EFFECTS OF FARMING SYSTEM ON ROOT-ZONE FUNGAL POPULATIONS IN WHEAT

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SUMMARY

Effects of farming system on the composition of the root-zone microbiota and on the occurrence of root and stem-base diseases in winter wheat cv. Rywalka were analysed. The wheat was grown in organic, integrated and conventional systems of production and in monoculture over three years. Using classical methods, saprotrophic and pathogenic microorganisms, mainly fungi, were isolated from the root zone and identified on the basis of morphology and, where possible, designated as potential pathogens or antagonists. In roots, rhizoplane, and rhizosphere and non-rhizosphere soil the total frequencies of dominants (with frequency >5%) was 80.7- 98.7% and subdominants (with frequency 1-5%) was 0.5-14.5% and were similar in all production systems. Potential major soil-borne pathogens included *Fusarium* + *Haematonectria*. Potential pathogen antagonists included *Chaetomium*, *Clonostachys*, *Gliomastix*, *Sarocladium* and *Trichoderma* species. The low ratio of fungal pathogens : antagonists (*Fusarium* + *Haematonectria* : *Clonostachys* + *Trichoderma* spp.) in soil associated with lowest incidence of root and stem-base diseases, lower occurrence of *Fusarium* and *Rhizoctonia* in root and stem-base region and highest yield of grain in a conventional system for production of wheat cv. Rywalka confirms the cultivar's suitability for high-input conventional farming. Benefits of microbiological approaches in the study of suitability of cultivar to a particular farming system is demonstrated.

Keywords: farming, fungi, production system, winter wheat.

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INTRODUCTION

In 2010, world production of wheat was 651 million tonnes, making it the third most-produced cereal after maize and rice (Anonymous, 2010). About 95% of wheat produced is common wheat (bread wheat) (*Triticum aestivum* L.). Common wheat is grown in conventional, integrated and organic systems and in monoculture. Although integrated and organic farming have become increasingly popular (700 000 ha; Willer and Kilcher, 2009), the conventional system is still dominating, mostly because of the quality of grain produced (higher protein and gluten contents, higher sedimentation and flour water absorption values, longer dough stability time, greater loaf volume (L-Baeckstrom *et al*., 2006; Krejčířová *et al*., 2006, 2007; Ceseviciene *et al*., 2012).

It is important to have cultivars well adapted to a conventional production system. Wheat breeding for conventional agriculture concentrates on yield, good winter hardiness, resistance to nutrient deficiencies and ability to compete with weeds, but also resistance to fungal diseases and cooperation with root/soil microorganisms (Christensen, 1995; Seavers and Wright, 1999; Leibl and Petr, 2000; Jończyk, 2002; Bertholdsson, 2005; Kuś *et al*., 2006; Baresel *et al*., 2008; Wolfe *et al*., 2008; Stalenga, 2009; Kolb and Gallandt, 2012; Feledyn-Szewczyk *et al*., 2013, 2014).

Microorganisms may help suppress or eliminate plant pathogens. Disease suppression may be specific (related to the action of one or a few antagonistic organisms) or general (linked to abiotic and biotic factors resulting from the total microbial activity) (Baker and Cook, 1974; Cook and Baker, 1983; Termorshuizen and Jeger, 2008). The two kinds of suppression can occur simultaneously. Various mechanisms drive the phenomenon of disease suppression. Most of them are the results of interactions between pathogens and antagonists either by competition, antibiosis, or hyperparasitism. An additional biocontrol mechanism in cereals is the activation of induced disease resistance (Kogel and Langen, 2005).

Specific crop management can increase the natural soil suppressive potential.

Increases in crop yield in wheat and other cereals may be 5-43% (up to 80%) (Rodgers-Gray and Shaw, 2000; Nguyen *et al*., 2003; Cao *et al*., 2006; Chen *et al*., 2010). The

effect is often attributed to specific microbial populations linked to the kind and amount of organic matter (Mazzola, 2002; Bonilla *et al*., 2012).

Saprotrophic and plant-growth promoting rhizosphere (PGPR) bacteria, root-colonizing pseudomonads and arbuscular mycorrhizal (AM) fungi are the disease-suppressive organisms most often studied (Zhu *et al*., 2001; Gu and Mazzola, 2003; Oehl *et al*., 2004; Behl *et al*., 2007; Sari *et al*., 2008). Endophytic, often symbiotic fungi have been studied rarely (Larkin *et al*., 1996; Berg *et al*., 2005; Rengel and Marschner, 2005; Lenc *et al*., 2015).

In Poland, Łukanowski (2000) and Lemańczyk and Sadowski (2002) showed that the composition of the fungal community in wheat roots, including pathogenic *Gaeumannomyces graminis* (Sacc.) Arx & D.L. Olivier var. *tritici* Walker, depended considerably on the previous crop. The lowest incidence of pathogens occurred in crops grown after a mixture of oats and barley, oats and pea or lupin as a pure stand. Lenc *et al*. (2015) showed that the fungal antagonists of pathogens in roots, rhizoplane and rhizosphere of winter wheat cv. Zyta were most frequent in the organic system and least frequent in monoculture, suggesting that these systems had the most and least disease-suppressive habitats, respectively. Such studies can contribute to the development of strategies for manipulating the soil microbial environment as a viable crop management technique.

Plants, independently of habitat (resulting from the production system), evolve strategies for stimulating and supporting specific groups of antagonistic microorganisms in the rhizosphere (Smith *et al*., 1999; Cook, 2006). Differences in capacity of different plant genotypes to support specific groups of resident and introduced microbial antagonists have been reported (Larkin *et al*., 1993; Berg *et al*., 2002; Mazzola and Gu, 2000; Mazzola *et al*., 2004). More importantly, a genetic basis for specialization between plant host and microbial antagonists has been demonstrated (Smith *et al*., 1997, 1999).

The objectives of this study were to compare microbial communities (mostly fungi) in the root zone of winter wheat cv. Rywalka grown in different farming systems and to use the data to assess the suitability of cultivar to a particular (i.e. conventional) farming system. The study included evaluation of occurrence of: (i) root/soil microbiota in organic, integrated and conventional systems and monoculture, (ii) wheat fungal pathogens and their antagonists in the root/soil habitat, (iii) diseases caused by foot and root rot pathogens. Effects of the soil chemical properties and climate on the abundance of fungi was analysed additionally. The major pathogens considered were *Colletotrichum graminicola* (anthracnose), *Gaeumannomyces* (take-all), *Oculimacula* (eyespot), *Fusarium* + *Haematonectria*, *Pythium* (root and crown rot) and *Rhizoctonia* (sharp eyespot) (Pitt, 1964; Weise, 1987; Hornby and Bateman, 1998; Turner *et al*., 2001; Agrios, 2005; Bockus *et al*., 2010). The major antagonists include *Clonostachys* and *Trichoderma* spp. (Harman and Kubicek, 1998; Xue, 2002; Hue *et al*., 2009). Other fungi might act as minor pathogens or antagonists depending on several environmental and cultural factors (Mangan, 1967; Soytong *et al*., 2001; Choi *et al*., 2009; Amarlou *et al*., 2010).

The ultimate objective is eventually to choose the production system that optimizes the soil microbial environment for reduced disease, increased production, and long-term sustainability. Using the classical method of microbial community profiling (based on pure- culture isolation and morphotyping) allows quantification and qualification of the viable and active components of the microbial communities.

MATERIALS AND METHODS

Site description. Winter wheat (*T. aestivum*) cv. Rywalka was grown on experimental fields of the Institute of Soil Science and Plant Cultivation at Osiny, near Puławy, Poland (51° 52' 02" N, 22° 05' 25" E) in three growing seasons (2008-2010). The soil was uniformly sandy loam with pH in H_2O = 6.22-7.05, humus content = 1.09-1.63%, extractable soil nitrogen ($NO_3^- + NH_4^+$) = 43-170 mg kg^{-1} (Kjeldahl method), phosphorus = 8.33-21.6 mg kg^{-1} (Egner-Riehm method), potassium = 4.30-16.90 mg kg^{-1} (Egner-Riehm method), magnesium = 6.03-10.93 mg kg^{-1} (Schachtschabel method). The average temperature during the flowering and ripening stages of wheat growth (June and July) in 2008-2010 was 18.4-20.2°C, with the highest in 2010. Precipitation in June and July 2008-2010 was 90.4-164.8 mm, with 2009 being the wettest year.

In 1998 the 13-ha field was divided into blocks of 5, 4, 3 and 1 ha, under organic, integrated and conventional systems, and monoculture of wheat, respectively. In 2008, for experimental purposes, each block was sub-divided into 1-ha whole plots and each plot sub-divided into four replicate sub-plots (2500 m^2). This non-randomized block design was necessitated by practical constraints. The number of whole plots was determined by the number of crops in the rotation sequence. The type of rotation, preceding crops, cover crop, tillage, organic and inorganic fertilizers, pesticides and weed removal applied in each system are presented in Table 1. The winter wheat seed was sown one week after winter ploughing. Phased-in crop sequences ensured that one whole plot of winter wheat in each system was available for sampling in each harvest year 2008-2010.

Collection of samples. Each year (2008-2010), in each system, roots of wheat plants were collected from 100 randomly sampled plants at the soft dough growth stage (GS 85; Zadoks *et al*., 1974); 25 plants were collected along a diagonal transect in each of the four replicate sub- plots. Roots were shaken for collection of rhizosphere soil and the soil was then bulked. Each year (2008-2010), before harvest, 20 sub-samples of non-rhizosphere soil were

Table 1. Crop management procedures used in different systems of wheat production at Osiny in 2008-2010

Treatment	Organic	Integrated	Conventional	Monoculture
Crop rotation	Extended	Extended	Limited	Limited
Rotation	1. potato 2. spring wheat 3. white clover (*Trifolium repens* L.) + forage grasses 4. clover + forage grasses 5. winter wheat	1. potato 2. spring wheat 3. faba bean (*Vicia faba* L.) 4. winter wheat	1. spring wheat 2. oilseed rape (*Brassica napus* L.) 3. winter wheat	winter wheat
Cover Crop	2008: narrow-leaved lupin (*Lupinus angustifolius* L.) (70 kg ha^{-1}) + white mustard (*Sinapis alba* L.) (20 kg ha^{-1}) + buckwheat (*Fagopyrum esculentum* Moench) (20 kg ha^{-1}) applied after wheat harvest 2009: narrow-leaved lupin (120 kg ha^{-1}) + buckwheat (40 kg ha^{-1}) 2010: narrow-leaved lupin (100 kg ha^{-1}) + buckwheat (20 kg ha^{-1})			
Tillage	Autumn: first plough (12-14 cm deep), disc harrowing, shredding of the cover crop, post-harvest tillage (grubber), disc harrowing, winter plough (24-26 cm deep)		Autumn: post-harvest tillage (grubber), disc harrowing, winter plough (24-26 cm deep)	
Organic fertilizers	Compost[a]: under potato, in October, 30 t ha^{-1}			
Inorganic fertilizers	Autumn - Potassium sulphate 2007-2010: 75 kg K_2O ha^{-1} Ground rock phosphate 2008, 2009: 36+36 kg PO_4 ha^{-1} 2010: 27 kg PO_4 ha^{-1}	Autumn - Polifoska (NPK + Mg + S) or ammonium nitrate (NH_4NO_3 + $CaCO_3$ + $MgCO_3$)		
		2007-2010: 116 + 107 + 98 + 113 NH_4 kg ha^{-1} (in 3-4 doses)	2007-2010: 173 + 150 + 140 + 151 NH_4 kg ha^{-1} (in 3-4 doses)	2007-2010: 157 + 145 + 160 + 136 NH_4 kg ha^{-1} (in 3-4 doses)
		2007-2010: 58 + 60 + 60 + 60 P_2O_5 kg ha^{-1}	2007-2010: 64 + 70 + 75 + 70 P_2O_5 kg ha^{-1}	
		2007-2010: 80 + 60 + 78 + 90 K_2O kg ha^{-1}	2007-2010: 96 + 75 + 98 + 105 K_2O kg ha^{-1}	
Pesticides		Propiconazole + fenpropidin (Tilt Plus 400 EC) in April-May 2008: 1 l ha^{-1}; 2010: 0.9 l ha^{-1}		
		Cyprodinil (Unix 75 WG) in May 2008: 0.7 l ha^{-1}		
		Protioconazol + tebuconazol (Prosaro 250 EC) in May-June		
		2008-2009: 1.0 + 0.8 l ha^{-1}	2008: 1 l ha^{-1}	2008-2009: 1.0 + 1.0 l ha^{-1}
		Trinexapac-ethyl (Modus 250 EC) in April-May		
		2009: 0.3 l ha^{-1}	2008: 0.4 l ha^{-1}	
		Protioconazol + spiroxamine (Input 460 EC) in May		
		2009: 0.8 l ha^{-1}		2009: 1.0 l ha^{-1}
		2.4D + dicamba (Aminopielik 450 SL) in May		
		2009-2010: 3.0 + 3.0 l ha^{-1}	2010: 3.0 l ha^{-1}	
		Thiophanate-methyl + tetraconazole (Yamato 303 SE) in June 2010: 2.0 l ha^{-1}		
			Fluroxypyr (Starane 250 EC) in May 2008: 0.5 l ha^{-1}	
			Fenoxaprop-P-ethyl (Puma Uniwersal 069 EW) in April-May	
				2009-2010: 1.0 + 1.0 l ha^{-1}
			Fenpropimorph (Corbel 750 EC) in April 2010: 1.0 l ha^{-1}	
Removal of weeds	Harrowing or manually during vegetation	Harrowing		

[a]Compost included solid cattle manure enriched with grasses and clover and provided 2.8 dt ha^{-1} of organic matter and 0.1 t ha^{-1} of N, 0.04 t ha^{-1} of P, 0.1 t ha^{-1} of K, 0.25 t $ha^{-1}$of Ca, 0.27 t $ha^{-1}$of Mg, 0.005 t $ha^{-1}$of Na.

collected using a 5 cm diameter soil-core sampler from the A horizon of the ploughed soil (0-20 cm deep) along a diagonal transect in each of the four replicate sub-plots in each system. Soil was mixed thoroughly by rotating for 12 h.

Isolation and identification of fungi. Sixty root pieces of 1 cm length (1 g) were taken from 60 plants in each of the four replicate sub-plots in each system. The roots were rinsed successively in 10 flasks, each containing 70 ml of sterile distilled water, for 2 min each time. The ninth flask in the series also contained 30 g of sterile quartz sand. Roots removed from the 10th flask were dried in sterilized blotting paper, cut into 5-mm lengths and placed on potato dextrose agar (PDA; 39 g Difco PDA l^{-1}, pH 5.5). Aliquots from the second and 10th flask were used for isolation of fungi from the rhizosphere soil and rhizoplane (the external surface of roots together with closely adhering soil particles and debris), respectively: 0.1 ml was poured into an empty Petri dish and covered with liquid (50°C) Johnson-Martin's agar (JMA; glucose 10 g l^{-1}, peptone 5 g l^{-1}, KH_2PO_4 1 g l^{-1}, $MgSO_4.7H_2O$ 0.5 g l^{-1}, rose Bengal 0.03 g l^{-1}, aureomycin 0.0025 g l^{-1}, agar 20 g l^{-1}). The non-rhizosphere soil fungi were isolated after dilution of 1 g of soil in 74 g of sterile quartz sand; 0.02 g of the mixture was then put into a Petri dish and covered with liquid JMA. Eight replicates were made from each sample, making 32 replicates from each system. All plates were incubated for 20 days at 25°C.

All colonies on each plate were examined macro- and microscopically and distinguished on the basis of colour, growth rate, hyphal characteristics and sporulation.

Colonies of each species were pre-identified and counted, and representatives were identified on PDA, synthetic nutrient agar (SNA; KH_2PO_4 1 g l^{-1}, KNO_3 1 g l^{-1}, $MgSO_4.7H_2O$ 0.5 g l^{-1}, KCl 0.5 g l^{-1}, glucose 0.2 g l^{-1}, sucrose 0.2 g l^{-1}, agar 20 g l^{-1}), Czapek yeast autolysate agar (CYA; sucrose 30 g l^{-1}, powdered yeast extract 5 g l^{-1}, KH_2PO_4 1 g l^{-1}, Czapek concentrate 10 ml l^{-1}, agar 15 g l^{-1}) and 2% malt extract agar (MEA; powdered malt extract 20 g l^{-1}, glucose 20 g l^{-1}, peptone 1 g l^{-1}, agar 20 g l^{-1}). Identification was made mainly according to Domsch *et al.* (1980) and Klich and Pitt (1992) and fungal taxonomy was updated according to the CBS Fungal Biodiversity Centre nomenclature.

Abundance of fungi was defined as the number of colony-forming units in a sample. Frequency was defined as the proportion of isolates of an individual species in the total number of isolates from one habitat. Diversity was defined as the number of species in a sample. A species, or group of related species of fungi was considered as: (i) dominant, with frequency > 5%, (ii) subdominant, with frequency 1-5%, or (iii) subrecedent, with frequency < 1% (Tischler, 1949).

Assessment of wheat disease and yield of grain. Diseases were assessed on 100 plants collected randomly along a diagonal transect across each of the four replicate sub-plots in each system, at the dough growth stage (GS 80). After separating stems from leaf sheaths and washing, plants, including stem base and roots, were assessed for disease occurrence. Each disease was expressed as incidence. Isolation was used to verify the pathogens associated with disease symptoms. Pieces of root (0.5 cm long) and of stem bases (1 cm long) were surface-sterilized for 1 min in aqueous 10% ethanol, then in 8% sodium hypochlorite, rinsed twice with sterile distilled water, dried on sterile filter paper and placed on 2% PDA + 160 mg l^{-1} streptomycin sulphate. For *G. graminis* var. *tritici* and *Oculimacula* spp., root and stem pieces were placed on Ggt semi-selective medium (Juhnke *et al.*, 1984) and 2.0% Bacto agar + 50 mg l^{-1} rifampicin (Murray, 1992), respectively. Plates were incubated for 10 days at 15°C under near-ultraviolet light (NUV). No distinction was made between the closely-related *O. yallundae* (Wallwork & Spooner) Crous & W. Gams and *O. acuformis* (Boerema, R. Pieters & Hamers) Crous. Isolations were also made from control plants with no disease symptoms. Average yield of grain (at 15% moisture) was determined from eight 25 m^2 areas in each system. Number of ears was determined on 1 m^2 in each sub-plot. Thousand-kernel weight was determined from randomly distributed plants in each sub-plot.

Statistical analysis. Abundance and diversity of fungi were compared across the four systems (organic, integrated, conventional and monoculture) and three years (2008, 2009 and 2010) by χ^2 tests. All analyses were done separately for roots, rhizoplane, rhizosphere soil and non-rhizosphere soil. The analysis included main effects and involved constructing single models corresponding to the hypotheses. Relationships between abundances of fungi and soil chemical properties or weather variables were estimated by Pearson's correlation coefficient.

RESULTS

Fungi and Oomycota in each farming system were grouped into dominance classes (Tables 2 and 3). Dominants and subdominants included Zygomycota and Ascomycota fungi. In roots, rhizoplane, and rhizosphere and non-rhizosphere soil the total frequencies of microorganisms in two main classes were: (i) dominants (represented by 40 taxa) 80.7-98.7% and similar in all systems and years; (ii) subdominants (represented by 25 taxa) 0.5-2.1% in roots, 3.7-9.5% in the rhizoplane, 3.7-7.3% in rhizosphere soil and 7.1-14.5% in non-rhizosphere soil and also similar in all systems and years. There were 25 taxa of fungal and oomycetous subrecedents with frequency < 1%, including *Acremoniella fusca* (Kunze & J.C. Schmidt) Sacc., *Acrostalagmus luteoalbus* (Link) Zare. W. Gams & Schroers

Table 2. Frequency (%) of dominant fungi (>5% in at least one habitat) in winter wheat cv. Rywalka at Osiny in 2008-2010.

Group	Production system	Habitat			
		Roots	Rhizoplane	Rhizosphere soil	Non-rhizosphere soil
Zygomycota					
Absidia glauca Hagem + *A. spinosa* Lendn. + *Actinomucor elegans* (Eidam) C.R. Benj. & Hesselt. + *Cunninghamella elegans* Lendn. + *Mucor circinelloides* Tiegh. + *M. hiemalis* Wehmer + *M. moelleri* (Vuill.) Lendn. + *M. mucedo* Fresen + *Rhizopus arrhizus* A. Fisch. + *R. stolonifer* (Ehrenb.) Vuill.)	O	1.2^{a}	4.6^{ab}	9.3^{a}	1.5
	I	5.0	13.3^{a}	14.2	0.4
	C	4.0	6.6	6.8^{b}	1.4
	M	7.5^{a}	13.9^{b}	25.3^{ab}	2.8
Umbelopsis vinacea (Dixon-Stew.) Arx	O				
	I		6.6	0.3	
	C				
	M	0.5			2.4
Ascomycota					
Alternaria alternata (Fr.) Keissl.[2]	O		5.6^{a}	2.8	0.2
	I	2.0	0.4^{a}	0.7	0.1
	C		1.7	2.1	0.1
	M		3.1	0.2	
Cladosporium herbarum (Pers.) Link[2]	O		9.6^{a}	0.5	0.4
	I		2.8^{abc}	0.1	1.0
	C		10.2^{b}	0.1	0.7
	M		17.0^{c}	3.2	0.2
Clonostachys rosea f. *catenulata* (J.C. Gilman & E.V. Abbott) Schroers + *C. rosea* f. *rosea* (Link) Schroers. Samuels. Seifert & W. Gams + *C. solani* f. *solani* (Harting) Schroers & W. Gams[3]	O	5.4	5.0	5.3^{a}	3.6
	I	1.0^{a}	1.8	0.7^{ab}	1.8
	C	6.5^{ab}	1.7	7.1^{bc}	2.4
	M	0.9^{b}	1.5	1.1^{c}	1.7
Fusarium cerealis (Cooke) Sacc. + *F. culmorum* (W. G. Sm.) Sacc. + *F. oxysporum* Schlecht. emend. Snyd. et Hans. + *F. poae* (Peck) Wollenw. + *Fusarium sporotrichioides* Sherb. + *Gibberella avenacea* R.J. Cook + *G. intricans* Wollenw. + *G. pulicaris* (Fr.) Sacc. + *G. tricincta* El-Gholl, McRitchie, Schoult. & Ridings + *G. zeae* (Schwein.) Petch + *Haematonectria haematococca* (Berk. & Broome) Samuels & Rossman[1]	O	57.9	42.3^{ab}	31.8^{a}	19.7^{ab}
	I	54.8	53.9^{cd}	58.7^{abc}	12.7
	C	39.8	26.1^{ac}	32.1^{b}	8.0^{a}
	M	48.8	26.5^{bd}	36.8^{c}	6.7^{b}
Penicillium spp. including *P. glaucoalbidum* (Desm.) Houbraken & Samson[2]	O	5.0	19.9^{a}	18.7^{a}	38.0
	I	7.0	10.9^{b}	11.2^{b}	50.3^{a}
	C	5.0	22.4^{bc}	23.3^{bc}	38.7
	M	5.6	8.0^{ac}	6.9^{ac}	29.8^{a}
Periconia macrospinosa Lefebvre & Aar.G. Johnson[2]	O	12.4	0.4		
	I	12.6			
	C	6.5			0.3
	M	6.6			
Phoma eupyrena Sacc. + *P. glomerata* (Corda) Wollenw. & Hochapfel[2]	O		1.1	1.6	3.2
	I		0.4	0.4	0.6^{a}
	C		4.2	0.6	5.5^{a}
	M	0.9	0.5		2.0
Trichoderma hamatum (Bon.) Bain. + *T. harzianum* Rifai + *T. koningii* Oudem. + *T.polysporum* (Link ex Pers.) Rifai + *T. viride* Pers. ex Gray [3]	O	16.2^{a}	6.2^{ab}	20.9^{a}	18.1^{ad}
	I	15.6^{b}	2.6^{cd}	7.4^{abc}	13.8^{bc}
	C	36.9^{ab}	17.5^{ac}	22.5^{b}	31.8^{ab}
	M	24.4	19.9^{bd}	20.0^{c}	40.7^{cd}
Frequency of Ascomycota dominants	O	96.9	90.1	81.6	83.2
	I	93.0	72.8	79.2	80.3
	C	94.7	83.8	87.8	87.5
	M	87.2	76.5	68.2	81.1
Total frequency of dominants (Zygomycota + Ascomycota)	O	98.1	94.7	90.9	84.7
	I	98.0	92.7	93.7	80.7
	C	98.7	90.4	94.6	88.9
	M	95.2	90.4	93.5	86.3
Number of dominant species	O	6	6	6	5
	I	5	5	6	3
	C	7	7	7	4
	M	5	6	6	3

O = organic system; I = integrated system; C = conventional system; M = monoculture.

[a, b, c, d] the same letter in a column shows statistically significant difference ($P = 0.05$ or $P = 0.001$) between systems according to χ^2 tests.

[1] major pathogens; [2] minor pathogens; [3] antagonists.

Table 3. Frequency (%) of subdominant fungi (1-5% in at least one habitat) in winter wheat cv. Rywalka at Osiny in 2008-2010.

Species or group (explanation in Results)	Production system	Habitat			
		Roots	Rhizoplane	Rhizosphere soil	Non-rhizosphere soil
Zygomycota					
Mortierella alpina Peyronel + *M. hyalina* (Harz) W. Gams + *M. zonata* Linnem. ex W. Gams + *Mortierella* spp.	O		1.2	2.6	0.5
	I		2.6	0.3	1.0
	C		2.0	0.8	0.5
	M		0.3	0.4	3.7
Ascomycota					
Arthrinium phaeospermum (Corda) M.B. Ellis [2]	O				0.4
	I		0.1		
	C				
	M		3.8	2.1	0.2
Aspergillus fumigatus Fresen. + *A. niger* Tiegh. + *Aspergillus* spp. [2]	O				
	I		0.1		0.2
	C		0.1		0.1
	M	1.0			
Chaetomium cochliodes Palliser + *C. funicola* Cooke + *Chaetomium* spp. [3]	O		0.1		4.0
	I			0.1	2.9
	C		0.1	0.6	2.3
	M			0.9	2.6
Colletotrichum graminicola (Ces.) G.W. Wilson [1]	O				
	I	1.0			0.1
	C		0.3	0.3	
	M	0.5			0.2
Epicoccum nigrum Link [2]	O		0.7	0.9	
	I	0.5	0.1	0.1	
	C	0.5	0.3		0.3
	M		3.1	0.6	
Gibellulopsis nigrescens (Pethybr.) Zare. W. Gams & Summerb.	O		0.2		1.4
	I			0.1	3.1
	C		0.9	0.6	2.1
	M			0.2	1.1
Gliomastix cerealis (P. Karst.) C.H. Dickinson + *G. murorum* (Corda) S. Hughes	O		0.2	1.1	1.4
	I				1.3
	C		0.5		0.1
	M				0.4
Humicola fuscoatra Traaen + *H. grisea* Traaen	O		1.1	1.4	0.9
	I		0.5	0.7	0.9
	C		0.1	0.4	
	M		0.2		0.2
Paecilomyces variotii Bainier + *Paecilomyces* spp.	O		0.1	0.9	0.1
	I				3.3
	C			0.3	0.4
	M		0.1		1.1
Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken,Hywel-Jones & Samson	O	2.1	0.1	0.2	0.1
	I	0.5	2.5	3.7	0.8
	C		0.6	0.3	0.3
	M		1.8	0.4	0.4
Sarocladium strictum (W. Gams) Summerb.[3]	O			0.2	0.7
	I		0.3	0.4	0.6
	C		1.6	0.4	0.7
	M		0.1	0.6	1.1
Torulomyces indicus (S.B. Saksena) M.H. Hashmi, W.B. Kendr. & E.B.G. Jones [2]	O				0.7
	I		0.3	0.1	0.3
	C				0.3
	M		0.1	0.4	1.1
Frequency of Ascomycota subdominants	O	2.1	2.5[a]	4.7	9.7
	I	2.0	3.9	5.2	13.5
	C	0.5	4.5	2.9	6.6
	M	1.5	9.2[a]	5.2	8.4
Total frequency of subdominants (Zygomycota + Ascomycota)	O	2.1	3.7	7.3	10.2
	I	2.0	6.5	5.5	14.5
	C	0.5	6.5	3.7	7.1
	M	1.5	9.5	5.6	12.1
Number of subdominant species	O	0	9	13	7
	I	3	8	4	11
	C	1	11	8	9
	M	3	12	6	12

O = organic system; I = integrated system; C = conventional system; M = monoculture.
[a,b,c,d] the same letter in a column shows statistically significant difference ($P = 0.05$ or $P = 0.001$) between systems according to χ^2 tests.
[1] major pathogens; [2] minor pathogens; [3] antagonists.

Table 4. Abundance and diversity of Fungi + Oomycota in winter wheat cv. Rywalka in four production systems.

System	No. colony-forming units in sample				No. species			
	2008	2009	2010	Mean	2008	2009	2010	Mean
Roots								
O	97[1]	76[a]	69[1]	81	12	6	7	8
I	79[1]	51[a1]	69	66	13	6	6	8
C	73	67	61	67	10	8	5	8
M	73	67	73	71	12	10	11	11
Mean	81	65	68		12	8	7	
Rhizoplane								
O	345[ad1]	343[a2]	553[12]	414[ab]	25	25	28[a]	26
I	382[b12]	524[abc13]	603[a23]	503[acd]	28	26	10[a]	21
C	423[cd12]	356[b13]	499[a23]	426[ce]	27	27	19	24
M	1039[abc12]	309[c13]	516[23]	621[bcde]	28	22	17	22
Mean	547	383	543		27	25	19	
Rhizosphere soil								
O	225[ab12]	102[abc13]	315[ab23]	214[a]	25	13	19	19
I	265[c1]	156[ad12]	285[cd2]	235[b]	26[1]	17	13[1]	19
C	283[ad12]	154[be13]	239[ace23]	225[c]	30	18	18	22
M	172[bcd1]	99[cde12]	198[bde]	156[abc2]	21	13	11	15
Mean	236	128	259		26	15	15	
Non-rhizosphere soil								
O	383[abc12]	265[a13]	200[abc23]	283[abc]	40[a1]	30[a]	21[1]	30
I	304[ade12]	332[abc]	350[ade1]	329[ade]	30	23	28	27
C	148[bd12]	243[b1]	276[bdf2]	222[bdf]	27	23	18	23
M	157[ce1]	226[c12]	159[cef2]	181[cef]	25[a1]	12[a1]	19	19
Mean	248	267	246		31	22	22	

O = organic system; I = integrated system; C = conventional system; M = monoculture.
[a, b, c, d, e, f] the same letter in a column shows statistically significant difference between systems according to χ^2 tests at $P = 0.05$ or $P = 0.001$.
[1, 2, 3] the same number in a row shows statistically significant difference between years according to χ^2 tests at $P = 0.05$ or $P = 0.001$.

(coprophilous), *Botrytis cinerea* Pers., *Cephalotrichum stemonitis* (Pers.) Nees (coprophilous), *Coniothyrium cerealis* E. Müll., *Dendryphion nanum* (Nees) S. Hughes (endophytic), *G. graminis*, *Geotrichum candidum* Link, *Geotrichum* spp., *Gonytrichum macrocladum* (Sacc.) S. Hughes, *Gymnoascus reessii* Baran., *Metacordyceps chlamydosporia* (H.C. Evans) G.H. Sung, J.M. Sung (entomopathogenic), *Microascus brevicaulis* S.P. Abbott (entomopathogenic), *Monilia pruinosa* Cooke & Massee, *Myrothecium roridum* Tode, *M. verrucaria* (Alb. & Schwein.) Ditmar, *Papulaspora irregularis* Hotson., *Preussia aemulans* (Rehm) Arx (mycoparasite), *Pythium* sp., *Sporothrix* spp., *Stachybotrys cylindrospora* C.N. Jensen, *Stemphylium* spp. (minor pathogen), *Thielavia terricola* (J.C. Gilman & E.V. Abbott) C.W. Emmons, *Torula* sp. and *Trichocladium asperum* Harz.

The least abundance of fungi was recorded in the conventional system. There were differences in abundance between years, often with least abundance in 2009 (Table 4).

Fungi were affiliated, where possible, as pathogens or antagonists. Major pathogens included *Fusarium* + *Haematonectria* spp., *G. graminis*, *Oculimacula* spp. and *Rhizoctonia* spp. Antagonists included *Chaetomium*, *Clonostachys*, *Gliomastix*, *Sarocladium* and *Trichoderma* species (Tables 2 and 3).

Potential minor pathogens included *Alternaria* (leaf spot), *Arthrinium* (damping off), *Aspergillus* spp. (storage moulds), *B. cinerea* (damping off), *Cladosporium* (leaf spot), *C. cerealis*, *C. graminicola* (anthracnose), *E. nigrum* (leaf spot, root and crown rot), *Penicillium* spp. (storage moulds), *Periconia*, *Phoma* (leaf spot), *Pythium* spp. and Zygomycota (storage moulds).

Major pathogens were less abundant and antagonists were more abundant, often significantly, in the conventional system than in other systems (Tables 5 and 6). Mean ratio of the major fungal pathogens : antagonists (*Fusarium* + *Haematonectria* : *Clonostachys* + *Trichoderma* spp.) was low in the conventional system and highest in integrated system (Table 6).

Symptoms of root and stem-base diseases were observed on 35.6% (conventional system) to 70.3% (monoculture) plants (Table 6). *Fusarium* was isolated from similar numbers of plants in all systems and *Rhizoctonia* from the smallest number of plants in the conventional system. *Oculimacula* was most frequent in monoculture (Table 6). Most occurrence of *Oculimacula* in the root and stem-base region was associated with least abundance of *Penicillium* in soil. *Gaeumannomyces graminis* var. *tritici* and *Pythium* were scarce in soil (subrecedents).

The smaller abundance of fungi in the conventional system was usually associated with lower soil acidity. Abundance of fungi: (i) in roots and rhizoplane, increased with higher soil nitrogen content ($r = 0.93$-0.94; $P < 0.0001$),

Table 5. Abundance of pathogens and antagonists in winter wheat cv. Rywalka in 2008-2010.

	Cropping system	Roots	Rhizoplane	Rhizosphere soil	Non-rhizosphere soil
Abundance of pathogens (no. colony-forming units)	O	68^{ab}	247^{abc}	81^{a}	69^{abc}
	I	56^{c}	291^{ad}	141^{abc}	49^{ad}
	C	35^{ac}	183^{bde}	77^{b}	34^{be}
	M	43^{b}	336^{ce}	67^{c}	17^{cde}
Total frequency of pathogens (%)	O	70.3	59.9	37.9	24.5
	I	70.9	58.0	60.3	14.8
	C	47.3	43.0	34.4	15.3
	M	58.7	54.1	42.9	9.3
Diversity of pathogens (no. species)	O	7	15	12	17
	I	8	16	13	12
	C	6	14	14	13
	M	6	18	11	8
Abundance of antagonists (no. colony-forming units)	O	18	48^{abc}	59^{ab}	79
	I	11^{a}	24^{ade}	20^{ac}	68
	C	28^{a}	92^{bdf}	69^{cd}	83
	M	18	134^{cef}	35^{bd}	84
Total frequency of antagonists (%)	O	21.6	11.5	27.5	27.8
	I	16.6	4.7	8.6	20.6
	C	42.3	21.5	30.6	37.3
	M	25.3	21.5	22.6	46.5
Diversity of antagonists (no. species)	O	6	10	6	13
	I	6	7	8	11
	C	5	11	8	10
	M	5	7	5	9
Ratio of pathogen: antagonist abundance	O	3.8	5.1	1.4	0.8
	I	5.1	12.1	7.1	0.7
	C	1.25	2.0	1.1	0.4
	M	2.4	2.5	1.9	0.2

O = organic system; I = integrated system; C = conventional system; M = monoculture.
^{a,b,c,d,e,f} the same letter in a column shows statistically significant difference between systems according to χ^2 tests at $P = 0.05$ or $P = 0.001$.

(ii) in roots, decreased with higher soil potassium content ($r = -0.85$; $P < 0.0001$), (iii) in rhizoplane and rhizosphere soil, moderately increased and decreased, respectively, with higher soil magnesium content ($r = 0.80$- -0.78; $P < 0.0001$), and (iv) in rhizoplane, rhizosphere soil and non-rhizosphere soil, increased with higher temperature in June-July ($r = 0.65$-0.99; $P < 0.0001$) and decreased with higher precipitation in June-July ($r = -0.50$--0.99; $P < 0.0001$).

Yields of grain significantly differed between years, with the lowest in 2010 (data for individual years not shown). The highest and lowest yields of grain, numbers of ears per m^2 and thousand-kernel weights (g) were recorded in the conventional and organic systems, respectively (Table 6). There is no clear indication that yields were affected by diseases.

DISCUSSION

The results presented here show effects of environment, created in organic, integrated and conventional farming systems and in monoculture, on the abundance and diversity of root/soil microbiota, on potential soil suppressiveness to the foot and root disease complex, and on yield in one winter wheat cultivar (= genotype), Rywalka. The potential soil suppressiveness was measured as the ratio of fungal pathogens : antagonists (*Fusarium* + *Haematonectria* : *Clonostachys* + *Trichoderma* spp.) in soil.

Cultivar Rywalka (class A), chosen for this study, is commonly grown in Poland, partly because of its high resistance to the foot and root disease complex and to leaf and head diseases, and its relatively high protein content (Rydzak *et al*., 2012). In the experiments in 2008-2010 it had an average grain yield of 6.9 t ha^{-1} in the conventional system, which was better than the yields of three other winter wheat cultivars tested in comparison (Feledyn-Szewczyk *et al*., 2014). The highest yield was associated with: (i) lower incidence of root and stem-base diseases, (ii) smaller occurrence of *Fusarium* and *Rhizoctonia* spp. in root and stem-base region, (iii) smallest abundance of wheat pathogens (*Fusarium* + *Haematonectria* spp.) and greater abundance of antagonists (*Clonostachys* + *Trichoderma*) in soil shown by the lower ratio of pathogens : antagonists (Table 6). The relatively high grain yield in the conventional system suggests that the plant-fungus-environment relationship in this system may help increase nutrient uptake, which may be dependent on vigorous

Table 6. Fungal, disease and crop yield characteristics in four production systems used for winter wheat cv. Rywalka cultivation.

Characteristics	Production system			
	Organic	Integrated	Conventional	Monoculture
Mean* abundance of Fungi (no. colony-forming units)	992[a]	1133[ab]	940[b]	1029
Mean** ratio of fungal pathogens: antagonists (*Fusarium* + *Haematonectria* : *Clonostachys* + *Trichoderma* spp.)	1.6	4.3	0.9	0.8
Mean proportion of plants with symptoms of root and stem-base diseases	44.3	50.6	35.6	70.3
Mean proportion of plants infected with *Fusarium* spp.	25.6	31.8	29.7	30.4
Mean proportion of plants infected with *Oculimacula* spp.	25.6[a]	28.9[b]	32.0[c]	50.7[abc]
Mean proportion of plants infected with *Rhizoctonia* spp.	13.1[a]	9.9[b]	1.9[abc]	14.2[c]
Mean** proportion of *Fusarium* + *Haematonectria* spp.	31.2	41.8[a]	22.0[a]	23.3
Mean** proportion of *Penicillium* spp.	25.5	24.1	28.1[a]	14.9[a]
Mean** proportion of *Clonostachys* + *Trichoderma* spp.	19.7	9.7[ab]	24.3[a]	28.3[b]
Yield of grain (t ha^{-1})+	3.37[abc]	6.66[ade]	6.90[bdf]	5.23[cef]
Number of ears in 1 m^2	350.0[abc]	453.0[a]	493.7[be]	441.7[ce]
Thousand kernels weight (g)	37.93	47.77	49.40	45.67

* Mean from roots + rhizoplane + rhizosphere soil + non-rhizosphere soil.

** Mean from rhizoplane + rhizosphere soil + non-rhizosphere soil.

[a, b, c, d, e, f, g, h] the same letter in a row shows statistically significant difference between systems according to $\chi 2$ tests at $P = 0.05$ or $P = 0.001$.

+ Yield of grain was analysed after conversion to kilograms.

root growth (partly through elimination of pathogens), higher mineralization via root exudates and a higher level of mycorrhization.

Effects of naturally occurring fungal antagonists on decreasing the occurrence of diseases or elimination of pathogens have been observed previously. Suppression of wheat soil-borne root pathogens (*F. culmorum*, *G. graminis*, *Rhizoctonia*) and foliar pathogens (*Zymoseptoria tritici* (Desm.) Quaedvl. & Crous, *Puccinia recondita* Dietel & Holw.) by soil microbiota were reported by Lemańczyk and Łukanowski (2000), Mazzola and Gu (2000), Lemańczyk and Sadowski (2002), Roberti *et al.* (2008), Sari *et al.* (2008) and Donn *et al.* (2014).

The positive phytopathological effects observed in the conventional system in 2008-2010, in comparison with the organic or integrated systems or monoculture, was associated with 5-17% decrease in overall abundance of fungi. This was associated with decreased soil acidity and smaller soil nitrogen and magnesium contents, probably a result of no organic fertilizers or limited amounts of inorganic fertilizers being applied. The behaviour of the microbiota resulted from fungal preferences for lower pH and probably from fungal response to nutrient availability. Intensive inorganic fertilization usually decreases the preference of rhizosphere microbiota for root-derived substrates, leading to a simpler microbe association (Ai *et al.*, 2015).

The decreased abundance of fungi in the conventional system seems not to have resulted from the type or doses of pesticides applied (pesticides were similar, and doses even smaller, than in monoculture, where greater abundance of fungi was observed) but may, however, have resulted from competition by antagonistic fungi, i.e. dominating *Clonostachys* + *Penicillium* + *Trichoderma* species.

Cultivar Rywalka was bred for conventional farming systems and the results presented show that the desired traits were expressed mostly in that system. Such performance is not unusual. A similarly greater performance of wheat cv. Zyta (bred for organic farming) in the organic production system was reported by Lenc *et al.* (2015). Benefits and potential negative side-effects of growing particular plant cultivars (including wheat) in preferred and non-preferred production systems were also reported by Lammerts van Bueren *et al.* (2011).

The microbiota detected is mostly an effect of habitat created by the production system. Effects of plant genotype (cultivar) cannot be excluded, however. Cultivar contributes to creating the environment in which soil microorganisms respond. Cultivar-specific effects on the spectrum of microbiota have been observed by Hetrick *et al.* (1995), Smith and Goodman (1999), Engelhard *et al.* (2000), Mazzola and Gu (2000), Germida and Siciliano (2001), Rengel (2002), Mazzola *et al.* (2004), Singh *et al.* (2012), Donn *et al.* (2015) and Sapkota *et al.* (2015). These effects result from long evolution over generations and specific physiological adaptation to particular conditions (Crowley and Rengel, 1999; Uren, 2007). Root exudates (selective substances, including signaling compounds) resulting from specific biosynthetic processes and cross-membrane transport play the most important role in the mechanisms of microbial response (Hartmann *et al.*, 2009). Discrimination between pathogens and beneficial mutualists or commensals can also be controlled by the plant's immune system (Jones and Dangl, 2006; Shade and Handelsman, 2012).

The present study and those of Wissuva *et al.* (2009) and Lenc *et al.* (2015) show that the microbial interactions in the root/soil zone may be used in selection of a wheat cultivar for production in a particular set of conditions or in breeding programmes.

In conclusion, the low ratio of fungal pathogens : antagonists (*Fusarium* + *Haematonectria* : *Clonostachys* + *Trichoderma* spp.) in soil associated with lowest incidence of root and stem-base diseases, lower

occurrence of *Fusarium* and *Rhizoctonia* in the root and stem-base region and highest yield of grain in a conventional system for production of wheat cv. Rywalka confirms the cultivar's suitability for high-input conventional farming.

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