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The Effectiveness of Tebuconazole and Chitosan in Inhibiting the Growth of *Fusarium* Species on Winter Wheat Grain under Field Conditions

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***Abstract*—**A three-year field experiment (2010-2012) was conducted to determine the abundance of epiphytic and endophytic filamentous fungi colonizing the grain of winter wheat cv. Bogatka. Wheat spikes were protected with tebuconazole or chitosan at the watery ripe stage. Untreated plants served as control. Tebuconazole exerted an inhibitory effect primarily on *F. culmorum* and *F. graminearum,* and its effectiveness was determined by the pressure from pathogens that infected wheat spikes during the growing season. Chitosan did not suppress the growth of *Fusarium* species and *Alternaria alternata.*

***Keywords*—**Winter wheat, tebuconazole, chitosan, *Fusarium.*

I. Introduction

FUSARIUM Head Blight (FHB) is the most dangerous fungal diseases of wheat worldwide. The most common necrotrophic pathogens of the genus *Fusarium* causing FHB are *F. graminearum, F. culmorum* (teleomorph: *Gibberella zeae*), *F. poae*, *F. avenaceum* (teleomorph: *G. avenacea*) and *F. sporotrichioides* [5], [11]-[13]. FHB reduces wheat grain yield and quality, mostly due to the production of numerous mycotoxins by *Fusarium* species. The maximum levels of *Fusarium* toxins in cereal grains have been set in Commission Regulation (EC) No. 856/2005 of 6 June 2005. The efficacy of fungicide application to control FHB epidemics in winter wheat remains controversial [15]. Tebuconazole reduces the severity of FHB symptoms, but it does not always decrease the mycotoxin contamination of grain [13]. Plant resistance inducers are often applied to improve the health status of crops [7], [9]. Chitosan, an elicitor of defense responses in plants, can effectively reduce the infection of winter wheat spikes by *F. culmorum* as well as grain contamination with the mycotoxin deoxynivalenol (DON) [1], [9]. The aim of this study was to determine the effectiveness of tebuconazole and chitosan in inhibiting the growth of *Fusarium* species on winter wheat grain.

II. Methods

A. Field-Plot Experiment

A field-plot experiment was conducted in 2010-2012 in north-eastern Poland, in a randomized design with four replications. Winter wheat (*Triticum aestivum* L.) cv. Bogatka, sown in 25m^2 plots, was treated with chemicals at three growth stages: stem elongation (BBCH 31), heading (BBCH 55) and watery ripe (BBCH 71). The fungicide Corbel 750 EC (fenpropimorph) was applied at the stem elongation stage, and the fungicide Opera Max 147.5 SE (pyraclostrobin, epoxiconazole) – at the heading stage. Wheat spikes were protected with the fungicide Tarcza Łan 250 EW (tebuconazole). Corbel 750 EC and Tarcza Łan 250 EW were applied at 1l per ha, and Opera Max 147.5 SE – at 2l per ha. Organically grown wheat plants were treated with the growth promoter Asahi SL (ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol) at 0.6l per ha and twice with the resistance inducer Biochicol 020 PC (chitosan) at 2.5l per ha. Untreated plants served as control.

B. Abundance of Microorganisms

The abundance of microorganisms colonizing wheat kernels was estimated at harvest and after six months' storage at 11°C. Disinfected and non-disinfected kernels were transferred to potato glucose agar [6]. The colonies of filamentous fungi were identified to the species level based on their sporulation characteristics [4], [8]. The biodiversity of filamentous fungi was estimated by Margalef index, which is the number of species – 1/ln (abundance of fungal communities).

III. Results

A total of 433 colonies of epiphytic filamentous fungi representing 16 genera and species were isolated in 2010-2012 at harvest and after six months of wheat grain storage (Table I, Fig. 1). The fungal community, typical of the analyzed ecological niche, was characterized by low species diversity and the dominance of *Fusarium* species which accounted for 38.37% and 27.82% of all colonies at harvest and after six months' storage, respectively. Fungicide treatment reduced the total abundance of *Fusarium* species only in the third year of the study (Table I).

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TABLE I
EPIPHYTIC FILAMENTOUS FUNGI FROM THE SURFACE OF WINTER WHEAT GRAIN

Species of filamentous fungi	Control			Cor+OpM+TŁ			As+Bio+Bio			Total
	CFU on 24 kernels after harvest									
Year 20..	10	11	12	10	11	12	10	11	12	
Acremoniella atra	1	2	2	1	1	1		3	2	13
Alternaria alternata	1	11	8		4	6		7	10	47
Epicoccum purpurascens		5	7	1	7	3		3	2	28
Fusarium spp.	2	9	12	3	11	4	5	12	13	71
Other saprotroph	4	2		4	3		1	2		16
Other pathogens					1					1
Non-sporulating colonies	8	1								9
Total colonies	16	30	29	9	27	14	6	27	27	185
Number of species	6	9	7	5	9	5	3	9	6	24
Biodiversity (Margalef index)*	1.80	2.35	1.78	1.82	2.43	1.52	1.12	2.43	1,52	4.41
Species of filamentous fungi	Control			Cor+OpM+TŁ			As+Bio+Bio			Total
	CFU on 24 kernels after six months' storage									
Year 20..	10	11	12	10	11	12	10	11	12	
Acremoniella atra				1			7	1		9
Alternaria alternata	12	12	10	13	13	11	7	15	20	113
Epicoccum purpurascens		6			4		1	5	3	19
Fusarium spp.	5	5	13		9	9	10	10	8	69
Other saprotroph	1		1	4	1	12			4	23
Other pathogens										0
Non-sporulating colonies	1	2				6	6			15
Total colonies	19	25	24	18	27	38	31	31	35	248
Number of species	5	4	6	4	5	5	5	5	6	17
Biodiversity (Margalef index)*	1.36	0.93	1.57	1.04	1.21	1.10	1.16	1.16	1.41	2.90

Cor+OpM+TŁ - Corbel 750 EC (fenpropimorph) + Opera Max 147.5 SE (pyraclostrobin, epoxiconazole) + Tarcza Łan 250 EW (tebuconazole), As+Bio+Bio - Asahi SL (ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol) + Biochicol 020 PC (chitosan) + Biochicol 020 PC (chitosan)

Other saprotroph: *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium* spp., *Rhizopus nigricans*, *Mucor* spp., *Trichothecium roseum*. Other pathogens: *Botrytis cinerea.*

* - Calculated using the formula given in the Methods section.

Tebuconazole exerted an inhibitory effect primarily on *F. graminearum* - on both dates of analysis and on *F. culmorum* – on the second date (Fig. 1). The effect of teboconazole was maintained over six months of grain storage. Chitosan did not suppress the growth of *Fusarium* species and *Alternaria alternate* (Table I, Fig. 1). More abundant populations of *F. culmorum* and *F. poae* were isolated from the grain of wheat plants protected with ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol and chitosan, and this adverse trend was observed throughout grain storage (Fig. 1).

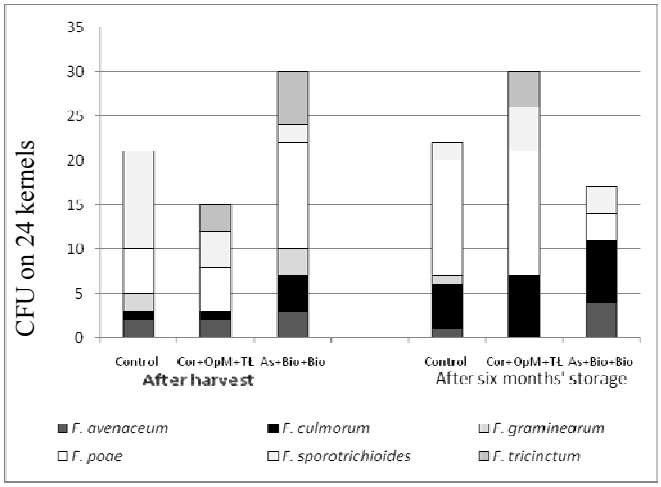

Fig. 1 Epiphytic filamentous fungi of the genus Fusarium from winter wheat grain (total in 2010-2012)
Cor+OpM+TŁ - Corbel 750 EC (fenpropimorph) + Opera Max 147.5 SE (pyraclostrobin, epoxiconazole) + Tarcza Łan 250 EW (tebuconazole), As+Bio+Bio - Asahi SL (ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol) + Biochicol 020 PC (chitosan) + Biochicol 020 PC (chitosan)

The abundance of endophytic pathogens of the genus *Fusarium* was significantly lower, compared with epiphytes. Endophytes accounted for 23.20% and 17.09% of all isolates

at harvest and after six months' storage, respectively (Table II). Fungicide treatment provided only partial protection against infections caused by *Fusarium* species. Immediately after harvest, teboconazole offered effective protection only against *F. poae* (Fig. 2). Only in the first year of the study, after six months of grain storage, fungicides prevented penetration of *F. culmorum* into the interior of wheat kernels (Table II).

TABLE II
ENDOPHYTIC FILAMENTOUS FUNGI FROM THE SURFACE OF WINTER WHEAT GRAIN

Species of filamentous fungi	Control			Cor+OpM+TŁ			As+Bio+Bio			Total
	CFU on 24 kernels after harvest									
Year 20..	10	11	12	10	11	12	10	11	12	
Alternaria alternata	2	7	12		5	9	2	3	12	52
Epicoccum purpurascens		6	5	1	6			6	5	29
Fusarium spp.	1	7	5	3	5	5	2	8	6	42
Other saprotroph	1	1		4	1	2	1			9
Non-sporulating colonies	4	1								7
Total colonies	8	22	22	9	17	16	5	17	23	139
Number of species	3	9	5	5	6	5	4	5	5	18
Biodiversity (Margalef index)*	0,96	2,59	1,29	1,82	1,76	1,44	1,86	1,41	1,41	3,45
Species of filamentous fungi	Control			Cor+OpM+TŁ			As+Bio+Bio			Total
	CFU on 24 kernels after six months' storage									
Year 20..	10	11	12	10	11	12	10	11	12	
Alternaria alternata	3	15	13	2	12	10	12	12	16	95
Epicoccum purpurascens		11			7		1	7	4	29
Fusarium spp.	3	2	7		2	12	12	4	5	47
Other saprotroph	4	3		3	1	5	12		3	31
Non-sporulating colonies	10	1			4		6	2		17
Total colonies	20	32	20	5	26	27	31	25	28	219
Number of species	3	5	4	2	5	4	5	3	9	13
Biodiversity (Margalef index)*	0,67	1,15	1,00	0,62	1,23	0,91	1,16	0,62	2,40	2,23

* - Calculated using the formula given in the Methods section.

Cor+OpM+TŁ - Corbel 750 EC (fenpropimorph) + Opera Max 147.5 SE (pyraclostrobin, epoxiconazole) + Tarcza Łan 250 EW (tebuconazole), As+Bio+Bio - Asahi SL (ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol) + Biochicol 020 PC (chitosan) + Biochicol 020 PC (chitosan)

Other saprotroph: *Acremoniella atra*, *Arthrinium phaerospherum*, *Cladosporium cladosporioides*, *C. herbaru*m, *Penicillium* spp., *Rhizopus nigricans*, *Mucor* spp., *Trichothecium roseum.* Other pathogens*: Botrytis cinerea.*

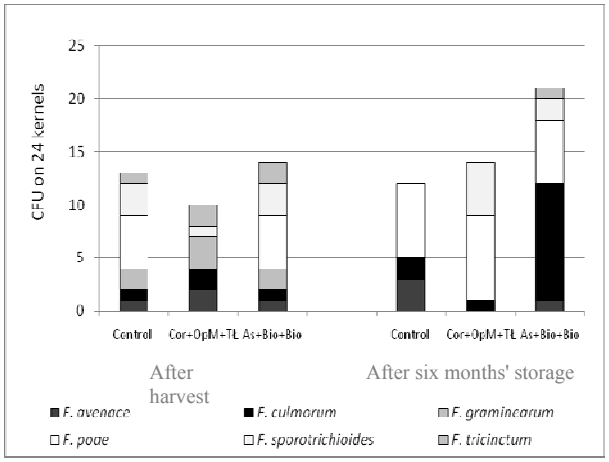

Fig. 2 Endophytic filamentous fungi of the genus Fusarium from winter wheat grain (total in 2010-2012)
Cor+OpM+TŁ - Corbel 750 EC (fenpropimorph) + Opera Max 147.5 SE (pyraclostrobin, epoxiconazole) + Tarcza Łan 250 EW (tebuconazole), As+Bio+Bio - Asahi SL (ortho-nitrophenol, para-nitrophenol, 5-nitroguaiacol) + Biochicol 020 PC (chitosan) + Biochicol 020 PC (chitosan)

IV. DISCUSSION

FHB severity is determined by temperature and humidity conditions at flowering [3], [14]. In the present study, the abundance of *Fusarium* pathogens was by 9.6% higher on average that that reported by Berghofer et al. [2]. Under field conditions, chitosan - an elicitor of defense responses in plants, had no inhibitory effect on grain contamination with toxin-producing *Fusarium* species. Previous research has demonstrated that chitosan reduced the infection of winter wheat spikes by *F. culmorum* as well as DON contamination of wheat grain [1], [9]. In our experiment, the toxin-producing species *F. culmorum* tended to accumulate on the surface of winter wheat kernels, in particular those collected from plants sprayed with chitosan-based products, which could pose serious health risks.

Tebuconazole reduced the abundance of *Fusarium* pathogens only on some dates of analysis, and its effect was directed only towards selected species, mostly *F. culmorum* and *F. graminearum*. Lack of inhibitory effect of single tebuconazole treatment on the epiphytic species *F. poae* was also noted by other authors [11]. According to Müllenborn et al., fungicides can disturb homeostasis between *Fusarium* pathogens and the saprotrophs colonizing winter wheat grain. Fungicides have a direct impact on the health of wheat spikes

when they suppress the growth of pathogens and in indirect influence when they interact with saprotrophs. Some saprotrophs that colonize cereal grain significantly reduce the growth of pathogens [11]. Moradi et al. demonstrated that spike inoculation with *F. poae* reduces the occurrence of other *Fusarium* species [10].

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