lipki Meteorological Station for the growing season, April-August inclusive, in the three years 1982-1984 is provided in Table 1.

Table 1

Weather characteristics during the growing season (April-August) in 1982-1984

	Year	April	May	June	July	August
Rainfall (mm)	1982	23	63	60	13	16
	1983	86	89	23	23	49
	1984	23	73	125	100	32
Mean temperature °C	1982	6.2	12.6	16.5	18.5	18.9
	1983	8.6	13.3	16.0	19.2	18.3
	1984	7.2	13.0	14.0	16.0	17.6

Main differences in the weather conditions during the three-year study occurred in June and July. The rainfalls in June and July of 1984 were 2.1 to 7.7 times higher than those in 1982 and 1983. June and July of 1983 were exceptionally dry. The mean temperatures of June and July were lower by 2.0-3.2°C than those of 1982 and 1983.

General characteristics. During the three-year study, 2196 fungal colonies were isolated from the leaf fragments collected (Tabs. 2-4). Most fungal isolates were recovered in 1982 (839), then in 1983 (725) and 1984 (632). The number of fungi associated with leaves increased as the season progressed. The number of fungi obtained from leaves collected at 11.1.2-4 was twice as high as that representing fungal populations of leaves from plants at 10.5.1. A similar tendency occurred in the number of fungal species.

The fungal species present throught the growing season were *A. alternata*, *A. pullulans*, *B. cinerea*, *C. cladosporioides*, *C. herbarum*, *C. macrocarpum*, *E. purpurascens*, *H. sativum*, *H. triseptatum*, *P. arundinis*, *S. botryosum*, and a yeast-like pink fungus. Additionally, non-sporulating fungi were found on leaves taken from all combinations during the three-year study.

Effects of fungicides on mycoflora of leaves. Stage 10.5.1. A total of 451 fungal colonies with 12 species were isolated (Tab. 2). The highest number of isolates (225) was noted in 1984 samples (225), followed by those in 1982 (127) and 1983 (102). The number of fungal species ranged from 0 to 9, depending on the year of study and the fungicide used.

The fungal species of the highest frequency of occurrence in the study years and the fungicide combinations compared were *A. alternata* and *C. herbarum* (both found in 83.3 % of year x fungicide combinations), *C. macrocarpum* (66.7 %), *E. purpurascens* (41.7 %), a yeast-like pink fungus (58.3 %), and non-sporulating fungi (100 %).

Independent of the years of study and the fungicides examined, the fungi dominating in the isolated fungal populations were non-sporulating fungi (159 isolates), followed by *C. herbarum* (143), *A. alternata* (50), and yeast-like pink fungus (44).

The fungicide which highly reduced the number of fungi associated with leaves of *T. aestivum* was Funaben K (56.6 % an average of three years), followed by Dithane M-45 (40.5 %) and Bayleton 25 WP (27.3 %). The highest decline in the number of fungi was noted after plant treatment with Funaben K in 1984 (by 94.1 %).

The total number of species was reduces during the three years of study the most by Funaben K (by 55.6 % on average). In 1984, Funaben K eliminated all species, except for seven colonies of non-sporulating fungi.

Stage 10.5.4. Leaf fragments collected at 10.5.4 yielded 774 fungal colonies representing 18 species (Tab. 3). The highest number of isolates was obtained in 1982 (338), then in 1983 (282) and 1984 (154). The number of species occurring in the fungal populations ranged from 3 to 12 species, depending on the growing season and the fungicide used.

Table 2

The effect of fungicides on the occurrence of fungi associated with leaves of *Triticum aestivum* collected at 10.5.1

Fungus	Bayleton 25 WP			Dithane M-45			Funaben K			Control		
	1982	1983	1984	1982	1983	1984	1982	1983	1984	1982	1983	1984
Acremonium sp.	–	1	–	–	–	–	1	–	–	–	–	–
Alternaria alternata (Fr.) Keissler	–	1	1	2	2	4	1	6	–	8	13	12
Aureobasidium pullulans (de Bary) Arn.	–	1	1	–	1	2	–	–	–	–	–	4
Botrytis cinerea Pers. ex Fr.	–	1	–	–	–	–	–	–	–	–	–	2
Chaetomium sp.	–	1	–	–	–	–	–	–	–	–	–	–
Cladosporium cladosporioides (Fres.) de Vries	–	–	1	–	–	–	–	–	–	1	–	1
C. herbarum Link ex Fr.	12	10	30	8	3	38	4	2	–	1	–	35
C. macrocarpum Preuss	–	1	3	6	–	1	3	–	–	1	1	2
Epicoccum purpurascens Link	–	1	–	–	1	–	–	1	–	1	1	–
Helminthosporium sativum P. K. B.	–	–	–	–	–	–	–	–	–	6	–	–
H. triseptatum Drechsl.	–	–	–	–	–	–	–	–	–	1	–	–
Mucor plumbeus Bonorden	–	–	1	–	–	–	–	–	–	–	–	–
Papularia arundinis (Corda) Fr.	1	–	–	–	–	–	–	–	–	2	–	–
Penicillium spp.	–	1	–	–	–	–	–	–	–	1	–	–
Stemphylium botryosum Wallr.	–	–	–	–	–	–	–	3	–	–	1	–
Trichoderma viride Pers. ex Fr.	–	–	–	–	–	–	–	–	–	1	–	–
Yeast-like pink	1	1	18	–	1	11	–	–	–	–	1	11
Pycnidium-forming sp.	–	–	–	1	–	–	–	–	–	–	–	1
Non-sporulating	10	11	12	9	9	14	17	12	7	28	17	13
Total	24	30	67	26	14	70	26	24	7	51	34	81
No. of species	2	6	5	3	4	4	3	4	–	9	4	6

Table 3

The effect of fungicides on the occurrence of fungi associated with leaves of *Triticum aestivum* collected at 10.5.4

Fungus	Bayleton 25 WP			Dithane M-45			Funaben K			Control		
	1982	1983	1984	1982	1983	1984	1982	1983	1984	1982	1983	1984
Acremoniella sp.	–	–	–	–	–	–	1	–	–	–	–	–
Alternaria alternata (Fr.) Keissler	35	22	2	38	31	1	29	30	4	40	33	1
Aspergillus flavus Link	–	1	–	–	–	–	–	–	–	1	–	–
Aureobasidium pullulans (de Bary) Arn.	1	15	2	–	4	–	2	3	–	–	3	4
Botrytis cinerea Pers. ex Fr.	3	–	2	–	–	–	–	–	–	1	–	–
Chaetomium globosum Kunze ex Fr.	–	–	–	–	–	–	1	–	–	–	–	–
Cladosporium cladosporioides (Fres.) de Vries	6	3	–	–	–	–	3	–	–	–	–	–
C. herbarum Link ex Fr.	15	10	13	11	–	14	6	–	2	4	1	37
C. macrocarpum Preuss	–	–	2	–	–	1	–	–	–	–	–	1
Epicoccum purpurascens Link	1	2	–	–	3	–	–	–	1	3	4	–
Fusarium culmorum (W. G. Smith) Sacc.	1	–	–	–	–	–	–	–	–	2	–	–
F. poae (Peck) Wollenw.	2	–	–	–	–	–	–	–	–	3	–	–
Fusidium sp.	–	–	–	–	1	–	–	–	–	–	–	–
Helminthosporium sativum P. K. B.	–	–	–	–	–	1	1	–	–	–	–	–
H. triseptatum Drechsl.	–	1	–	1	–	–	–	1	–	–	–	–
Mucor hiemalis Wehmer	1	–	–	1	–	–	–	–	–	3	–	–
Nigrospora oryzae (P. Br.) Petch	–	–	–	–	–	–	–	–	–	–	–	1
Papularia arundinis (Corda) Fr.	–	–	–	–	–	–	1	–	–	1	–	–
Penicillium spp.	1	–	1	–	–	–	1	–	–	–	–	–
Rhizopus nigricans Ehrenb.	1	–	–	–	–	–	–	–	–	3	–	–
Stemphylium botryosum Wallr.	4	1	–	2	–	–	2	3	–	5	–	–
Torula herbarum Pers. ex Fr.	–	–	–	–	–	–	–	–	–	–	–	1
Ulocladium botrytis Preuss	1	–	–	–	–	–	–	–	–	2	–	–
Yeast-like pink	10	14	3	–	2	8	–	1	–	1	2	3
Yeast-like yellow	–	–	–	–	1	–	–	–	–	–	–	–
Non-sporulating	17	16	9	20	28	14	30	23	4	20	23	22
Total	99	85	34	73	70	39	77	61	11	89	66	70
No. of species	11	8	5	5	3	4	8	4	3	12	4	6

The fungi most frequently occurring were *A. alternata* and non-sporulating fungi (both present in 100 % of year x fungicide combinations), followed by *C. herbarum* (83.3 %), a yeast-like pink fungus (75.0 %), *E. purpurascens*, and *S. botryosum* (both 50.0 %).

Considering jointly the number of isolations made from leaves of all the fungicide combinations used, *A. alternata*, *A. pullulans*, *C. herbarum*, a yeast-like pink fungus, and non-sporulating fungi predominated in the fungal communities obtained, being isolated 266, 34, 113, 44, and 226 times, respectively.

Funaben K reduced the most the number of foliar mycoflora (by 35.1 % on average of three years). The percentage reductions caused by Dithane M-45 and Bayleton 25 WP were 18.7 % and 3.8 %, respectively. The highest level of reduction occurred following the use of Funaben K in 1984 (by 84.3 %).

Dithane M-45 was the most harmful fungicide having reduced the number of species by 38.9 % of compared with the control leaves. Bayleton 25 WP increased the number of species by 58.3 %.

Stage 11.1.2-3. Nine hundred and sixty-eight fungal colonies were isolated from fragments of leaves collected at 11.1.2-4 (Tab. 4). The number of colonies isolated in the years 1982-1984 were 374, 341, and 253, respectively. The fungal communities included 27 species. The number of species occurring in fungal populations recovered from a particular year x fungicide combination ranged from 2 to 17 species.

The most frequently occurring fungi were *A. alternata* and non-sporulating fungi (both present in 100 % of year x fungicide combinations), followed by *A. pullulans* and *E. purpurascens* (both 75.0 %), *C. herbarum* (66.7 %), and *S. nodorum* (50.0 %).

Considering jointly the number of colonies of individual species obtained from leaves of all fungicide combinations, the predominating fungi were *A. alternata* (359 colonies), non-sporulating fungi (192), *A. pullulans* (125), a yeast-like pink fungus (52), *E. purpurascens* (34), and *C. herbarum* (25). Species which also occurred abundantly were *Fusarium* spp. (40), *S. nodorum* (24), *H. sativum* (21), and *S. botryosum* (16).

The fungicide which reduced the most the number of fungi was Funaben K (by 24.6 % on average of three years). Dithane M-45 and Bayleton 25 WP reduced the number of populations by 14.7 % and 13.1 %, respectively. The highest decline in the number of fungi was noted after spraying of plants with Funaben K in 1982 (43.9 %).

During the three growing seasons, the mean level of reduction in the number of species was highest following the use of Funaben K (47.9 %). Dithane M-45 and Bayleton 25 WP reduced the number of species by 47.4 % and 34.0 %, respectively.

Effects of fungicides on fungi dominating in mycoflora of leaves. The occurrence of fungi dominating on the examined leaf fragments depended on the fungicide used and the time of collection (Tab. 5).

Table 4

The effect of fungicides on the occurrence of fungi associated with leaves of *Triticum aestivum* collected at 11.2-3

Fungus	Bayleton 25 WP			Dithane M-45			Funaben K			Control		
	1982	1983	1984	1982	1983	1984	1982	1983	1984	1982	1983	1984
Acremoniella sp.	–	–	–	–	–	–	1	–	–	–	–	–
Acremonium sp.	–	–	–	–	–	–	–	–	–	1	–	–
Alternaria alternata (Fr.) Keissler	33	34	7	36	38	22	32	36	15	37	37	32
Aureobasidium pullulans (de Bary) Arn.	18	24	1	20	23	–	6	8	–	10	15	–
Botrytis cinerea Pers. ex Fr.	–	–	–	–	–	–	–	–	–	4	–	–
Chaetomium globosum Kunze ex Fr.	–	–	–	–	–	–	1	–	–	–	–	–
Cladosporium cladosporioides (Fres.) de Vries	3	–	–	–	–	–	2	–	–	1	–	–
C. herbarum Link ex Fr.	4	–	2	2	–	–	1	1	–	4	6	5
C. macrocarpum Preuss	–	–	–	–	–	–	–	–	–	4	–	–
Epicoccum purpurascens Link	3	5	6	1	1	–	1	–	–	2	1	19
Fusarium avenaceum (Corda ex Fr.) Sacc.	–	6	–	–	–	–	–	–	–	1	–	–
F. culmorum (W. G. Smith) Sacc.	–	3	–	2	–	–	–	–	1	2	–	–
F. graminearum Schwabe	–	3	–	–	1	–	–	–	–	–	3	–
F. poae (Peck) Wollenw.	1	–	–	–	–	–	–	–	–	2	–	–
F. semitectum Berk. et Rav.	–	8	–	–	2	–	–	2	–	–	2	–
F. sporotrichoides Sherb.	–	–	–	–	–	–	–	–	–	–	1	–
Fusidium sp.	–	1	–	–	–	–	–	2	–	–	2	–
Helminthosporium sativum P. K. B.	–	–	–	–	–	1	–	–	–	19	–	1
H. triseptatum Drechsl.	1	–	–	–	–	–	1	–	–	2	–	–
Mucor hiemalis Wehmer	2	–	–	1	–	–	1	–	–	1	–	–
M. plumbeus Bonorden	–	–	–	–	2	–	–	3	–	–	–	1
Nigrospora oryzae (P. Br.) Petch	–	–	–	–	–	–	–	–	–	–	1	–
Papularia arundinis (Corda) Fr.	–	–	–	1	–	–	1	–	–	–	–	–
Penicillium notatum Westling	–	–	11	–	–	–	–	–	–	–	–	1
Penicillium spp.	–	–	1	1	–	–	–	–	–	–	–	–
Rhizopus nigricans Ehrenb.	–	–	5	–	–	–	–	–	–	–	–	4
Septoria nodorum Berk.	5	–	–	3	–	–	–	–	1	10	2	3
Sordaria fimicola (Roberge) Ces. et de Not.	–	–	–	–	1	–	–	–	–	–	–	–
Stemphylium botryosum Wallr.	–	–	–	–	–	–	1	–	–	15	–	–
Trichoderma viride Pers. ex Fr.	–	–	–	–	–	–	–	–	–	1	–	–
Ulocladium botrytis Preuss	–	–	–	1	1	–	–	2	–	3	2	–
Yeast-like pink	3	–	23	–	–	9	–	–	17	–	–	–
Pycnidium-forming sp.	–	–	–	–	–	1	–	–	–	–	–	–
Non-sporulating	13	5	6	14	17	32	26	30	19	13	10	7
Total	86	89	62	82	86	65	74	84	53	132	82	73
No. of species	9	7	6	9	8	2	10	6	3	17	10	8

Table 5

The effect of fungicides on the proportion of seeds colonized by fungi (means for 1982-1984)

Fungi	Bayleton 25 WP	Dithane M-45	Funaben K	Control
Stage 10.5.1				
Alternaria alternata	1.7 b	6.7 b	5.8 b	27.5 a
Aureobasidium pullulans	1.7 a	2.5 a	0.0 a	3.3 a
Cladosporium spp.	15.8 a	15.6 a	2.5 a	11.1 a
Epicoccum purpurascens	0.8 a	0.8 a	0.8 a	1.7 a
Yeast-like pink	16.7 a	10.0 a	0.0 a	10.0 a
Non-sporulating	27.5 b	26.7 b	30.0 b	48.3 a
Stage 10.5.4				
Alternaria alternata	49.2 a	28.3 b	52.5 a	31.7 b
Aureobasidium pullulans	15.0 a	3.3 a	4.2 a	5.8 a
Cladosporium spp.	13.6 a	7.2 a	3.1 a	11.9 a
Epicoccum purpurascens	2.5 a	2.5 a	0.8 a	5.8 a
Yeast-like pink	22.5 a	8.3 ab	0.8 b	5.0 b
Non-sporulating	35.0 b	51.7 ab	47.5 ab	54.2 a
Stage 11.2-3				
Alternaria alternata	69.7 b	80.0 b	69.2 b	88.3 a
Aureobasidium pullulans	35.8 a	35.8 a	11.7 b	20.8 ab
Cladosporium spp.	2.5 a	0.6 a	1.1 a	5.6 a
Epicoccum purpurascens	11.7 ab	1.7 b	0.8 b	18.3 a
Fusarium spp.	4.2 a	0.8 a	0.5 a	1.8 a
Helminthosporium spp.	0.4 a	0.4 a	0.4 a	9.2 a
Septoria nodorum	4.2 a	2.5 a	0.8 a	12.5 a
Yeast-like pink	21.7 a	7.5 ab	14.2 ab	0.0 b
Non-sporulating	20.0 b	52.5 a	62.5 a	25.0 b

Means followed by the same letter do not differ significantly at the 5 % level acording to Tukey test.

All fungicides significantly reduced the number of *A. alternata* species on leaves collected at 10.5.1 and 11.2-3. At 10.5.4, Funaben K and Bayleon 25 WP significantly increased the proportion of leaves with *A. alternata*. Significant differences in the occurrence of *A. pullulans* were marked only at 11.2-3 after Bayleton 25 WP and Dithane M-45 applications. None the fungicide significantly changes the proportion of leaves with *Cladosporium* spp. at the three plant developmental stages. In comparison with the control leaves, those treated with fungicides yielded significantly less. *E. purpurascens* colonies, but only when collected at 11.2-3 stages. Bayelton 25 WP significantly increased the occurrence of a yeast-like pink fungus. All fungicides significantly reduced the number of non-sporulating fungi on leaves from the 10.5.1 and 10.5.4 collections. At 11.2-3, a significantly higher proportion of leaves with these fungi was found in collections from Funaben K – and Dithane M-45--treated plants. *Fusarium* and *Helminthosporium* spp., as well as *S. nodorum* occurred in appreciable numbers only at 11.2-3. When comparing the above mycoflora with that of leaves from control plots no statistical significant differences were found.

DISCUSSION

Fungicide applications had of profound effect on fungi associated with leaves of *Triticum aestivum*, reducing populations in the field in some cases by up to 84.3 %. These results are in accordance with similar studies on *T. aestivum* and other hosts (Andwers, Kenerley, 1978; Fokkema, Nooil, 1981; Hill, Lacey, 1983; Magan, Lacey, 1986; De Cal, Melgarejo, 1992).

Sprays with Funben K considerably reduced both the total number of fungi and the number of species in populations. Large reductions of epiphytic microorganisms after carbendazimgenerating fungicide applications were previously reported (Błaszkowski, 1991, in press; DeCal, Melgarejo, 1992; Hill, Lacey, 1983) and probably resulted from a broad spectrum of toxicity of these fungicides against fungi (Webster, Cook, 1979).

The reduction of the total mycoflora of *T. aestivum* laeves was largely due to the decline in the number of *A. alternata* and non-sporulating fungi. Large reductions in the occurrence of *Alternaria* spp. were observed by, e.g., De Cal and Melgarejo (1992) after chemical applications. High susceptibility of non-sporulating fungi to fungicides were also found by Błaszkowski (1991) and Truszkowska (1984).

None of the fungicide changed significantly the proportion of leaves with *Cladosporium* spp. This contradicts the findings of Błaszkowski (in press) concerning the studies of the effect of the same fungicides on mycoflora of *T. aestivum* seeds and those of other investigators (e.g., Hill, Lacey, 1983; De Cal, Melgarejo, 1992) who found *Cladosporium* spp. to be highly sensitive to a range of fungicides.

In the present study a significant increase in the proportion of leaves with *A. alternata* at 10.5.4 was accompanied by a decrease in the number of non-sporulating fungi similarly, the considerable proportion of leaves colonized by a yast-like pink fungus and not-sporulating fungi at 11.2-3 was associated with a significant decrease in the occurrence of *A. alternata*. This suggests that fluctuations in the occurrence of fungi mentioned above may have been due to their highly competitive nature and the ability of these fungi exploit any ecological niche left vacant rather than to any tolerance to fungicides, as De Cal and Melgarejo (1992) and Mills and Wallace (1968) suggested. The increased proportions of *A. alternata* and yeast-like pink fungus may be of importance as these fungi have been reported to be antagonistic to several pathogenic fungi (Fokkema, 1971; Fokkema, Van Der Meulen, 1976).

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