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POPULATIONS OF FUNGI AND BACTERIA ON EARS OF CONVENTIONALLY AND ORGANICALLY GROWN WINTER WHEAT

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Abstract

In 2006 effects of two cropping systems: conventional and organic on population of bacteria, yeasts and filamentous fungi colonizing ears, at different stages of their development, of two winter wheat cultivars ('Roma' and 'Zyta') were compared. Populations of saprotrophic fungi, particularly yeasts, on ears grown under conventional cropping system were not significantly reduced by fungicides applied in the system as compared to the organic one. At the milk kernel stage total numbers of bacteria, yeasts and filamentous fungi, including *Fusarium* spp., on ears in the conventional system were even significantly higher than on the ears in the organic one. In the organic system wheat stands were thinner but taller (due to lack of any mineral fertilizers and plant growth regulators) and in consequence ears in the system kept moisture shorter than in the conventional system. It seems that this was the most important factor differentiating proliferation of microorganisms on ears under the two cropping systems.

Key words: farming system, winter wheat, ear, fungi, bacteria

Introduction

The above-ground parts of plants are normally colonized by diverse and dense communities of microorganisms including bacteria, yeasts, filamentous fungi and algae. The majority of them, called epiphytes, have no detectable effect on plant growth and function, some of them are beneficial whereas others, able to infect plant tissues, may be damaging to the host plant (Magan and Lacey 1986, Dik et al. 1992, Lindow and Brandl 2003). Epiphytes encounter rather harsh conditions on

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plant surfaces, where they are exposed to fluxes of UV radiation, rapidly fluctuating temperature and humidity, as well as to a strong competition for limited nutrient sources (Lindow and Brandl 2003). Densities and composition of microbial populations on the aerial parts of field grown crops depend also on agricultural practices, such as mineral fertilization or spraying with plant protection chemicals. Fokkema et al. (1983, 1987) and Dik et al. (1991, 1992) in their extensive studies proved that pink and white yeasts, predominating in the saprotrophic phyllosphere communities colonizing wheat leaves, were effective in removing aphid honeydew and other nutrients from wheat leaves, which resulted in the suppression of necrotrophic pathogens and higher crop yields. Magan and Lacey (1986) reported that flag leaves and ears of spring and winter wheat were colonized by high populations of yeasts and yeast-like fungi, while within filamentous fungi predominated *Cladosporium* spp., *Alternaria alternata, Verticillium lecanti* and *Fusarium* spp. The authors have also shown that some fungicides could significantly reduce fungal populations on flag leaves and ears, with yeasts and *Cladosporium* spp. being the most sensitive fungi to such treatments. In Poland Łukanowski and Sadowski (2005) have found more *Fusarium* spp. on kernels of winter wheat grown under conventional and integrated cropping systems than under organic one. It seems possible that fungicide application for fungal diseases control in conventional cropping systems reduces populations, and thus competitive potential of saprotrophic fungi, e.g. yeasts, and thus facilitating infection of ears (and grains) with other fungi, including toxigenic *Fusarium* spp.

To check this we have examined populations of microorganisms on ears of winter wheat, based on the field experiment described by Łukanowski and Sadowski (2005). We were particularly interested in populations of yeasts and filamentous fungi colonizing winter wheat ears at various stages of their development as influenced by farming systems (conventional *versus* ecological).

Material and methods

Field experiment

The studies were based on a long-term field experiment (established in 1994) located on a brown soil (Albic Luvisol) at the Experimental Station of the Institute of Soil Science and Plant Cultivation – State Research Institute in Osiny (51°27' N, 22°2' E, Lublin province). In the experiment various crops are grown under three different cropping systems: conventional, integrated and organic (Martyniuk et al. 2001). The objects of these studies were ears of two conventionally and organically grown winter wheat cultivars ('Roma' and 'Zyta'). In the conventional cropping system (CCS) the crop rotation is: winter rape – spring barley – winter wheat. In this system winter wheat was grown according to the high input technology generally used by farmers in Poland, which included two applications of fungicides. The first spray of winter wheat plants with Sarfun 500 SC + Artea 330 EC was per-

formed on May 15 (GS 37–39) and the second application of Artea 330 EC took place on June 22 (GS 69–70). In the organic cropping system (OCS) the crop rotation includes: potato – spring wheat – grass/red clover mixture (two years) – winter wheat. All crops, including winter wheat, in this system are grown without application of any synthetic mineral fertilizers or plant protection chemicals.

Sampling ears and microbial analyses

Ears were sampled four times during 2006: I – June 1, booting (GS 45–47, acc. to Zadocks), II – June 8, heading and flowering (GS 59–60), III – June 29, milk kernel (GS 73–75) and IV – July 20, ripening (GS 91–93). Three samples, each consisting of three ears, were randomly collected from fields of both cultivars ('Roma' and 'Zyta'). Ear samples were placed in disinfected plastic bags and within 2 h brought to the laboratory and refrigerated at 4°C. Next day each sample (three ears) was placed in a 300 ml glass bottle containing 90 ml of sterile distilled water with the addition of 0.01% Tween 80 (Dik et al. 1992). The content of the bottles was shaken for 30 min on a rotary shaker at 200 rev per 1 min. The obtained suspensions were than serially diluted (10-fold dilutions) and aliquots of 0.1 ml from each dilution were inoculated onto the surface of agar media in Petri plates to assess numbers of yeasts and filamentous fungi occurring on the examined ears. Populations of yeasts and filamentous fungi were assessed on basal yeast agar (BYA) medium containing 20 g glucose, 1 g yeast extract (Difco), 10 g proteose peptone (Difco), 15 g agar and 10^6 i.u. streptomycin sulphate per 1 l (Dik et al. 1992). Fungal colonies were counted after seven days of incubation at 27°C. Populations of bacteria colonizing the ears collected at the first three sampling dates have also been assessed. Bacterial colonies were counted on 1/10 strength TSA medium (tryptic soy agar, Difco) after three days of incubation at 27°C. Numbers of each group of microorganisms were expressed as colony forming units (cfu) per 1 g of ear d.m. Colonies of filamentous fungi were transferred onto fresh BYA slants and identified using the following manuals: Domsch et al. (1980), Kwaśna et al. (1991), Leslie and Summerell (2006).

One-way analysis of variance (ANOVA) and Tukey's test ($\alpha = 0.05$) were used to determine the significance of differences between the farming systems for each cultivar.

Results

At the booting stage (GS 45–47) ears of both cultivars ('Roma' and 'Zyta') were generally free from any microorganisms (Tables 1, 2). Population of fungi on the heads at GS 59–60 (heading and flowering) ranged from 2.7×10^3 to 5.4×10^3 cfu/g and those of bacteria were even markedly higher, ranging from 0.45×10^5 to 45.5×10^5 cfu/g. At this growth stage numbers of fungi on the ears of both tested cultivars grown under CCS were lower than on those grown under OCS, but the

Table 1

Total number of fungi and yeasts (in brackets) on winter wheat (cvs. 'Roma' and 'Zyta') ears as influenced by their development stages and cropping system (cfu/g d.m.)

Cropping system	Development stages			
	GS 45–47 booting	GS 59–60 heading and flowering	GS 73–75 milk kernels	GS 91–93 ripening
'Roma'				
Conventional	N.d.	2.70×10^3 a	4.77×10^6 a	2.54×10^7 a
		(0.45×10^3 a)	(1.45×10^6 a)	(2.28×10^7 a)
Organic	N.d.	3.31×10^3 a	1.02×10^6 b	2.64×10^7 a
		(0.48×10^3 a)	(0.23×10^6 b)	(2.28×10^7 a)
'Zyta'				
Conventional	26	4.92×10^3 a	4.10×10^6 a	3.28×10^7 a
		(1.46×10^3 a)	(1.03×10^6 a)	(3.01×10^7 a)
Organic	N.d.	5.40×10^3 a	2.78×10^6 b	3.41×10^7 a
		(0.80×10^3 b)	(0.35×10^6 b)	(3.10×10^7 a)

N.d. – not detected.

differences were not statistically significant (Table 1). Numbers of bacteria on the ears of both cultivars at GS 59–60 grown under CCS were significantly higher as compared to those on the ears in OCS (Table 2). At the milk kernels stage (GS 73–75) the ears in CCS were colonized by populations of bacteria only slightly more dense as compared to those on the ears in OCS, but there were marked differences between cultivars (Table 2). Number of bacteria on the ears of cv. 'Zyta' was almost three times higher than on the ears of cv. 'Roma'. At this growth stage colony number of fungi, including yeasts, on the ears of both cultivars grown under

Table 2

Number of bacteria on winter wheat (cvs. 'Roma' and 'Zyta') ears as influenced by their developmental stages and cropping system (cfu/g d.m.)

Cropping system	Developmental stages		
	GS 45–47 booting	GS 59–60 heading and flowering	GS 73–75 milk kernels
'Roma'			
Conventional	43	45.5×10^5 a	1.58×10^7 a
Organic	N.d.	0.66×10^5 b	1.35×10^7 a
'Zyta'			
Conventional	N.d.	8.9×10^5 a	5.60×10^7 a
Organic	N.d.	0.45×10^5 b	4.97×10^7 a

N.d. – not detected.

CCS were significantly higher than those on the ears in OCS and amounted to 1.02–2.78 × 10^6 cfu/g on the ears in OCS and to 4.10–4.77 cfu/g on the ears in CCS (Table 1). At ripening (GS 91–93) no significant differences were found in the numbers of filamentous fungi and yeasts on the ears of both cultivars grown under the compared cropping systems (Table 2). At this stage the total numbers of fungi on the ears further increased, as compared to the earlier stages, and their populations ranged from 2.54 × 10^7 to 3.41 × 10^7 cfu/g.

The qualitative composition of filamentous fungi on the examined ears is shown in Table 3. At the heading and flowering stage only *Alternaria alternata* and *Cladosporium herbarum* were isolated from the ears. At the milk kernel stage and at ripening *C. herbarum* was also the dominating species but only on milk kernels it was more numerous on the ears of both cultivars in OCS than in CCS (Table 3).

Two *Fusarium* species, *F. poae* and *F. tricinctum*, were found on the ears of the examined cultivars. The results presented in Table 3 clearly indicate that these spe-

Table 3

Number of filamentous fungi isolates* of obtained from winter wheat (cvs. 'Roma' and 'Zyta') ears as influenced by their developmental stages and cropping system (C – conventional, O – organic)

Fungus/Cultivar	'Roma' C	'Roma' O	'Zyta' C	'Zyta' O
Heading and flowering				
Alternaria alternata	1	1	–	1
Cladosporium herbarum	7	8	10	11
Milk kernels				
Acremonium sp.	–	1	3	2
Alternaria alternata	–	–	3	–
Cladosporium herbarum	28	57	75	96
Fusarium poae	20	–	2	–
Fusarium tricinctum	5	–	–	–
Penicillium sp.	–	5	–	1
Not sporulating fungi	6	10	1	3
Ripening				
Acremonium sp.	–	1	1	–
Alternaria alternata	6	–	–	1
Cladosporium herbarum	47	50	60	64
Fusarium poae	–	–	3	1
Penicillium sp.	–	2	1	11
Phoma sp.	1	3	–	–
Rhizopus nigricans	–	–	1	–
Not sporulating fungi	2	3	7	4

*Isolates obtained on BYA medium inoculated with the initial suspension (0.1 ml) diluted 100-fold at heading and flowering and 1000-fold at the other stages.

cies were more frequently isolated from the ears of both cultivars grown under CCS than under OCS, particularly at the milk kernel stage.

Discussion

Ears of both cultivars ('Roma' and 'Zyta') examined at the booting stage (GS 45–47, shortly before the fag leaf sheath opening) were generally free from any microorganisms. At this stage ears were still covered with the swollen leaf sheaths of flag leaves protecting them from air contaminations. Only in the case of cv. 'Zyta' grown in the conventional cropping system (CCS) and cv. 'Roma' in the organic cropping system (OCS) low numbers of fungal or bacterial colonies, respectively, were found on the agar media inoculated with the washings from the heads of these cultivars. It seems, however, that these colonies were just contaminants originating from laboratory air. Next sampling was done one week later (at GS 59–60) when heading was complete and it revealed that the ears had already been colonized with relatively high populations of microorganisms. At GS 59–60 numbers of fungi on the ears of both cultivars grown under CCS were lower than those on the ears grown under OCS, but the differences were not statistically significant. This slight reduction in the numbers of fungi on winter wheat ears in CCS could be attributed to fungicides sprayed onto leaves of winter wheat in CCS at GS 37–39. The fungicidal treatment was applied more than three weeks before ears were sampled and this was probably the reason for a weak inhibitory effect of the fungicides applied on the total numbers of fungi, including saprotrophic yeasts. Yeast populations were even significantly higher on the ears of cv. 'Zyta' in CCS than in OCS (Table 1). At this sampling date (GS 59–60) numbers of bacteria on the ears of both cultivars grown under CCS were significantly higher as compared to those on the ears in OCS. These marked differences between the cropping systems could be explained, at least partially, by lower populations of fungi colonizing winter wheat ears in CCS than in OCS. However, it should be mentioned that the stands of both cultivars in CCS were denser and shorter than those in OCS (Kuś et al. 2007). In dense stands ears preserve moisture (e.g. dew droplets) for a longer period of time during the day than ears in sparse stands, like those in OCS, and this was probably the main factor beneficially influencing bacterial proliferation and thus higher numbers of this group of microorganisms on the ears in CCS. The same explanation could also be applied to the effect of the farming systems on microbial populations colonizing the ears of both cultivars at GS 73–75 (milk kernels). While at this stage the ears in CCS were colonized by only slightly higher populations of bacteria as compared to the ears in OCS, numbers of fungi, including yeasts, on the ears of both cultivars grown under CCS were significantly higher than those on the ears in OCS (Table 1). These results indicate that the second application of Artea (at GS 69–70) did not harm saprotrophic microorganisms occurring on winter wheat ears in CCS. Tables 1 and 2 show that the numbers of the tested groups of microorganisms on ears increased more than 100 times during three weeks (between flower-

ing and milk kernels) of ears development. At ripening (GS 91–93) only fungal populations were counted and no significant differences were found in the numbers of filamentous fungi and yeasts on the ears of both cultivars grown under the compared cropping systems. There was, however, a substantial difference concerning the share of yeasts in the total fungal populations colonizing the ears at ripening in comparison to the earlier developmental stages. While yeasts constituted about 13–30% of the total populations of fungi on the ears examined at the earlier stages (flowering and milk kernels), this group of fungi predominated (86–92%) on the ears at the ripening stage. Results of our study concerning quantitative changes of fungal populations on the ears of winter wheat cultivars at different stages of their development as influenced by cropping systems, indicate that fungicides applied in the conventional cropping system (CCS) did not affect harmfully saprotrophic fungi, particularly yeasts colonizing winter wheat ears. Magan and Lacey (1986) reported that fungicides generally reduced populations of some filamentous fungi (*Cladosporium* spp.) and yeasts on leaves and ears of winter wheat, but they compared fungicide treated and untreated winter wheat crops grown only in the intensive (conventional) cropping system. In our studies effects of two different cropping systems (conventional *versus* organic) were compared. As we mentioned earlier, winter wheat stands in these two systems differed significantly with respect to their density and height. In the organic system stands were thinner but taller (due to lack of mineral fertilizers and plant growth regulators) and in consequence winter wheat ears in this system kept moisture shorter than in the conventional system. In our opinion this was an important factor differentiating proliferation of fungi, particularly of yeasts, on winter wheat ears. It should also be mentioned, in support of this opinion, that at all the examined developmental stages of ears, the share of yeasts in the total fungal populations was higher in CCS than in OCS (Table 2).

The qualitative composition of filamentous fungi occurring on the examined ears was also influenced by the studied factors. At the heading and flowering stage, one weak after opening of the fag leaf sheaths, only *A. alternata* and *C. herbarum* were isolated from the ears, but the latter species was isolated more frequently than the former one. At the milk kernel stage *C. herbarum* was also the dominating species and it was more numerous on the ears of both winter wheat cultivars in OCS than in CCS, probably due to the fungicides applied in CCS. Magan and Lacey (1986) also reported that *Cladosporium* spp. were sensitive to fungicides sprayed to control foliar diseases of winter wheat. *Cladosporium herbarum* is an ubiquitous fungus commonly colonizing various soils and dead plant material. Its numerous air-borne conidia contaminate the phyllosphere of almost all plant species (Domsch et al. 1980, Lindow and Brandl 2003).

At ripening *C. herbarum* was again the most frequently isolated fungus, but since the activity of the fungicides in CCS ceased, its populations on the ears were similar in both farming systems. The most important group of fungi occurring on ears are those belonging to the genus *Fusarium*. Many species of the genus under favourable conditions are able to infect heads of cereals and to produce various toxins contaminating the infected grain (Domsch et al. 1980, Magan and Lacey 1986,

Kwaśna et al. 1991, Łukanowski and Sadowski 2005). In our studies two *Fusarium* species, *F. poae* and *F. tricinctum*, were found on the ears of the examined winter wheat cultivars and they were more frequently isolated from both cultivars grown under CCS than under OCS, particularly at the milk kernel stage. These results are in accordance with those presented by Łukanowski and Sadowski (2005), who found more *Fusarium* spp. on kernels of winter wheat grown under CCS than OCS. Generally more *Fusarium*, particularly *F. poae*, were isolated from winter wheat ears at the milk kernel stage than at the ripening stage. Weather conditions might have been responsible for this difference, e.g. hot and dry June and similar weather conditions during the first decade of July in 2006, were unfavourable for development of *Fusarium* spp. on winter wheat ears at ripening stages.

Concluding, populations of saprotrophic fungi, particularly yeasts, on winter wheat ears grown under conventional (intensive) farming system were not significantly reduced by fungicides applied in this system as compared to the organic one. At the milk kernel stage of development total numbers of bacteria, yeasts and filamentous fungi, including *Fusarium* spp., on winter wheat ears under the conventional system were even significantly higher than on the ears under the organic system.

Streszczenie

POPULACJE GRZYBÓW I BAKTERII NA KŁOSACH PSZENICY OZIMEJ UPRAWIANEJ W SYSTEMACH KONWENCJONALNYM I EKOLOGICZNYM

W 2006 roku badano wpływ systemu uprawy (konwencjonalnego i ekologicznego) na zespoły bakterii, drożdżaków i grzybów strzępkowych zasiedlających kłosy, w czterech fazach ich rozwoju, u dwóch odmian pszenicy ozimej ('Roma' i 'Zyta'). Fungicydy stosowane w systemie konwencjonalnym (dwukrotne opryskiwanie) nie spowodowały istotnego zmniejszenia liczebności badanych grup mikroorganizmów na kłosach pszenicy ozimej uprawianej w tym systemie w porównaniu z uprawą w systemie ekologicznym (bez fungicydów). W stadium mlecznej dojrzałości zespoły bakterii i grzybów, w tym rodzaju *Fusarium*, były nawet liczniejsze na kłosach pszenicy w systemie konwencjonalnym niż w systemie ekologicznym. W systemie konwencjonalnym łan pszenicy był bardziej zwarty i niższy niż w systemie ekologicznym, co mogło sprzyjać m.in. dłuższemu utrzymywaniu się wilgoci na kłosach i w związku z tym rozmnażaniu się zasiedlających je mikroorganizmów, co w konsekwencji niwelowało ewentualny ujemny wpływ fungicydów na grzyby epifityczne.

Literature

Dik A.J., Fokkema N.J., Van Pelt J.A., 1991: Consumption of aphid honeydew, a wheat yield reducing factor, by phyllosphere yeasts under field conditions. Neth. J. Plant Pathol. 97: 209–232.

Dik A.J., Fokkema N.J., Van Pelt J.A., 1992: Influence of climatic and nutritional factors on yeast population dynamics in phyllospere of wheat. Microbiol. Ecol. 23: 41–52.

Domsch K.H., Gams W., Anderson T.-H., 1980: Compendium of soil fungi. Academic Press, London.

Fokkema N.J., Dik A.J., Daamen R.A., 1987: Use of carbendazim and carbendazim-resistant yeasts to create different yeast densities on wheat leaves for studies on biological control. Neth. J. Plant Pathol. 93: 273–283.

Fokkema N.J., Riphagen I., Poot R., De Jong C., 1983: Aphid honeydew, a potential stimulant of *Cochliobolus sativus* and *Septoria nodorum* and the competitive role of saprophytic mycoflora. Trans. Br. Mycol. Soc. 81: 355–363.

Kuś J., Jończyk K., Kawalec A., 2007: Factors limiting the yields of winter wheat in different crop production systems. Acta Agrophys. 10, 2: 407–417.

Kwaśna H., Chełkowski J., Zajkowski P., 1991: Grzyby (*Mycota*). T. 22. Grzyby niedoskonałe (*Deuteromycetes*), Strzępczakowe (*Hyphomycetes*), Gruzełkowate (*Tubercul ariaceae*), Sierpik (*Fusarium*). Instytut Botaniki PAN, Warszawa.

Leslie J.F., Summerell B.A., 2006: The *Fusarium* laboratory manual. Blachwell, Ames, USA.

Lindow S.L., Brandl M.T., 2003: Microbiology of phyllosphere. Appl. Environ. Microbiol. 69: 1875–1883.

Łukanowski A., Sadowski Cz., 2005: Wykorzystanie metody PCR do badania jakości ziarna pszenicy ozimej uprawianej w systemach ekologicznym, integrowanym, konwencjonalnym oraz monokulturze w aspekcie fitopatologicznym. Acta Agrobot. 59, 2: 55–69.

Magan N., Lacey J., 1986: The phylloplane microflora of ripening wheat and effect of late fungicide applications. Ann. Appl. Biol. 109, 1: 117–128.

Martyniuk S., Gajda A., Kuś J., 2001: Microbiological and biochemical properties of soils under cereals grown in the ecological, conventional and integrated systems. Acta Agrophys. 52: 185–192.

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