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FUNGI COLONIZING THE GRAIN OF SPRING WHEAT GROWN IN THE CONVENTIONAL AND ORGANIC SYSTEMS

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Abstract

The abundance and species composition of fungi colonizing grain of spring wheat cvs. 'Koksa' and 'Torka', grown in the conventional and organic farming systems, was assessed in 2004–2006. More colonies of *Alternaria alternata, Aureobasidium pullulans, Botrytis cinerea* and *Fusarium* spp., *Gibberella* spp. were isolated from wheat grain obtained from conventional farms. Intensive chemical control did not reduce the abundance and species diversity of *Fusarium* spp., *Gibberella* spp. A considerably higher number of *Epicoccum nigrum* colonies were isolated in the organic farming system, as compared with the conventional system.

Key words: grain, fungi, spring wheat, conventional and organic systems

Introduction

The intensification of agricultural production has contributed to the loss of crop diversity. Modern farms focus on mass production of two or three technologically similar species. As a result, the share of cereals in the cropland structure has exceeded 70%. Crop species loss is accompanied by gradual habitat degradation, changes in the proportions and physicochemical properties of grain components, and in the abundance and species composition of kernel-colonizing microorganisms (Truszkowska et al. 1986, Łacicowa and Pięta 1994, Grabowski et al. 2008). The above often leads to domination of pathogenic fungi and selective elimination of natural microbial populations that protect plants against pathogens.

In conventional intensive farming systems, natural biological processes play a limited role in eliminating pathogenic agents, which is why crop protection chemicals are widely used (Kuś and Stalenga 2006). Recent years have witnessed

Phytopathologia 63: 39–50

ISSN 2081-1756

an increasing environmental awareness of the adverse side affects of chemical control method, and a growing interest in organic food produced using biocontrol methods that help to prevent the mineral fertilization and chemical protection in agriculture (Solarska et al. 2003). Plant health is maintained by creating a supportive environment for the growth of saprotrophic fungi which reduce pathogen populations through competition or hyperparasitism (Kuś 1997, Tyburski and Żakowska-Biemans 2007). In organic farming, effective disease control requires a thorough understanding of interactions between microorganisms (Teich 1994). The sanitary condition of grain, an important link in the food chain, needs to be regularly monitored, therefore the objective of this study was to determine the abundance and species composition of fungi colonizing the grain of two spring wheat cultivars grown in the conventional and organic farming systems.

Material and methods

A large-area experiment was conducted in 2004–2006 to compare the health status of spring wheat cvs. 'Koksa' and 'Torka', grown in the conventional and organic farming systems. Two organic farms (in Budziszewo – 53°36'N, 19°13'E and Zgniłobłoty – 53°28'N, 19°23'E), approved by the "Ekoland" Organic Food Producers Association, and two conventional farms (in Jabłonowo – 53°4'N, 19°15'E and Łasin – 53°53'N, 19°10'E) were selected for the study, based on the similarity of soil, habitat and wheatear conditions, which provided comparable results. The area of experimental plots delineated within each field was 400 m^2. In the first year of the study (2004), only wheat cv. 'Koksa' was grown in the conventional system. Kernels were sampled randomly, immediately before harvest. Phytopathological analysis of spring wheat grain was performed by the method proposed by Narkiewicz-Jodko (1986). Samples of 200 kernels were collected randomly from each treatment. A total of 2000 and 2400 kernels were collected in the conventional and organic system, respectively. The analyzed material was divided into kernels lots: showing disease symptoms and visually healthy ones. Half of the kernels was surface disinfected (in 50.0% ethanol and 0.1% sodium hypochlorite), and the other kernels were rinsed in sterile water. Grains were placed on potato dextrose agar (PDA) plates and incubated for four days at a temperature of 21°C. Fungal colonies were transferred to PDA slants, and fungi were identified to the species using relevant keys and monographs (Gilman 1957, Ellis 1971, Kwaśna et al. 1991). The statistical significance of differences in number of isolates in two cultivars in farming systems was determined by a χ^2-test (see Tables 1 and 2: a – the ratio for disinfected grain with disease symptoms *versus* disinfected healthy grain is significantly different at $p = 0.05$, b – the ratio for non-disinfected grain with disease symptoms *versus* non-disinfected healthy grain is significantly different at $p = 0.05$, c – the ratio for cultivars is significantly different at $p = 0.05$).

Results

A total of 4491 fungi were isolated from spring wheat grain, including 2041 colonies obtained from 2000 kernels collected in the conventional system (Tables 1 A and 1 B) and 2450 colonies obtained from 2400 kernels collected in the organic system (Tables 2 A and 2 B). In both farming systems, the highest number of fungi was isolated in the second year of the study. In 2005 and 2006 the frequency of fungal colonies was higher on 'Koksa' kernels than on 'Torka' kernels. The grain of spring wheat was colonized by fungi belonging to 35 taxa and non-sporulating colonies in the organic system, and by fungi representing 37 taxa and non-sporulating colonies in the conventional system. Regardless of the farming system, the predominant species was *Alternaria alternata* (47.1% and 48.2% isolates in the organic and conventional system, respectively). *Epicoccum nigrum* was also isolated frequently, but a considerably higher number of colonies was obtained from organic treatments (20.8%), compared with conventional treatments (14.0%).

Species of the genera *Fusarium* and *Gibberella* were isolated in great abundance, accounting for 10.4% and 17.4% of all isolates in the organic and conventional system, respectively. Higher species diversity of *Fusarium* spp. was noted in conventional farms, in comparison with organic farms. The most abundant species were *F. poae*, *F. culmorum*, *G. avenacea* and *F. oxysporum*. In both farming systems, *Fusarium* and *Gibberella* fungi were more frequently isolated from kernels with disease symptoms than from healthy looking ones. 'Koksa' kernels were more frequently colonized by *Fusarium* spp. and *Gibberella* spp. than 'Torka' kernels. Despite the intensive use of chemical control measures, the abundance of *Aureobasidium pullulans* and *Botrytis cinerea* was over five-fold and over three-fold higher, respectively, in conventional farms than in organic farms. *Trichoderma harzianum*, a natural antagonist of many pathogens, was isolated from wheat grain samples collected from organic treatments (0.6%) only.

Comparing the number of empirical values with the theoretical values relative to *A. alternata*, *E. nigrum* and *Fusarium*, *Gibberella* spp. showed the significance of differences between cultivars 'Koksa' and 'Torka' clearly perceptible in 2006 year. In some research years there was no clear effect of the research material disinfection on the number and species composition of fungi inhabiting wheat grains.

Fungi of the genera *Chaetomium* (5.0% and 6.5%) and *Rhizopus* (3.5% and 2.3%), and non-sporulating mycelia (2.4% and 5.4%) were also relatively abundant in the organic and conventional system, respectively. Fungi of the genus *Rhizopus* were isolated primarily from non-disinfected kernels.

Discussion

A comparison of the health status of crops produced in conventional and organic farming is an important consideration, especially while organic food is growing in popularity and the number of subsidized organic farms in increasing.

Table 1 A

Fungi isolated from grain of spring wheat cultivated in the conventional system – Łasin

Species of fungus	2004				2005								2006								Sum
	‘Koksa’				‘Koksa’				‘Torka’				‘Koksa’				‘Torka’				
	C		Z		C		Z		C		Z		C		Z		C		Z		
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Acremoniella atra																1					1
Alternaria alternata	24	12	24	14	4a	40	36a	42	38	33	44	28	20ac	20c	36ac	29c	13c	24c	18c	15c	514
Aspergillus brasiliensis										1											1
Aureobasidium pullulans																2					2
Botrytis cinerea								2													2
Chaetomium spp.					22		2	2	2		1	12									41
Cladosporium cladosporioides			1										1	4							6
Cochliobolus sativus	1	1		2																	4
Epicoccum nigrum	8	10	10	8	3a	9	12a	6	9	12	7	7	12a	1b	3a	13b	8a	2	24a		164
Fusarium chlamydosporum	1																				1
Fusarium culmorum	8			3				14		4											29
Fusarium oxysporum	7	5	1	5																	18
Fusarium poae		5	1		16	1	1	5	1	3		4				1		16	1	14	69
Fusarium sporotrichioides	2													1						13	16
Fusarium spp.	2	3	3	5		3						3				2					21
Gibberella avenacea						2			2												4
Gibberella pulicaris										3											3
Gibberella tricincta						1															1
Gibberella zeae	1																				1
Fusarium/Gibberella total	21a	13	5a	13	16ac	7bc	1ac	19bc	3c	10c		7c		1c		3c		16c	1c	27c	–
Gilmaniella humicola					1																1
Ilyonectria radicicola	1		2											7	1						11
Monographella nivalis		1																			1

Table 1 A – cont.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Mortierella sp.		1																			1
Mucor hiemalis										1											1
Oidiodendron cereale	1																				1
Penicillium sp.		1	1							1			1	1		3		2		5	15
Pleospora herbarum					4																4
Pseudogymnoascus roseus																				12	12
Rhizopus stolonifer														7							7
Trichothecium roseum	1		1																		2
Non sporulating fungi		6	5	14			1		2			4	5	2	5	2					46
Total	57	45	49	51	50	56	52	71	54	58	52	58	39	43	45	53	21	44	43	59	1000

C – grain with disease symptoms, Z – healthy grain, D – disinfected grain, N – non-disinfected grain.
a, b, c – the ratio is significantly different at $p = 0.05$.

Table 1 B

Fungi isolated from grain of spring wheat cultivated in the conventional system – Jabłonowo

Species of fungus	2004				2005								2006								Sum
	'Koksa'				'Koksa'				'Torka'				'Koksa'				'Torka'				
	C		Z		C		Z		C		Z		C		Z		C		Z		
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Alternaria alternata	28	24	29	21	29c	35bc	31c	18bc	5a	14	24a	22	17a	8b	33a	30b	21a	5b	40a	36b	470
Arthrinium phaeospermum					1				1												2
Aspergillus sp.				1																	1
Aureobasidium pullulans																		6		1	7

Table 1 B – cont.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Botrytis cinerea										1											1
Chaetomium spp.					4			19		17	12	20									72
Cladosporium cladosporioides		1															3	2			6
Cochliobolus sativus	1			1									3								5
Epicoccum nigrum	6	12	15	8	5	5	5	8	11	7	5	5	3a	1	13a		2	1	5	4	121
Fusarium culmorum														18	1	16					35
Fusarium oxysporum	4	2		1																	7
Fusarium poae	1	2			2	7	9	1	1	11		10	2	5			1	8		2	62
Fusarium sporotrichioides									2			5									7
Fusarium spp.	1	2		1	2	2										1					9
Gibberella avenacea					5	21	1	6	4	13											50
Gibberella intricans						3	1		2												6
Fusarium/Gibberella total	6	6		2	10	33b	11	8b	12	24		15	2c	23c	1c	17c	6c	8c		2c	–
Glomerella tucumanensis											1										1
Haematonectria haematococca					1			1	3								5				10
Monographella nivalis	1		4																		5
Mortierella alpina											3										3
Mucor hiemalis		2																	3		5
Penicillium spp.	2	2	2	13					1	1										1	22
Pleospora herbarum					2	4	1	2	13												22
Pseudogymnoascus roseus													4								4
Rhizopus stolonifer	3	7									2			14		14					40
Umbelopsis isabellina							1		2												3
Non sporulating fungi	3	1	4	6	1	3	14						15		2		6	3	4	3	65
Total	50	55	54	52	52	80	63	55	45	64	47	62	44	46	49	61	38	25	52	47	1041

C – grain with disease symptoms, Z – healthy grain, D – disinfected grain, N – non-disinfected grain.
a, b, c – the ratio is significantly different at p = 0.05.

Table 2 A

Fungi isolated from grain of spring wheat cultivated in the ecological system – Budziszewo

Species of fungus	2004								2005								2006								Sum
	‘Koksa’				‘Torka’				‘Koksa’				‘Torka’				‘Koksa’				‘Torka’				
	C		Z		C		Z		C		Z		C		Z		C		Z		C		Z		
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Alternaria alternata	27	31	32	18	27	29	30	31	35c	20bc	47c	50bc	16c	15bc	17c	41bc	22	15	15	23	15	15	17	27	615
Aureobasidium pullulans						1												5		5		17			28
Botrytis cinerea						2							1												3
Chaetomium spp.									5	28												5			38
Cladosporium cladosporioides	1				3														6	7					17
Epicoccum nigrum	16	13b	17	27b	21a	5b	10a	16b	6	5	4	3	5	12	2	12	15	15	19	2	21a	1	10a	6	263
Fusarium culmorum								1	13					1						1					16
Fusarium oxysporum	2	6			2	5	2	2																	19
Fusarium poae		1		1	1				9	5	5	2	26	24	21	1	1				1	1			99
Fusarium sporotrichioides													1												1
Fusarium spp.		1			1		1																		3
Gibberella intricans															1										1
Gibberella tricincta												1	1												2
Fusarium/Gibberella total	2	8b		1b	4	5	3	3	22a	5	5a	3	28	25b	23	1b	1	2		1	1	1			–
Glomerella tucumanensis											1														1
Haematonectria haematococca															1			2							3
Monodictys levis							1																		1
Monographella nivalis	1				1		1																		3
Mortierella lignicola					1									1											2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Mucor hiemalis	1																								1
Oidiodendron cereale			1				1																		2
Penicillium spp.				3																					3
Pleospora herbarum														1											1
Pseudogymnoascus roseus																	4	6			1		2	8	21
Rhizopus stolonifer	1					7	6					21										7			42
Non sporulating fungi					1	2	1		5				3		1			4	3		2	2	1	2	27
Total	49	52	50	49	58	51	53	50	73	58	57	77	53	54	43	54	42	47	43	38	40	48	30	43	1212

Table 2 B

Fungi isolated from grain of spring wheat cultivated in the ecological system – Zgniłobłoty

Species of fungus	2004								2005								2006								Sum
	'Koksa'				'Torka'				'Koksa'				'Torka'				'Koksa'				'Torka'				
	C		Z		C		Z		C		Z		C		Z		C		Z		C		Z		
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Alternaria alternata	20c	20c	33c	23c	16c	10c	23c	5c	24ac	49c	49ac	43c	32c	21c	30c	32c	9	10	20	8	20	5b	16	23b	541
Arthrinium phaeospermum									2		1		1		1										5
Aspergillus sp.		1																							1
Aureobasidium pullulans																				2				1	3
Botrytis cinerea					2																				2
Chaetomium spp.											1	2	2		3										8
Cladosporium cladosporioides		4			9	16	5	18														1	8		61
Cochliobolus sativus	2				9	16	5	18								1									51
Epicoccum nigrum	11c	21c	15c	28c	8c	18c	4c	14c		3	3	3	2	2	2	5	9c	5c	19c	6c	15c	19c	20c	14c	246
Fusarium culmorum				1												1	1								3

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Fusarium oxysporum	1				3	3	8	1																	16
Fusarium poae		1			1															1	7	8	5	2	25
Fusarium spp.		9		6	12	10	12	1			1			2		3									56
Gibberella avenacea															1										1
Gibberella intricans												1													1
Fusarium/Gibberella total	1c	10c		7c	16c	13bc	20c	2bc			1	1		2	1	4	1c			1c	7c	8c	5c	2c	–
Humicola fuscoatra																		1		7					8
Ilyonectria radicicola					1	1	4	10				1													17
Monographella nivalis					5	1	1																		7
Paecilomyces carneus	1																								1
Penicillium spp.		3			1		5			1								9		13	2	6			40
Pleospora herbarum									21			1	3	2	7	9									43
Rhizopus stolonifer		13						1						11		4		8						7	44
Trichoderma harzianum									3	4				7											14
Volutella ciliata										1		1	6		5										13
Non sporulating fungi	2	1	2	2				1			2		3	6	3		3	3		2			1		31
Total	37	73	50	60	67	75	67	69	50	58	57	52	49	51	52	55	22	36	39	39	44	39	50	47	1238

C – grain with disease symptoms, Z – healthy grain, D – disinfected grain, N – non-disinfected grain.
a, b, c – the ratio is significantly different at $p = 0.05$.

Organic agriculture refers to a holistic approach: farms are treated as a living whole system where particular factors do not act in isolation, which makes it difficult to analyze the system's components separately and to compare the results of studies on traditional and organic farming systems. It should also be noted that until 2009, conventionally-grown untreated seed could be used by organic farmers, which was a common denominator of the two systems, but also made it necessary to analyze kernels obtained from both systems.

The sanitary quality of grain needs to be monitored, because in Poland only 10% of seed stock comes from certified plantations (Wiewióra et al. 2009). It should be stressed that the quality of seeds, including microbial colonization, significantly affects the yield. In the present study, high abundance of the saprotrophic species *A. alternata* on spring wheat grain was found in both farming systems. Similar results were reported by Kundsen et al. (1995), Łukanowski and Sadowski (2005) and Wiewióra et al. (2009). As demonstrated by Baturo (2002), toxins produced by *A. alternata* inhibit root elongation and seedling growth. Baturo (2002) noted also high abundance of *E. nigrum* whose percentage was higher in the organic farming system. The above mentioned endophyte was often accompanied by *Cochliobolus sativus* (*B. sorokiniana*) which was less frequently isolated from conventionally-produced grain. According to published sources, *C. sativus* poses a serious problem to organic farmers. The frequency of its occurrence increases if hot weather continues through May, followed by rain and cooling in June (Agarwal and Sinclair 1997). *Cochliobolus sativus* may constitute an infection reservoir and decrease seed germination even by 25% in the subsequent year (Łacicowa 1982). Wiewióra et al. (2009) observed high abundance of *C. sativus* and *Fusarium*, *Gibberella* on organically-grown grains, which suggested that the quality of organic cereals needs to be closely examined. The mycological analysis of spring wheat grain, performed in this study, revealed a higher occurrence frequency of *Fusarium* spp. in kernels from conventional farms. This is in accordance with other authors' results. Despite chemical control, *F. poae*, *F. culmorum*, *G. avenacea* and *F. oxysporum* were isolated in large numbers. Łukanowski and Sadowski (2005) observed a correlation between weather conditions and the occurrence of *G. avenacea* and *F. poae*. The latter dominated over *G. avenacea* when high temperatures prevailed after flowering and primary infection. *Fusarium poae* was also dominant on grain harvested from different cultivars of wheat grown in organic system (Sadowski et al. 2010). Although the abundance of *Fusarium* spp. was lower in organic farms, it should be noted that organic farming without fungicides does not always lead to low mycotoxin contamination levels. Kirchmann and Thorvaldsson (2000) reported a high mycotoxin content of organic products. Due to their high genetic diversity and a wide degree of environmental tolerance, *Fusarium* spp. may successfully compete with saprotrophs for ecological niches, following biological and chemical control of other phytopathogens. Disruption of the dynamic balance of antagonistic interactions contributes to the occurrence of new pathogens and a reduction in the population size of natural enemies (Dubas 2007). Similarly, in study, used chemical protection had effect on the proportions of the species composition of different ecological groups of fungi. Typical saprotrophic storage fungi, such as *Penicillium*, were isolated from wheat grain produced in both systems. The genus *Rhizopus,* associated with allergies, was more common in grain samples collected from organic farms. It should be stressed that the natural antagonist *T. harzianum* was isolated only from "Ekoland"-approved crops, which indicates that crop protection chemicals may have an inhibitory effect on pathogen-antagonist interactions (Damszel et al. 2009).

The results of this study show that the populations of pathogenic microbes colonizing spring wheat kernels can be effectively reduced provided that the dynamic balance in agricultural ecosystems is maintained with the use of natural interactions between microorganisms.

Streszczenie

GRZYBY ZASIEDLAJĄCE ZIARNIAKI PSZENICY JAREJ UPRAWIANEJ W SYSTEMIE KONWENCJONALNYM I EKOLOGICZNYM

Wzrost zainteresowania płodami rolnymi pochodzącymi z upraw ekologicznych, w których dobry stan fitosanitarny roślin uzyskuje się poprzez tworzenie warunków sprzyjających rozwojowi grzybów saprotrofitycznych, a jednocześnie świadomość ograniczonej roli naturalnych procesów biologicznych w redukcji patogenów i kompensowanie ich poprzez stosowanie w produkcji konwencjonalnej środków ochrony roślin skłoniły autorów do porównania zbiorowisk grzybów zasiedlających ziarniaki produkowane w dwóch systemach uprawy. Określono liczebność i skład gatunkowy grzybów zasiedlających ziarno pszenicy jarej odmian 'Koksa' i 'Torka' uprawianej w systemie konwencjonalnym i ekologicznym.

Z ziarniaków pochodzących z gospodarstw konwencjonalnych izolowano więcej kolonii *Alternaria alternata, Aureobasidium pullulans, Botrytis cinerea* i *Fusarium* spp., *Gibberella* spp. Intensywna ochrona chemiczna nie ograniczyła liczebności ani różnorodności gatunkowej *Fusarium* spp. Z systemu ekologicznego izolowano licznie *Epicoccum nigrum.*

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Accepted for publication: 18.06.2012