Eur J Plant Pathol (2014) 140:491–502
DOI 10.1007/s10658-014-0483-9

Early PCR-based detection of *Fusarium culmorum*, *F. graminearum*, *F. sporotrichioides* and *F. poae* on stem bases of winter wheat throughout Poland

A. Kuzdraliński · H. Szczerba · K. Tofil · A. Filipiak · E. Garbarczyk · P. Dziadko · M. Muszyńska · E. Solarska

Accepted: 10 July 2014 / Published online: 16 August 2014

Abstract Foot rot and crown rot are fungal diseases of wheat caused by a complex of *Fusarium* species. They have a huge economic impact mainly due to yield reduction. A survey was conducted to identify four *Fusarium* species, occurring on wheat stem bases, using species-specific PCR assays in samples collected during spring of 2012. The dominant species was *F. graminearum*, which was identified in above 64 % of samples. *F. culmorum* was detected in 15.71 %, *F. poae* in 15.71 % and *F. sporotrichioides* in 5.71 % wheat fields. Most of the wheat fields in the eastern Poland were infected with at least one or two of *Fusarium* species, while in central Poland no *Fusarium* species were identified in most of the fields. The presence of *F. graminearum* tends to favor the presence of *F. culmorum* and this effect was visible also for *F. poae* and *F. sporotrichioides*. The frequency of *F. graminearum* and *F. culmorum* detections were highest where wheat crops were preceded by maize and in the samples from late sown fields. The opposite observation was made for *F. poae* and *F. sporotrichioides*, where the number of detections of these species was higher in samples from early sown fields. The number of detected *Fusarium* species was significantly lower in samples collected from fields protected with autumn herbicide in comparison to unprotected fields. The rate of autumn N fertilization did not affect the number of *Fusarium* detections.

Keywords *Fusarium* · Winter wheat · Species-specific PCR · Stem base

A. Kuzdraliński (✉) · H. Szczerba · K. Tofil · A. Filipiak · E. Garbarczyk · P. Dziadko · M. Muszyńska · E. Solarska
Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences in Lublin, ul. Skromna 8, 20-704 Lublin, Poland
e-mail: adamkuzdralinski@gmail.com

Introduction

The genus *Fusarium* contains many plant-pathogenic fungi, which are responsible for three main diseases of cereals: Fusarium head blight (FHB), foot rot (FR) and crown rot (CR). FHB is the best known *Fusarium* disease mainly due to the deterioration of grain quality through the production of mycotoxins (Parry et al. 1995; Champei et al. 2004; Gargouri et al. 2011). FR and CR are also a major problem in the production of wheat, causing significant yield losses each year (Smiley and Patterson 1996). CR can cause up to 89 % loss of wheat yield (Klein et al. 1991).

F. graminearum, *F. pseudograminearum* and *F. culmorum* have been identified as the predominant species associated with these diseases. Less frequently isolated species are *F. poae*, *F. sporotrichioides*, *F. acuminatum*, *F. avenaceum*, *F. crookwellense*, *F. oxysporum* and *F. equiseti* (Braithwaite et al. 1998; Bottalico and Perrone 2002; Monds et al. 2005). The relationship between FR, CR and FHB caused by the same fungi from the genus *Fusarium* is generally unclear (Parry et al. 1995). *Fusarium* spp. causing FR and CR survive in dead plant material or in the soil. Necrosis

Table 1 Samples collected from winter wheat fields in the spring of 2012 along with their geographic origin, forecrop, date of sowing and nitrogen level fertilization

Sample	Geographic region	Forecrop	Date of sowing	N fertilization [kg/ha]
1	central Poland	winter rape	mid-September	5
2	central Poland	winter rape	mid-September	0
3	central Poland	winter rape	mid-September	50
4	eastern Poland	maize	early October	22,2
5	eastern Poland	winter wheat	early September	58
6	north-westernPoland	winter rape	early October	0
7	north-westernPoland	winter rape	late September	16
8	north-westernPoland	winter rape	mid-September	40
9	north-westernPoland	maize	early October	0
10	north-westernPoland	winter wheat	mid-September	55
11	eastern Poland	winter wheat	mid-September	52
12	eastern Poland	winter wheat	early September	8
13	north-westernPoland	winter rape	mid-September	40
14	north-westernPoland	maize	late September	0
15	north-westernPoland	winter barley	mid-September	0
16	eastern Poland	white beet	early October	30
17	north-westernPoland	winter rape	late September	0
18	central Poland	winter wheat	mid-September	30
19	north-westernPoland	winter wheat	late September	0
20	north-westernPoland	winter rye	early October	0
21	north-westernPoland	winter barley	late September	0
22	central Poland	winter rape	late September	0
23	south-western Poland	maize	mid-October	0
24	south-western Poland	winter rape	late September	0
25	south-western Poland	winter rape	mid-September	12
26	south-western Poland	maize	early October	0
27	south-western Poland	winter rape	late September	28
28	south-western Poland	maize	early October	0
29	south-western Poland	maize	mid-October	0
30	south-western Poland	maize	mid-October	10
31	south-western Poland	maize	mid-October	34
32	eastern Poland	winter rape	mid-September	110
33	south-western Poland	winter rape	mid-September	0
34	south-western Poland	winter wheat	mid-October	0
35	south-western Poland	maize	early October	0
36	south-western Poland	winter rape	late September	18
37	north-westernPoland	lupine	late September	0
38	central Poland	nd	nd	nd
39	north-westernPoland	winter wheat	mid-September	0
40	central Poland	winter wheat	mid-September	0
41	central Poland	winter wheat	nd	18
42	south-western Poland	maize	late October	0
43	south-western Poland	winter wheat	mid-October	0

Table 1 (continued)

Sample	Geographic region	Forecrop	Date of sowing	N fertilization [kg/ha]
44	north-westernPoland	spring wheat	late September	nd
45	north-westernPoland	winter rape	late September	0
46	north-westernPoland	white beet	mid-September	0
47	eastern Poland	winter rape	late September	20
48	central Poland	winter rape	mid-September	20
49	south-western Poland	maize	late September	10
50	north-westernPoland	white beet	early September	15
51	south-western Poland	triticale	nd	nd
52	north-westernPoland	winter rape	nd	nd
53	eastern Poland	winter rape	mid-September	20
54	eastern Poland	winter wheat	mid-September	20
55	central Poland	winter wheat	early October	250
56	south-western Poland	winter rape	late September	0
57	south-western Poland	winter rape	nd	nd
58	south-western Poland	white beet	nd	nd
59	south-western Poland	winter rape	late September	0
60	north-westernPoland	winter wheat	early September	30
61	north-westernPoland	winter wheat	mid-September	30
62	eastern Poland	nd	nd	nd
63	eastern Poland	winter rape	late September	60
64	central Poland	winter wheat	mid-September	0
65	eastern Poland	winter wheat	mid-September	35
66	central Poland	maize	late October	15
67	eastern Poland	winter wheat	mid-September	18
68	eastern Poland	spring wheat	mid-September	18
69	central Poland	white beet	early November	0
70	south-western Poland	winter rape	late September	0

nd no data available

of the crown and stem base of wheat are the main symptoms of FR and CR. In some cases, severe disease occurrence contributes to premature death of the whole plants. Residual stubble has been identified as the source of primary infection but still little is known about the basis of the infection process (Burgess et al. 2001). Many authors highlight the fact that host residues for *Fusarium* spp. inoculum depends on cultivation method, cropping sequence and herbicide usage (Wiese 1987; Cromey et al. 2006). The few studies conducted in Poland have reported that the presence of *Fusarium* spp. on stem bases of winter wheat depends mainly on the weather and to a lesser extent on the crop rotation and weed infestation (Jaczewska-Kalicka 2001; Korbas 2004; Narkiewicz-Jodko et al. 2005).

Identification of fungi of the genus *Fusarium*, based on the morphology of mycelium and macroconidia, is a reliable method but it requires time and necessary skills. The polymerase chain reaction (PCR) technique is one of the most frequently used molecular tools for rapid and sensitive identification of *Fusarium* species (Niessen et al. 2004; Mulé et al. 2005; Demeke et al. 2005; Jurado et al. 2005, 2006).

In this study, we report the incidence of four important *Fusarium* species on stem bases of winter wheat in Poland. The selected species of *F. graminearum*, *F*

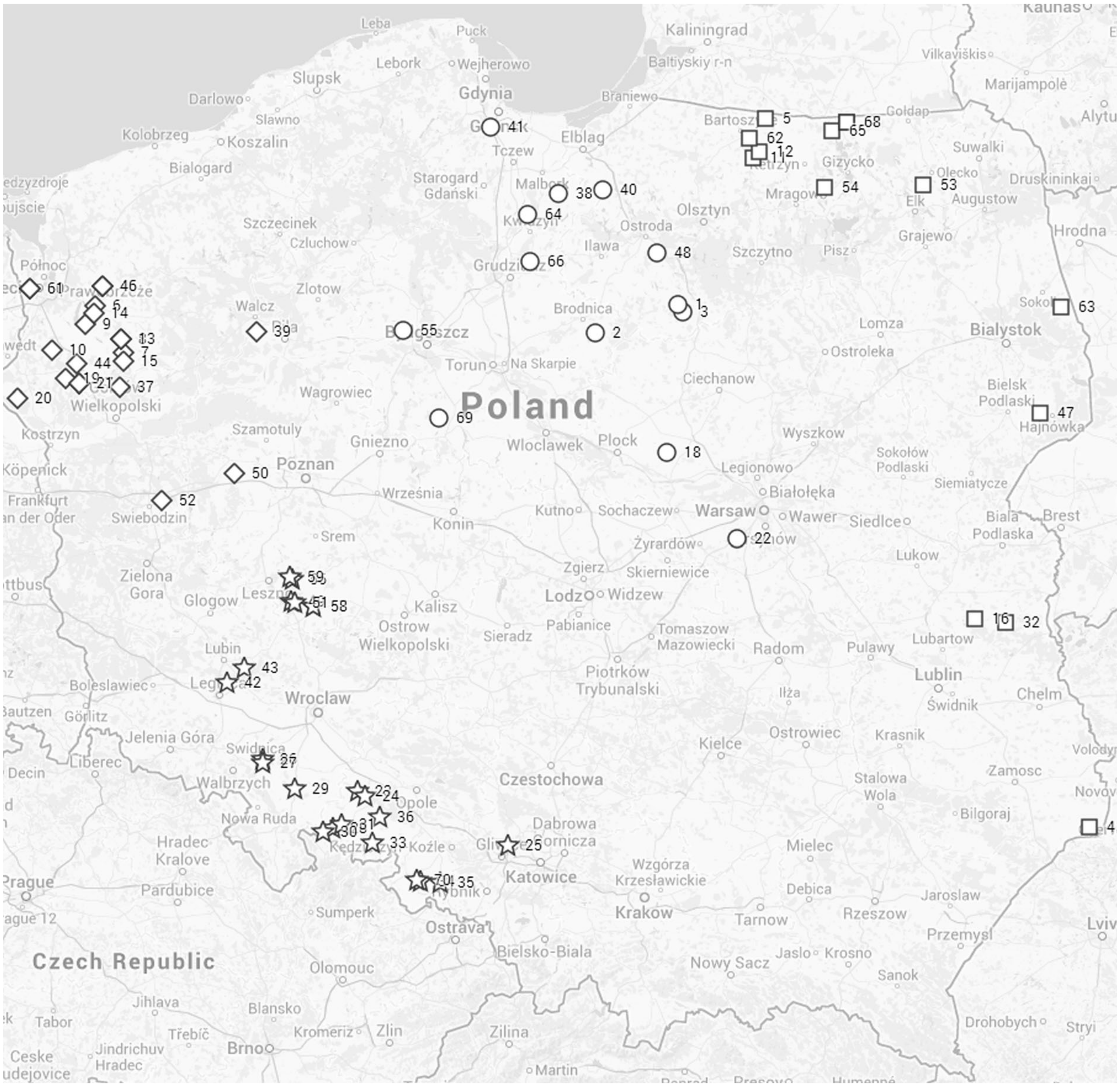

Fig. 1 Map of Poland (Google Maps Engine Lite, Liebert 2013) showing the location of sites of collection of winter wheat plants. Samples were divided into four groups with respect to geographic and climatic regions and marked with different icons: *square* - eastern Poland, *circle* - central Poland, *star* – south-western Poland and *diamond* – north-western Poland

.*culmorum*, *F. poae* and *F. sporotrichioides* are the prevalent causal agents of *Fusarium* diseases of roots and stem bases of wheat in Poland (Baturo 2006; Łukanowski 2009; Mielniczuk et al. 2012). We have determined their relationships with the following important factors: geographic region, previous crop, herbicide application, date of sowing and rate of autumn nitrogen fertilization. Lastly, we evaluate the interactions between *Fusarium* species tested in this work.

Materials and methods

Sample collection

Samples of winter wheat plants were collected in the spring of 2012 from 70 fields located in Poland during the tillering and just before the stem elongation stage of the crop growth (Table 1, Fig. 1). The number of plants collected varied slightly at each site, ranging from 10 to

Table 2 Species-specific primers used to identify *Fusarium* species

Target species	Primer name	Sequence 5′-3′	Amplicon size (bp)	Reference
F. graminearum	Fg16NF Fg16NR	ACAGATGACAAGATTCAGGCACA TTCTTTGACATCTGTTCAACCCA	280	Nicholson et al. 1998
F. culmorum	Fc01F Fc01R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	570	Nicholson et al. 1998
F. poae	Fp82F Fp82R	CAAGCAAACAGGCTCTTCACC TGTTCCACCTCAGTGACAGGTT	220	Parry and Nicholson 1996
F. sporotrichioides	FspITS2K P28SL	CTTGGTGTTGGGATCTGTGTGCAA ACAAATTACAACTCGGGCCCGAGA	288	Kulik et al. 2004

20 plants, depending on the size of the field. Growers were provided with a questionnaire to supply information on previous crop, sowing date, autumn herbicide and fertilizer applications. Each sample was transported to the laboratory where it was stored at −20 °C.

DNA extraction

The leaf sheaths of each plant were removed and stems were washed carefully with tap water to remove adhering soil. Single stem sections between the crown roots and the first node (0.5–1 cm in length) were removed from each stem and used for DNA isolation. DNA was extracted from 15- to 25-mg subsamples of wheat stem bases that were transferred to 2 ml Eppendorf tube and pestled with liquid nitrogen to a fine powder. DNA from stem bases was obtained using Plant and Fungi Kit (EURx, Poland) according to the manufacturer's instruction. DNA purity and concentration was determined spectrophotometrically (NanoDrop, ThermoScientific, USA). Until the analysis, DNA samples were stored at −20 °C.

PCR identification of *Fusarium* species

Species-specific PCR primers were used for identification of *F. graminearum*, *F. culmorum*, *F. poae*, and *F. sporotrichioides*. The sequences of the primers, the sizes of the amplicons and reference sources are shown in Table 2. Each PCR analysis included DNA of *F. graminearum*, *F. culmorum*, *F. poae*, and *F. sporotrichioides*, which served as positive controls and were obtained from internal *Fusarium* spp. strains collection of the Department of Biotechnology, Human Nutrition and Science of Food Commodities.

Samples were run in 25 μl reactions using 2 × PCR Master Mix (Thermo Scientific Fermentas, Lithuania) with 20 pmol of each primer and 20 ng of DNA on a SensoQuest Labcycler (SensoQuest GmbH, Germany). Thermal cycling conditions specific for each primer pairs were as follows: an initial step at 95 °C for 5 min and 5 cycles at 95 °C for 30 s, 66 °C for 30 s, and 72 °C for 30 s, 5 cycles at 95 °C for 30 s, 64 °C for 30 s, and 72 °C for 30 s, 25 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s followed by 72 °C for 8 min for *F. graminearum* and *F. culmorum*, respectively; an

Table 3 Incidence of *Fusarium* species detected in different sampling regions

	Fusarium species [%]			
Sampling region	*F. graminearum*	*F. culmorum*	*F. sporotrichioides*	*F. poae*
Eastern Poland (n=14)	64.29 (9 out of 14)	21.43 (3 out of 14)	14.29 (2 out of 14)	35.71 (5 out of 14)
Central Poland (n=13)	46.15 (6 out of 13)	7.7 (1 out of 13)	7.7 (1 out of 13)	23.08 (3 out of 13)
South-western Poland (n=22)	72.73 (16 out of 22)	22.73 (5 out of 22)	0 (0 out of 22)	9.09 (2 out of 22)
North-western Poland (n=21)	66,67 (14 out of 21)	9.52 (2 out of 21)	4.76 (1 out of 21)	4.76 (1 out of 21)
Total (n=70)	64.29 (45 out of 70)	15.71 (11 out of 70)	5.71 (4 out of 70)	15.71 (11 out of 70)

Table 4 Number of *Fusarium* species detected in different sampling regions

Sampling region	No. of *Fusarium* species detected					
	0	1	2	3	>1	>2
Eastern Poland (n=14)	3 (21.42 %)	4 (28.57 %)	6 (42.86 %)	1 (7.14 %)	11 (78.57 %)	7 (50 %)
Central Poland (n=13)	5 (38.46 %)	5 (38.46 %)	3 (23.08 %)	0 (0.0 %)	8 (61.54 %)	3 (23.08 %)
South-western Poland (n=22)	6 (27.27 %)	9 (40.9 %)	7 (31.82 %)	0 (0.0 %)	16 (72.72 %)	7 (31.82 %)
North-western Poland (n=21)	6 (28.57 %)	13 (61.9 %)	1 (4.76 %)	1 (4.76 %)	15 (71.43 %)	2 (9.52 %)
Total (n=70)	20 (28.57 %)	31 (44.29 %)	17 (24.29 %)	2 (2.86 %)	50 (71.42 %)	19 (27.14 %)

initial step at 95 °C for 5 min and 40 cycles at 95 °C for 30 s, 68 °C for 30 s, and 72 °C for 40 s followed by 72 °C for 8 min for *F. sporotrichioides*; an initial step at 95 °C for 3 min and 38 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 30 s followed by 72 °C for 8 min for *F. poae*.

Amplification products were separated by electrophoresis in 1.5 % (wt/vol) agarose gels stained with ethidium bromide in 1× TBE 1.5 h at 120 V. DNA bands were then visualized using GelDoc 2000 gel documentation system (BioRad, USA), and sizes of the PCR products were determined by comparison against the migration of GeneRuler 100 bp plus DNA Ladder (Thermo Scientific Fermentas, Lithuania).

Data analysis

Data analyses were carried out: (i) to determine the presence of interactions between species identified, and (ii) to determine whether the occurrence of *Fusarium* species depends on one of the known factors: geographic location, date of sowing, previous crop, herbicide application in autumn and autumn N fertilization. Relationships were statistically analyzed by Pearson correlation coefficient using Statgraphics Centurion XV (Stat Point, Inc.). Significance was assumed at $P \leq 0.05$. Charts were plotted using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

Results

Table 3 shows the incidence of *Fusarium* species identified by the PCR technique in the eastern, central, south-western and north-western regions of Poland. All four species were detected. Species-specific PCR for *F. graminearum* amplified the expected DNA fragment in 64.29 % of stem bases samples of winter wheat. The second most frequently detected species were *F. culmorum* (15.71 %) and *F. poae* (15.71 %), followed by *F. sporotrichioides* (5.71 %). *F. graminearum* was recorded four times more often in comparison to *F. culmorum* and *F. poae*. The highest number of *F. graminearum* samples was identified in the south-western Poland (72.73 %) in comparison to other regions. The proportion of samples, from all regions, positive for the *Fusarium* species were: 45:11:11:4 for *F. graminearum*, *F. culmorum*, *F. poae* and *F. sporotrichioides*, respectively.

Table 4 shows the number of samples with none, one or multiple detections of tested *Fusarium* species. There were more fields in the eastern Poland with more than

Table 5 Pearson correlation coefficients of *Fusarium* spp. incidence in stem bases of winter wheat

Fusarium species	*F.graminearum*	*F.culmorum*	*F.sporotrichioides*	*F.poae*
F. graminearum	–	0.324*	ns	ns
F. culmorum	0.324*	–	ns	ns
F. sporotrichioides	ns	ns	–	0.26*
F. poae	ns	ns	0.26*	–

ns not statistically significant

* correlation is significant at $P<0.05$

Table 6 *Fusarium* spp. incidence in stem bases of winter wheat with different forecrops

Previous crop (number of samples)	*Fusarium* spp. incidence				Crops with >1 species detected	Crops with >2 species detected	Crops with >3 species detected
	F. graminearum	*F. culmorum*	*F. sporotrichioides*	*F. poae*			
Winter rape (n=25)	64 % (16 out of 25)	16 % (4 out of 25)	8 % (2 out of 25)	12 % (3 out of 25)	68 % (17 out of 25)	20 % (5 out of 25)	8 % (2 out of 25)
maize (n=13)	69,23 % (9 out of 13)	38,46 % (5 out of 13)	7,69 % (1 out of 13)	15,38 % (2 out of 13)	84,62 % (11 out of 13)	46,15 % (6 out of 13)	0,0 % (0 out of 13)
Winter wheat (n=18)	61,11 % (11 out of 18)	5,56 % (1 out of 18)	0,0 % (0 out of 18)	16,67 % (3 out of 18)	61,11 % (11 out of 18)	22,22 % (4 out of 18)	0,0 % (0 out of 18)

one or two detections of *Fusarium* species on the stem bases of wheat (11 and 7 out of 14, respectively) than in other regions of Poland included in the study. The number of negative samples for *Fusarium* species tested was highest in central Poland. Moreover, the number of detected *Fusarium* species was lowest in the central and northwestern Poland in comparison to the eastern and south-western regions. In all samples considered collectively, only 28.57 % of crops were free of the tested species of *Fusarium*.

The presence of one species of *Fusarium* tended to favour the presence of other (Table 5). Overall, the presence of *F. graminearum* appeared to be related to the presence of *F. culmorum* and the presence of *F. poae* seemed to be associated with *F. sporotrichioides*. The correlations found were positive. Correlation coefficient between *F. culmorum* and *F. graminearum* showed slightly stronger interaction in comparison to *F. sporotrichioides* and *F. poae*. However, both correlations indicated rather weak but noticeable associations between these *Fusarium* species.

A limited range of preceding crops (forecrops) was present in the surveyed samples, mainly winter rape, maize and winter wheat. Among the 70 wheat fields examined, 35.7 % followed winter rape, 18.6 % maize and 25.7 % winter wheat. Previous crop was the factor associated with the occurrence of *Fusarium* spp. in wheat stem bases (Table 6). The frequency of *F. graminearum* and *F. culmorum* detections were highest where wheat followed maize. The number of samples with at least one or two *Fusarium* species identified was also highest in wheat grown after maize in comparison to rape and wheat forecrops. Only about 15 % of samples that followed maize as the previous crop were free of *Fusarium* species in comparison to 32 % for winter rape and 39 % for winter wheat as forecrops.

The incidence percentage of *Fusarium* species depended also on the date of sowing (Fig. 2). The frequency of *F. graminearum* and *F. culmorum* detections was highest in samples collected from late sown fields. However, the number of samples positive for *F. poae* and *F. sporotrichioides* was higher in crops sown earlier, in September, rather than in October.

The incidence of *F. graminearum*, *F. culmorum* and *F. sporotrichioides* in stem bases of winter wheat was lower when herbicide was applied in autumn 2012 (Table 7). The number of Fusarium-free samples was over 100 % higher if herbicide was applied in comparison to unprotected crops.

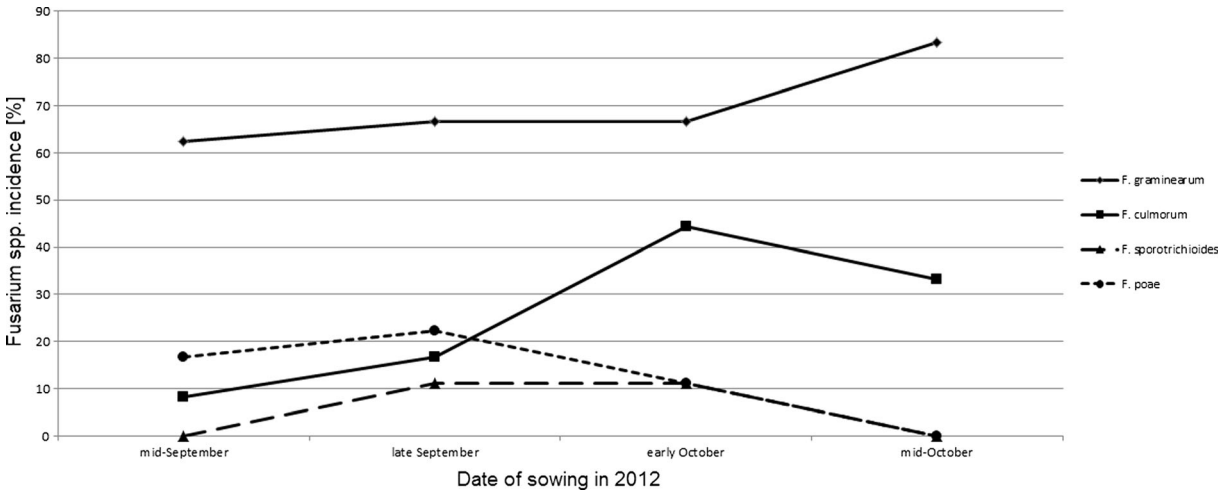

Fig. 2 The percentage of *Fusarium* spp. incidence in stem bases of winter wheat sown in different months. Early - 1st decade of the month, mid - 2nd decade, late - 3rd decade. Earlier and later dates of sowing were disregarded due to the low number of samples

The level of autumn nitrogen fertilization was also compared with the number of detected *Fusarium* species (Fig. 3). There was a slight trend detected, but not statistically significant, indicating the positive role of the level of N fertilization on the number of *Fusarium* species. About 36 % of samples originated from fields with no fertilization in autumn. Nitrogen fertilization rate below 50 kg/ha was recorded in about 39 % of the fields. More than 7 % of the samples originated from the fields with N fertilization rate above 50 kg/ha (data not shown).

Discussion

Wheat (*Triticum aestivum* L.) is one of the most extensively cultivated cereals around the world. *Fusarium* diseases of wheat are very important factors contributing to economic losses and deterioration in grain quality (McMullen et al. 2012). The PCR-based assays can be used for the routine detection and identification of pathogenic fungi from genus *Fusarium* without morphological determination (Murillo et al. 1998; Moeller et al. 1999; Mulé et al. 2004). The development and use of PCR assays could be also very helpful for early diagnosis and control of *Fusarium* population on wheat ear and stem base (Ben-Amar et al. 2012).

In this study, *F. graminearum* was the most frequently detected species occurring on wheat stem bases. The results obtained by other authors in years 1997 to 1999 and from 2000 to 2002 showed that *F. culmorum* and *F. poae* were the dominant *Fusarium* species isolated from wheat stem bases in Poland during those years (Narkiewicz-Jodko et al. 2005; Kurowski et al. 2008). The dominance of *F. culmorum* in stem bases of cereals in Poland was also observed from 2001 to 2006 and only few isolates of *F. graminearum* were obtained in 2001 from rye seedlings (Kiecana et al. 2008, 2009). The results obtained in this study suggest that the population structure of *Fusarium* spp. on wheat stem bases has changed in Poland, and that the predominant species in 2012 was *F. graminearum*. However, until now, *F. graminearum* species has been detected only occasionally and its high incidence in this study has never been previously observed in Poland on stem bases of cereals.

Table 7 *Fusarium* spp. incidence in wheat crops in relation to the application of autumn herbicide prior to sowing

Herbicide autumn application	*Fusarium graminearum* incidence [%]	*Fusarium culmorum* incidence [%]	*Fusarium sporotrichioides* incidence [%]	*Fusarium poae* incidence [%]	No *Fusarium* spp. detected [%]	Crops with 1 species incidence [%]	Crops with 2 species incidence [%]	Crops with 3 species incidence [%]
yes n=39	59	15.4	2.6	20.5	33.3	38.5	25.6	2.6
no n=25	76	20	12	8	16	56	20	4

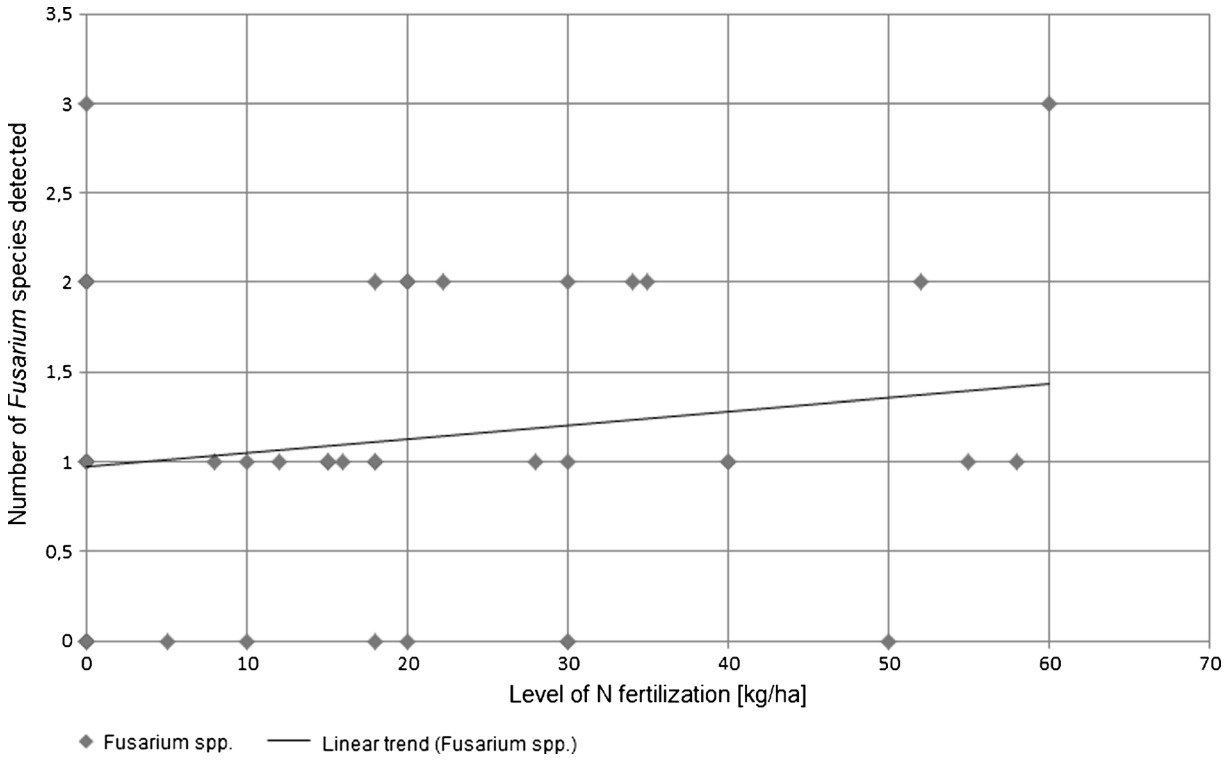

Fig. 3 Number of *Fusarium* spp. detected according to the level of autumn N fertilization with line indicating linear trend (n=61)

These results may explain the higher number of detections of *F. graminearum* observed lately on wheat kernels in Poland, Netherlands and Austria (Stępień et al. 2008; Weber and Kita 2010; Mielniczuk et al. 2012). *F. graminearum* is one of the most aggressive species in comparison to other fungi of the genus *Fusarium* and for this reason, a common prevalence of this species on stem bases of winter wheat in Poland should be taken seriously. The progressive dominance *of F. graminearum* over *F. culmorum* observed in Europe could be explained by climatic conditions during certain growing seasons, the observed changes in climate, or widespread use of feed maize in crop rotation (Scherm et al. 2013). Similarly, the presence of *F. sporotrichioides* in *Fusarium* population infecting seedlings, roots and stem bases has increased in Poland during recent years (Kiecana et al. 2008; Kiecana and Mielniczuk 2010).

In the current study, there have been also geographic differences in the *Fusarium* species occurrence, related to the region. The samples from the southwestern Poland had highest frequency of detections of all *Fusarium* spp. However, the number of samples with at least one or two detections of *Fusarium* species on the stem base of wheat was also highest in the eastern Poland. There are several reports describing the structure of *Fusarium* population in Poland, based on the regions of the country. Goliński et al. (2010) compared two locations, Cerekwica near Poznan (centralwestern Poland) and Sitaniec near Zamość (south-eastern Poland). This author observed highest rate of *Fusarium* infections and level of mycotoxin biosynthesis in the south-eastern Poland. However, no detailed studies have been published on the distribution of *Fusarium* spp. in Poland on wheat stem bases to compare with the results obtained in this study. Most of the studies focus on one location (Narkiewicz-Jodko et al. 2005; Kurowski et al. 2008; Kiecana et al. 2008, 2009).

The analysis of co-occurrence of all cases studied showed correlation of *F. graminearum* with *F. culmorum* and *F. poae* with *F. sporotrichioides*. Interaction between *F. graminearum* and *F. culmorum* can be explained by their coexistence as a complex of main species on the same plant (Nicholson et al. 1998). Interactions between species from the genus *Fusarium* has been observed previously, although most of them had a character of growth inhibition, e.g., *F. moniliforme* can suppress the growth of *F. graminearum*. Negative correlation has also been found between *F. moniliforme* and

both *F. graminearum* and *F. subglutinans* (Reid et al. 1999). In addition, *F. culmorum* was showed to suppress the growth of *M. nivale* (Simpson et al. 2004) and *F. graminearum* reduced the growth rate of *F. moniliforme* and *F. proliferatum* (Marin et al. 1998). These relationships are likely to be explained by the influence of other associated factors, i.e., forecrop and date of sowing that emerged from further analyses of samples. However, a very interesting co-incidence was also noted in these correlations of *Fusarium* species from the same *Fusarium* sections: *Discolor* (*F. graminearum* and *F. culmorum*) and *Sporotrichiella* (*F. poae* and *F. sporotrichioides*) (Watanabe et al. 2011).

Our data supports the findings that previous crop has an influence on *Fusarium* incidence (Wiese 1987). Among analyzed forecrops, maize promoted the occurrence of *Fusarium* spp. and this influence was highly significant in the case of *F. graminearum* and *F. culmorum*. This factor could positively affect co-occurrence of these species. Furthermore, *F. graminearum* is described as a major pathogen of maize stalks and ears (Waalwijk et al. 2003; Osborne and Stein 2007). The presence of maize in the structure of crop rotations in this study could explain the domination of *F. graminearum* over *F. culmorum* (Scherm et al. 2013).

Results of the current study indicate that the date of sowing of winter wheat could also influence the incidence of *Fusarium* in spring. Later date of sowing increased the incidence of *F. graminearum* and *F. culmorum*, while the number of samples positive for *F. sporotrichioides* and *F. poae* was highest in earlier sown crops. This could be the second factor responsible for the correlation between these *Fusarium* species. These findings confirmed results obtained by Subedi et al. (2007) who showed that the later the sowing date, the greater the incidence of *Fusarium* spp. Furthermore, the incidence of Fusarium-damaged kernels was also higher after later sowing (Ma et al. 2013). Since *F. graminearum* and *F. culmorum* represent a significant part of *Fusarium* spp. population present on wheat stem bases among species analyzed in this study, it should be recommended to avoid late dates of sowing.

The presence of weeds on the field might promote the occurrence of pathogenic fungi. Weeds are one of the potential sources of *Fusarium* inoculum (Altinok 2013). Our findings confirmed the negative impact of herbicide application in autumn on *Fusarium* spp. incidence in spring. However, the effect of herbicides on plant pathogens is not clear. The application of herbicides can result in a decrease of the severity of diseases but they can also trigger opposite effects (Velini et al. 2010; Lemańczyk 2012).

The level of autumn nitrogen fertilization had no effect on *Fusarium* spp. incidence. Several recent studies have shown that FHB infection and *Fusarium* mycotoxin contaminations were increased with higher rate of N application (Lemmens et al. 2004; Burgt et al. 2011), whereas other studies did not reveal any significant effects of N application on FHB (Váňová et al. 2008; Yoshida et al. 2008).

Conclusions

PCR-assay can be used for early detection of *Fusarium* spp. even if symptoms of fungi presence are not visible on plant. Among *Fusarium* species tested, *F. graminearum* was most frequently isolated from stem bases of wheat. The occurrence of *F. graminearum* and *F. culmorum* as well as *F. poae* and *F. sporotrichioides* was weakly correlated. Maize most highly promoted the incidence of *Fusarium* spp among forecrops analyzed. Later date of sowing increased the incidence of *F. graminearum* and *F. culmorum* in the spring and decreased the incidence of *F. poae* and *F. sporotrichioides*. Application of herbicides in autumn reduced the population of *Fusarium*. The rate of autumn N fertilization did not affect the number of *Fusarium* detections.

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