DISTRIBUTION OF TWO *FORMAE SPECIALES* OF *POLYMYXA GRAMINIS* IN THE CZECH REPUBLIC*

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It has been shown that two *formae speciales* of *P. graminis*, namely f. sp. *temperata* (ribotype Pg-I) and f. sp. *tepida* (ribotype Pg-II), are widely distributed throughout temperate areas of Europe. In this study, the presence of both forms of the temperate *Polymyxa* spp. was identified in soil samples from different locations of the Czech Republic during a survey performed in 2012 and 2013. Based on polymerase chain reaction results, of the total 58 tested samples, 67.2% contained at least one monitored *forma specialis*. Specifically, *P. graminis* f. sp. *temperata* was detected in 48.3% of soil samples, while *P. graminis* f. sp. *tepida* was detected in 44.8% of samples. Mixed populations were found in 25.9% of the tested areas. This plasmodiophorid was confirmed not only in crop fields but also in meadows and forests in all explored regions. Our results extend the knowledge on the distribution of both ribotypes of *P. graminis* and provide the first evidence of f. sp. *tepida* within the Czech Republic.

Plasmodiophoromycetes, temperate ribotypes, monitoring, PCR

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INTRODUCTION

The genus *Polymyxa* represents one of ten genera in the family *Plasmodiophoracea* (order *Plasmodiophorales*, phylum *Cercozoa*, and kingdom *Rhizaria*) (Simpson, Roger, 2004). Within the genus, two species have been recognized largely on the basis of host range, namely *P. graminis*, first described by Ledingham (1939) as a parasite on wheat (*Triticum aestivum* L.) roots in Canada, and *P. betae* (Keskin, 1964), described from roots of sugar beets (*Beta vulgaris* L.) in Europe. *P. graminis* infests only monocotyledonous species in the *Gramineae* (Barr, 1979; Kanyuka et al., 2003), whereas *P. betae* occupies dicotyledonous species in the *Chenopodiaceae* and the related plant families *Amaranthaceae*, *Portulacaceae*, and *Caryophyllaceae* (Barr, Asher, 1992; Legrève et al., 2002; Rush, 2003). However, there are some reports of *P. graminis* infection of dicotyledonous species (Ratna et al., 1991) and of *P. betae* infection of monocotyledonous species.

Legrève et al. (2002) proposed classifying *P. graminis* into five different *formae speciales*, including *P. graminis* f. sp. *temperata* (Pg-I), f. sp. *tepida* (Pg-II), f. sp. *tropicalis* (Pg-III_a or III_b), f. sp. *subtropicalis* (Pg-IV_a or IV_b), and f. sp. *colombiana* (Pg-V). These distinct groups (ribotypes) appear to be related in host range,

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temperature requirements, and geographical origin. Interestingly, one host plant can contain more than one ribotype of *Polymyxa* in its roots (Ward et al., 2005; Vaianopoulos et al., 2007; Smith et al., 2011). Later, Cox et al. (2014) described a separate group of isolates originating from western Africa and Australia, which they suggested should be detached to form the group Pg-VI.

P. graminis is ubiquitous and has been reported on a great number of cultivated and wild species (Ledingham, 1939; Britton, Rogers, 1963; Canova, 1964; Dale, Murdock, 1969; Inouye, Fujii, 1977; Barr, 1979; Thouvenel, Fauquet, 1980; Langenberg, 1984; Bastin et al., 1989; Skipp, Christensen, 1989; Ratna et al., 1991; Legrève et al., 2002) from different origins. *P. graminis* has been detected in many areas of the world. Nevertheless, information about the distribution of particular ribotypes of *P. graminis*, especially the temperate f. sp. *tepida* and f. sp. *temperata*, is still restricted due to lack of available data.

While the wide host range of *P. graminis* is worrying because of all the crop and wild grass species that have been confirmed as hosts (e.g. *Triticum* species, *Hordeum* species, *Secale cereale*, *Agrypyron repens*, *Bromus* species, *Sorghum* species, *Oryza sativa*, *Avena sativa*, *Zea mays*, *Pennisetum glaucum*, *Trifolium* species, *Cynodon dactylon*, *Agrostis stolonifera*, *Dactylis*, *Festuca*, *Poa*, *Phleum* species, *Cyperus rotundus*, *Eleusine coracana*, *Tridax procumbens* and *Arachis hypogaea*) and that might serve as the reservoirs for the parasite, the extent to which different isolates can infest all of them is largely unknown (Kanyuka et al., 2003). The presence of both f. sp. *tepida* and f. sp. *temperata* has been described only on barley, wheat, triticale, and rye (Legrève et al., 2002). Ward et al. (2005) confirmed that *P. graminis* f. sp. *tepida* predominantly infests wheat, whereas *P. graminis* f. sp. *temperata* is more often found on barley. Subsequently, these preferences were affirmed by Vaianopoulos et al. (2007) and Cox et al. (2014). Smith et al. (2013) revealed that *Poa* sp. and pearl millet are specific hosts of *P. graminis* f. sp. *temperata* and oat roots of *P. graminis* f. sp. *tepida*.

P. graminis is an obligate root-infecting organism. Though the parasite is non-pathogenic (or causes sporadically minor root necrosis along with other zoosporic root parasites (Wiese, 1977)), it attracted particular attention when it was shown to be involved in the transmission of economically important plant viruses in temperate areas (Maraite, 1991). All stages of the life cycle of *Polymyxa* can carry viruses *in vivo* (Rush, 2003). Viral pathogens are protected from the environment within *P. graminis* resting spores (cysts) (Driskel et al., 2004) that may remain dormant but are viable for decades (likely until a suitable host plant is encountered) (Kanyuka et al., 2003) and are released and carried upon germination of resting spores into swimming zoospores (Adams, 2002). At least fifteen different plant viruses classified into the genera *Bymovirus* (including e.g. *Barley yellow mosaic virus* (BaYMV), *Barley mild mosaic virus* (BaMMV), *Oat mosaic virus* (OMV), *Rice necrosis mosaic virus* (RNMV), *Wheat yellow mosaic virus* (WYMV), and *Wheat spindle mosaic virus* (WSSMV)), *Furovirus* (including e.g. *Soil-borne wheat mosaic virus* (SBWMV), *Soil-borne cereal mosaic virus* (SBCMV), *Chinese wheat mosaic virus* (CWMV), *Japanese soil-borne wheat mosaic virus* (JSBWMV), *Oat golden stripe virus* (OGSV), and *Sorghum chlorotic spot virus* (SCSV)), *Benyvirus* (*Rice stripe necrosis virus* (RSNV)) and *Pecluvirus* (including *Peanut clump virus* (PCV) and *Indian peanut clump virus* (IPCV) (Kanyuka et al., 2003; International Committee on Taxonomy of Viruses – http://www.ictvonline.org/virusTaxonomy.asp.), have been confirmed to be transmitted by *P. graminis*. Some of these cause serious diseases in cereal crop species and result in significant yield reductions. For example, the winter barley disease caused by BaYMV and/or BaMMV has spread in Europe, Japan, and China where it is of great concern to farmers and the agricultural industry. Yield losses of > 50% may occur when susceptible barley varieties are grown in severely infested soils (Plumb et al., 1986).

Epidemiological studies on an obligate parasite, such as *Polymyxa* spp., are comparatively difficult and time consuming. As mentioned above, there is only one brief record of *P. graminis* f. sp. *temperata* occurring in the Czech Republic (Ketta et al., 2011). In this paper, we report not only the identification of *P. graminis* f. sp. *temperata* but also *P. graminis* f. sp. *tepida* in the soil obtained from fields, meadows, and forests all over the country. Identification was carried out on the basis of PCR amplification and sequencing of *Polymyxa graminis*-specific ribosomal DNA from soil samples.

MATERIAL AND METHODS

Biological material

To monitor *P. graminis*, 58 different soil samples were collected separately from 43 cereal fields (wheat and barley), 13 meadows, and 2 forests located in 8 regions across the Czech Republic between the years 2012 and 2013 (Table 1, Fig. 1). Soil was harvested using a digging fork from approximately 10 cm depth and placed in large sealed polyethylene bags (the weight of each sample was approximately 3 kg). Each sample was composed of 20–30 subsamples taken from different geolocated parts of its area. Neither plants nor weeds were present among the soil samples. After sampling, soils were air-dried properly at room

Table 1. List of localities where the occurrence of both *formae speciales* of *Polymyxa graminis* in the Czech Republic was tested

No.	Region	Locality	Type of stand/crop	*P. graminis*		No.	Region	Locality	Type of stand/crop	*P. graminis*	
				tempe-rata	*tepida*					*tempe-rata*	*tepida*
1	Středočeský	Hořešovice	field/wheat	+	–	30	Ústecký	Březno 2	field/wheat	+	–
2		Polepy 1	field/barley	–	–	31		Březno 3	field/wheat	+	–
3		Polepy 2	field/barley	–	+	32	Královéhradecký	Březno 4	field/wheat	–	–
4		Libice 1*	field/wheat	–	+	33		Sobotka	field/oilseed rape	+	+
5		Libice 2	field/wheat	–	–	34		Holín	field/oilseed rape	–	–
6		Libice 3	field/wheat	–	–	35		Hradec Králové	field/oilseed rape	–	–
7		Byšice	field/oilseed rape	+	+	36		Praskačka	field/wheat	–	+
8		Mladá Boleslav	field/wheat	–	+	37		Výrava 1	meadow	+	–
9		Kolín	field/corn	–	–	38		Výrava 2	meadow	+	+
10		Starý Kolín	field/oilseed rape	–	+	39		Milovice u Hořic 1	meadow	+	–
11		Miličín	field/wheat	+	+	40		Milovice u Hořic 2	meadow	+	+
12		Osečany	field/wheat	–	–	41		Dobrá Voda u Hořic	meadow	–	–
13		Plaňany	field/wheat	–	–	42		Šárovcova Lhota 1	meadow	+	+
14		Čáslav	field/oilseed rape	+	–	43		Šárovcova Lhota 2	forest	–	+
15		Konopiště*	field/wheat	+	+	44		Holovousy	forest	+	+
16		Zahradnice	field/wheat	–	–	45		Stračov*	meadow	+	+
17		Zdice, Hředle	field/barley	–	–	46		Třebnoušovec	meadow	+	+
18		Slaný, Lotouš	field/wheat	–	–	47		Záměl	meadow	+	+
19		Hostouň	field/oilseed rape	–	–	48	Vysočina	U letiště*	field/oilseed rape	+	+
20		Sukorady	meadow	+	–	49		Havlíčkův Brod*	field/wheat	–	+
21	Praha	CULS	field/oilseed rape	–	–	50		Kámen*	field/barley	–	+
22	Ústecký	Chlumčany*	field/oilseed rape	+	–	51		Olšínky*	field/wheat	+	+
23		Dřemčice*	field/wheat	+	+	52		Věž	field/oilseed rape	+	–
24		Kochovice 1	field/wheat	–	+	53	Plzeňský	Svojkovice 1	field/wheat	+	–
25		Kochovice 2	field/wheat	–	+	54		Svojkovice 2	field/wheat	–	–
26		Počeplice	field/barley	+	–	55		Černovice	field/oilseed rape	–	+
27		Cítoliby	field/wheat	–	–	56	Pardubický	Moravská Třebová	meadow	–	–
28		Staňkovice	field/oilseed rape	–	–	57		Svitavy*	meadow	+	+
29		Březno 1	field/wheat	+	–	58	Olomoucký	Pavlov	meadow	+	–

CULS = Czech University of Life Sciences Prague, (+) presence or (–) absence of *P. graminis* in soil samples confirmed by PCR analysis

*isolates of *Polymyxa graminis* used for subsequent sequencing

temperature, crushed, sieved (2 mm) and thoroughly homogenized.

Nucleic acid extraction from soil

Total DNA was extracted from soil samples using the GeneMATRIX SOIL DNA Purification Kit (EURx, Gdańsk, Poland) according to the manufacturer's instructions. Approximately 0.25 g soil was placed in the Bead tube, 60 µl of Lyse SL solution was added, and the tube was vortexed horizontally at maximum speed for 10 min. The tube was centrifuged (2 min; 14 000 rpm), and 400 µl of the supernatant was transferred to a new tube with 400 µl PR buffer. After a brief vortexing and incubation on ice for 5 min, the tube was centrifuged (1 min; 14 000 rpm), and 600 µl of supernatant was transferred to a new tube along with 600 µl Sol SL solution and 200 µl of 96% ethanol. After vortexing, 600 µl of solution was transferred to a spin-column (which was prepared by adding 40 µl of activation buffer SL) and centrifuged (12 000 rpm; 30 s). The flow-through was discarded, the spin-column tube was replaced, and the step was repeated. 500 µl of Wash SLX1 buffer was added to the spin-column and centrifuged (1 min; 12 000 rpm). Then, 500 µl of Wash SLX2 buffer was added and centrifuged (2 min; 12 000 rpm). The spin-column was then placed in a new, clean tube, and 50 µl of Elution buffer heated to 70°C was added. The tube was incubated for 2 min at room temperature, and total DNA was obtained by centrifugation (30 s; 12 000 rpm). The quality and quantity of extracted total DNA was evaluated using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

PCR protocols for detection and distinction of the two forms of *P. graminis*

To determine whether *P. graminis* was present in soil, PCR was performed using four different sets of primers: *Polymyxa*-specific, *P. graminis*-specific, *P. graminis* f. sp. *temperata*-specific, and f. sp. *tepida*-specific; namely Psp1/Psp2rev (L e g r è v e et al., 2003) which amplifies a 404-bp fragment to 509-bp fragments of the *Polymyxa* spp. partial 18S rDNA, ITS1, and partial 5.8S rDNA; Pgfwd2/Pxrev7 (W a r d , A d a m s , 1998), which amplifies a 280-bp fragment of the *P. graminis* partial ITS1 and partial 5.8S rDNA; Pg.F1/Pg.R1 (W a r d et al., 2005), which amplifies a 292-bp fragment of *P. graminis* f. sp. *temperata* partial ITS1, 5.8S rDNA and partial ITS2; and Pg.F2/Pg.R2 (W a r d et al., 2005), which amplifies a 430-

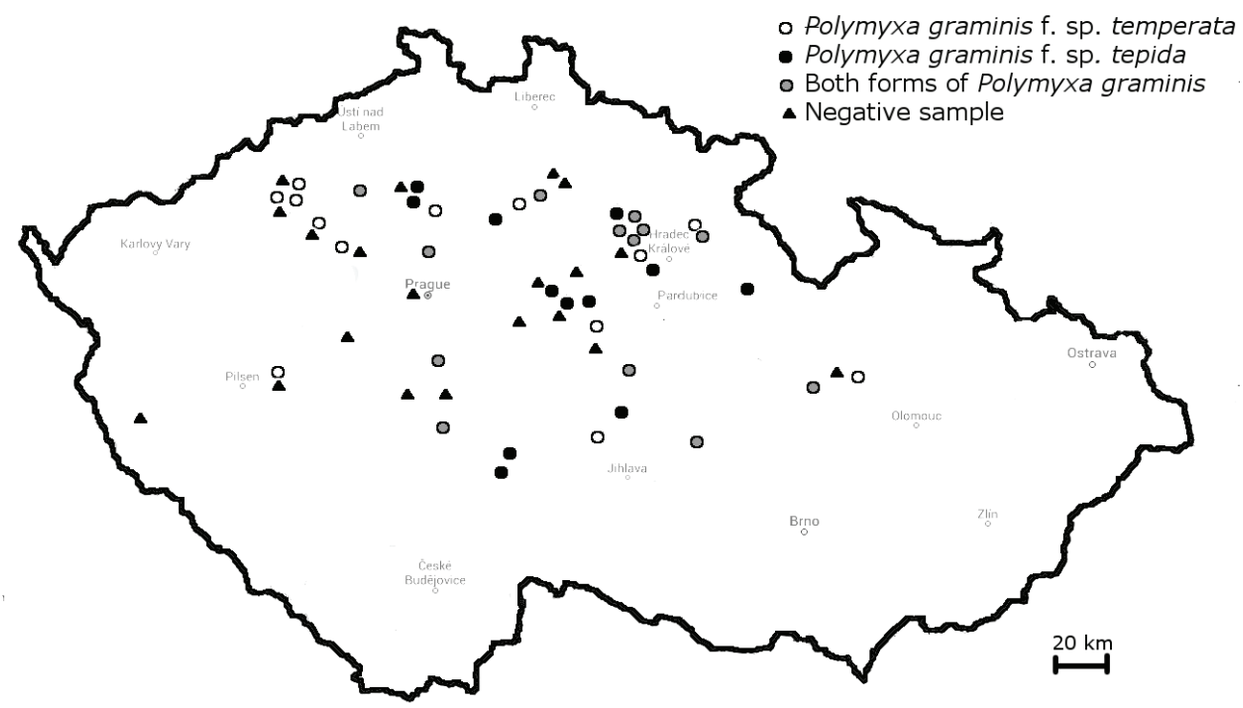

Fig. 1. Geographic localities throughout the Czech Republic where the occurrence of *Polymyxa graminis* f. sp. *temperata* and f. sp. *tepida* was surveyed

bp fragment of *P. graminis* f. sp. *tepida* partial ITS1, 5.8S rDNA, ITS2 and partial 28S rDNA.

Polymerase chain reaction (PCR) was performed using approximately 50 ng of total DNA in a 25-µl mixture containing 1× Dream*Taq*^{TM} Buffer, 0.1 mM of each deoxynucleoside triphosphate, 0.2 µM of each primer, and 1 U of Dream*Taq*^{TM} DNA Polymerase (Thermo Fisher Scientific). Cycling parameters were as follows: 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C (for primers Psp1/Psp2rev), 56°C (for primers Pgfwd2/Pxrev7), 66°C (for primers Pg.F1/Pg.R1) or 64°C (for primers Pg.F2/Pg.R2) for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 7 min. PCR-amplified fragments from 10 µl of the reaction mixture were visualized after electrophoresis in ethidium bromide stained 1.5% agarose gels.

Restriction fragment length polymorphism (RFLP) analysis for distinction of *P. graminis* forms

On the basis of sequences specific to two forms of *P. graminis*, the restriction enzymes *NruI* and *SalI* were selected to specifically cut rDNA of *P. graminis* f. sp. *temperata* in the ITS1 region, or rDNA of *P. graminis* f. sp. *tepida* in nuclear small rDNA 18S and were used to digest Psp1/Psp2rev PCR products (L e g r è v e et al., 2003). Reactions were performed using 10 µl of PCR product in 10.5 µl of double distilled water, 2.5 µl 2x Y+/Tango buffer (Thermo Fisher Scientific) and 2 µl restriction enzyme (Thermo Fisher Scientific). The mixture was incubated overnight at 37°C, and the next day enzymes were inactivated by heating to 65°C for 15 min. After this, 10 µl of product was separated and visualized by horizontal electrophoresis in ethidium bromide stained 1% agarose gels. The restriction enzyme *NruI* cut PCR products of *P. graminis* f. sp. *temperata* to 295-bp and 177-bp fragments, whereas *SalI* cut PCR products of *P. graminis* f. sp. *tepida* to 348-bp and 152-bp fragments (V a i a n o p o u l o s et al., 2007) (Fig. 2).

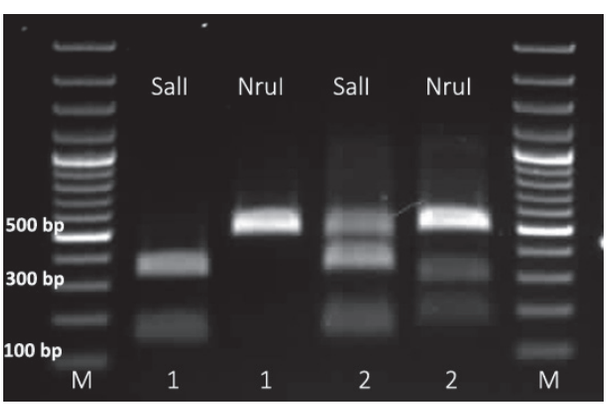

Fig. 2. Representative results for the detection of *Polymyxa graminis* by PCR-RFLP in several soil samples using Polymyxa-specific primers (Psp1/Psp2rev) and restriction enzymes (NruI and SalI), cutting PCR products of *P. graminis* f. sp. *temperata* (295-bp/177-bp), and *P. graminis* f. sp. *tepida* (348-bp/152-bp), respectively
lane M = MassRuler Low Range DNA Ladder (Thermo Fisher Scientific, Waltham, USA), 1 = PCR products from soil infected by *Polymyxa graminis* f. sp. *tepida*; 2 = PCR products from soil infected by *Polymyxa graminis* f. sp. *temperata* and *tepid*a

Sequence analysis for determination of *P. graminis* forms

PCR amplification was performed with Platinum® *Taq* DNA Polymerase High Fidelity (Invitrogen, Carlsbad, USA) using the primers Psp1/Psp2rev (L e g r è v e et al., 2003) or Pgfwd2/Pxrev7 (W a r d , A d a m s , 1998). The amplified PCR products were gel-purified using the MiniElute Gel Extraction Kit (Qiagen, Hilden, Germany), cloned into the pSC-A vector (Agilent Technologies, Santa Clara, USA) and transformed into competent cells (Agilent Technologies). DNA sequencing was performed by Macrogen, the Netherlands. Obtained nucleotide sequences were compared using BLAST (A l t s c h u l et al., 1997) and analyzed using the program BioEdit 7.0.9 (Ibis Biosciences, USA).

RESULTS

Evaluation of several PCR primers for the detection of *Polymyxa graminis*

In an attempt to reliably and specifically detect and subsequently distinguish particular forms of *P. graminis* from each other, four sets of *Polymyxa*-specific primers, namely Psp1/Psp2rev (L e g r è v e et al., 2003), Pgfwd2/Pxrev7 (W a r d , A d a m s , 1998), Pg.F1/PgR1 and Pg.F2/PgR2 (W a r d et al., 2005) were tested within our study. During the first monitoring trial in 2012, the presence of *P. graminis* f. sp. *tepida* was in several cases accidentally mistaken for the presence of *P. betae*. This confusion resulted from the unsuitable use of Psp1/Psp2rev primers together with RFLP analysis and was not revealed until sequencing of PCR fragments. It is well known that the primers Psp1/Psp2rev were designed to detect both *Polymyxa* species in any tested biological material. However, it was not expected that *P. betae* would be so abundant in cereal fields and that after digestion of its PCR products using *SalI* for RFLP analysis, two fragments of very similar sizes to the ones of *P. graminis* f. sp. *tepida* (approximately 150-bp and 350-bp) would be visible as well.

The utility of the other three sets of primers was determined to be very good (Fig. 3). Moreover, when the primers Pg.F1/PgR1 and Pg.F2/PgR2 were used during PCR amplification, no sequence analysis was

necessary to additionally determine the two *formae speciales*.

Monitoring of *Polymyxa graminis*

Altogether, 58 different localities situated in 8 regions of the Czech Republic were inspected for the presence of *P. graminis*. 58 soil samples were individually tested using PCR with the primers Pgfwd2/Pxrev7 (Ward, Adams, 1998), Pg.F1/PgR1 and Pg.F2/PgR2 (Ward et al., 2005) to detect and determine two forms of the studied plasmodiophorid. The results are shown in Table 1 and Fig. 1.

With regard to cereal field monitoring, 60.5% of the 43 total soil samples contained at least one monitored *forma specialis*. *P. graminis* f. sp. *tepida* was detected in 17 samples (39.5%), followed by *P. graminis* f. sp. *temperata* in 16 samples (37.2%). Mixed populations were found in 16.3% of tested soils.

For meadows and forests, of the 15 total areas, 13 soil samples (86.7%) contained at least one monitored *forma specialis*. *P. graminis* f. sp. *temperata* was confirmed in 80% of the tested areas and *P. graminis* f. sp. *tepida* in 60%. Mixed populations were found in 53.3% of tested soil samples.

The distribution of both forms appeared to be almost equal in all tested areas.

To confirm the presence of *P. graminis*, partial nucleotide sequences of 18S rDNA, ITS1 and 5.8S rDNA were determined for nine *P. graminis* f. sp. *temperata* isolates and one *P. graminis* f. sp. *tepida* isolate collected throughout the Czech Republic (Fig. 1). The ten high-quality partial sequences of *P. graminis* f. sp. *temperata* and f. sp. *tepida* were deposited in GenBank under the accession numbers LT221857–LT221866.

DISCUSSION

Because *Polymyxa* sp. is an obligate biotroph and can only develop in the roots of host plants, the majority of methods used to detect this parasite in a given area have historically been based solely on the host plants naturally growing in the tested soil or on bait plants growing on tested soils. Bait plant techniques are expensive and time-consuming, taking more than 3–4 weeks to produce zoospores and approximately 2–3 months to produce resting spores (Kanyuka et al., 2003). Traditionally, subsequent detection and estimation of *Polymyxa* spp. in roots of infected natural host/bait plants has relied on light microscopy. However, new methods have been developed to facilitate rapid detection of *Polymyxa* spp., including immunological (Delfosse et al., 2000; Mutasa-Gottgens et al., 2000) and molecular techniques (Ward, Adams, 1998; Legrève et al., 2002; Legrève et al., 2003; Vaianopoulos et al., 2007 a.o.), but until recently they had not been applied to detection in soil.

Several methods have been described for extracting microbial DNA from soil, all varying in their

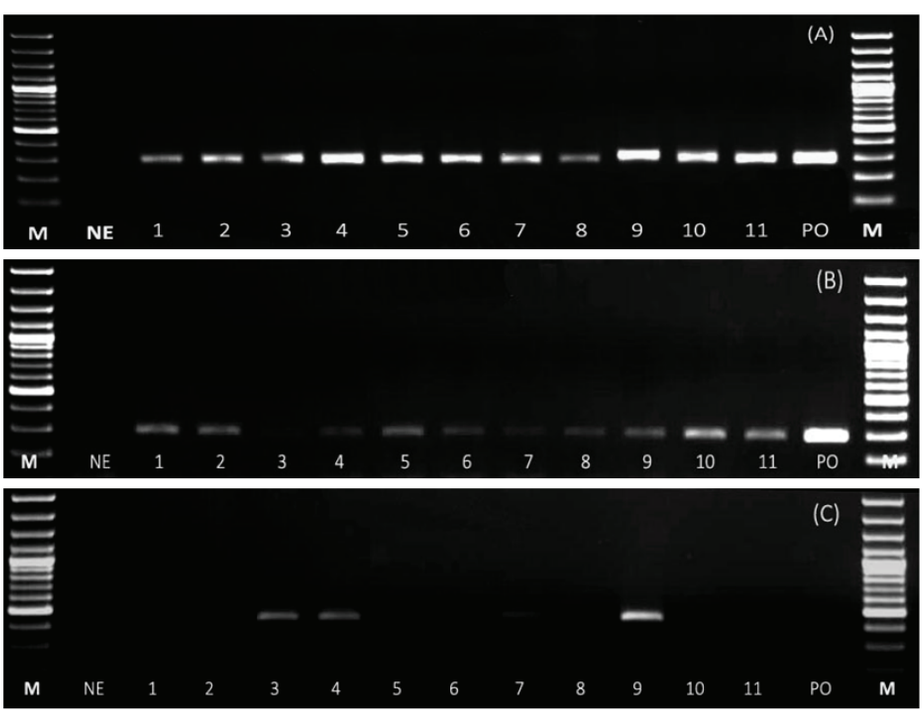

Fig. 3. Representative results for the detection of *Polymyxa* spp. by PCR in several soil samples using three different sets of primers: *P. graminis*-specific, *P. graminis* f. sp. t*emperata*-specific, and f. sp. *tepida*-specific (A) Pgfwd2/Pxrev7 primers amplifying a 280-bp fragment of *P. graminis*, (B) Pg.F1/Pg.R1 primers amplifying a 292-bp fragment of *P. graminis* f. sp. *temperata*, and (C) Pg.F2/Pg.R2 primers amplifying a 430-bp fragment of *P. graminis* f. sp. *tepida* lane M = MassRuler Low Range DNA Ladder (Thermo Fisher Scientific, Waltham, USA), lane NE = PCR product of healthy control, lanes 1–11 = PCR products of various healthy/infected samples, lane PO = PCR product of positive *Polymyxa graminis* infected control

complexity. Because microorganisms are distributed unevenly in soil and can be easily attached to soil particles or aggregated with organic matter, the mode of sampling for successful pathogen detection is essential (Gherbawy, Voigt, 2010). Moreover, diverse particles, e.g., humid acid, polysaccharides, and metal ions, may be contained within soil that may complicate PCR reactions (Robe et al., 2003).

Proper primer selection was a crucial step in *P. graminis* detection and subsequent determination. At the start of our monitoring, the presence of *P. graminis* f. sp. *tepida* was mistakenly inferred due to the presence of *P. betae* when Psp1/Psp2rev primers (Legrève et al., 2003) were used in PCR analyses, and this confusion was not revealed until sequencing of the PCR fragments. Because the former Czechoslovakia was one of the biggest producers of sugar beets in Europe and the world in the last century, *P. betae* infestation of the fields was and very likely is still relevant because resting spores (cysts) of *Polymyxa* may remain dormant but viable even for decades. For that reason, the use of these 'universal' primers in PCR together with subsequent RFLP analysis as a diagnostic technique is not highly recommended in our region, even within fields and meadows where the sugar beet has not been grown for many years, as *P. betae* can also be perpetuated by many chenopodiaceous weeds.

On the other hand, the use of the other three primers pairs, Pgfwd2/Pxrev7 (Ward, Adams, 1998), Pg.F1/PgR1 and Pg.F2/PgR2 (Ward et al., 2005) was found to be very convenient. However, their narrow specificity can also lead to lower sensitivity in *P. graminis* detection, which Ward, Adams (1998) confirmed in the case of Indian *P. graminis* isolates. Nevertheless, all of the above-mentioned primers were able to detect both forms of *P. graminis* occurring in temperate areas of the Czech Republic.

The first report of *Polymyxa graminis* in the Czech Republic was described by Ketta et al. (2011, 2012). In this study, the presence of both forms of temperate *P. graminis* (f. sp. *tepida* and f. sp. *temperata*), occurring either separately or together in mixed populations in the tested areas, was confirmed. These findings agree with results published by Ziegler et al. (2015) in Germany and Poland, by Vaianopoulos et al. (2007) in France and Belgium, by Ward et al. (2005) in the United Kingdom, and recently by Cox et al. (2014) for Southwest Australia. Interestingly, according to our findings, both forms of *P. graminis* were detected not only in crop fields but also in soils obtained from natural meadows and forests at even higher percentage. Its presence in the former biotope may easily be explained by the close rotation of highly susceptible cereals such as wheat and barley; nevertheless, its prevalence in forest soil was surprising. However, considering the wide range of hosts for *Polymyxa graminis*, including not only cultivated crops, such as wheat, barley, rice, oat, rye, *Zea* and *Sorghum* species (Thouvenel, Fauquet, 1980; Langenberg, 1984), but also various wild grass species (Britton, Rogers, 1963; Canova, 1964; Dale, Murdock, 1969), which form larger proportions of meadows and forest undergrowth, their frequent occurrence in uncultivated areas is understandable. High densities of plants and the permanent presence of suitable hosts make these sites an even better environment for *P. graminis* than fields. Higher biodiversity in these ecosystems can also explain both higher percentages of mixed populations and the predominance of *P. graminis* f. sp. *temperata* compared to fields, as the genus *Poa* seems to be the preferred host of this form (Smith et al., 2013).

CONCLUSION

Although *Polymyxa graminis* is non-pathogenic, it has attracted attention due to its involvement in virus transmission in economically important cereal crops in temperate areas. The occurrence of two forms of *P. graminis*, namely *P. graminis* f. sp. *temperata* and *P. graminis* f. sp. *tepida*, in diverse biotopes across the country was confirmed in the Czech Republic. While their distributions were approximately equal in crop fields, higher occurrences of both *P. graminis* f. sp. *temperata* and mixed populations were found in non-cultivated ecosystems such as meadows and forests, suggesting their natural reservoirs.

REFERENCES

Adams MJ (2002): The mosaic viruses of barley: problems and prospects. In: Proc. of the BCPC Conference: Pests and Diseases, Brighton, UK, 105–112.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997): Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25, 3389–3402. doi: 10.1093/nar/25.17.3389.

Barr DJS (1979): Morphology and host range of *Polymyxa graminis*, *Polymyxa betae* and *Ligniera pilorum* from Ontario and some other areas. Canadian Journal of Plant Pathology, 1, 85–94. doi: 10.1080/07060667909501468.

Barr KJ, Asher MJC (1992): The host range of *Polymyxa graminis* (Plasmodiophoromycetes). Canadian Journal of Botany, 60, 2496–2504.

Bastin V, Boute C, Maraite M (1989): Inoculum potential and host range of *Polymyxa graminis*. EPPO Bulletin, 19, 541–546. doi: 10.1111/j.1365-2338.1989.tb00430.x.

Britton MP, Rogers DP (1963): *Olpidium brassicae* and *Polymyxa graminis* in roots of creeping bent in golf putting greens. Mycologia, 55, 758–763. doi: 10.2307/3756478.

Canova A (1964): Research on *Graminaceae* virus diseases I. Mosaic of wheat transmissible through the soil. Phytopathologica Mediterranea, 3, 86–94. (in Italian)

Cox BA, Luo H, Jones RAC (2014): *Polymyxa graminis* isolates from Australia: identification in wheat roots and soil, molecular characterization, and wide genetic diversity. Plant Diseases, 98, 1567–1575. doi: *10.1094/PDIS-02-14-0128-RE*.

Dale JL, Murdock CL (1969): *Polymyxa* infection of Bermuda grass. Plant Disease Report, 53, 130–131. doi: 10.1046/j.1364-3703.2003.00177.x.

Delfosse P, Reddy AS, Legrève A, Thirumala Devi K, Abdurrahman MD, Maraite H, Reddy VR (2000): Serological methods for detection of *Polymyxa graminis*, an obligate root parasite and vector of plant viruses. Virology, 90, 5, 537–545. doi: 10.1094/PHYTO.2000.90.5.537.

Driskel BA, Dross P, Littlefield LJ, Walker NR, Verchot-Lubicz J (2004): *Soilborne wheat mosaic virus* movement protein and RNA and *Wheal spindle streak mosaic virus* coat protein accumulate inside resting spores of their vector *Polymyxa graminis*. Molecular Plant Microbe Interaction, 17, 739–748. doi: 10.1094/MPMI.2004.17.7.739.

Gherbawy Y, Voigt K (2010): Molecular identification of fungi. Springer-Verlag, Berlin Heidelberg.

Inouyee T, Fujii S (1977): *Rice necrosis mosaic virus*. CMI/AAB Descriptions of Plant Viruses, 172, 4.

Kanuyuka K, Ward E, Adams MJ (2003): *Polymyxa graminis* and the cereal viruses it transmits: a research challenge. Molecular Plant Pathology, 4, 393–406. doi: 10.1046/j.1364-3703.2003.00177.x.

Keskin B (1964): *Polymyxa betae* n. sp., a parasite in the roots of *Beta vulgaris* Tournefort, especially during the youth development of sugar beet. Archieves für Mikrobiologie, 49, 348–374. (in German)

Ketta H, Zouhar M, Rysanek P (2011): First report of *Polymyxa graminis* f. sp. *temperata*, a vector of soilborne cereal viruses in the Czech Republic. Plant Diseases, 95, 353. doi: 10.1094/PDIS-05-10-0388.

Ketta H, Rysanek P, Zouhar M (2012): Detection of *Polymyxa graminis* in a barley crop in the Czech Republic. Plant Protection Science, 48, 65–71.

Langenberg WG (1984): No resistance found to *Polymyxa graminis*, vector of *Soil-borne wheat mosaic virus*, in sorghum, corn and wheat cultivars. Annual of Wheat Newsletter, 30, 145–146.

Ledingham GA (1939): Studies on *Polymyxa graminis* n. gen. n. sp., a plasmodiophoraceous root parasite of wheat. Canadian Journal of Research, Section C: Botanical Sciences, 17, 38–51. doi: 10.1139/cjr39c-005.

Legrève A, Delfosse P, Maraite H (2002): Phylogenetic analysis of *Polymyxa* species based on nuclear 5.8S and internal transcribed spacers ribosomal DNA sequences. Mycological Research, 106, 138–147. doi: 10.1017/S0953756201005391.

Legrève A, Delfosse P, van Hese V, Bragard C, Maraite H (2003): Broadspectrum detection of *Polymyxa* species and form species by polymerase chain reaction. In: Proc. 5th Symposium of the International Working Group on Plant Viruses with Fungal Vectors, Zürich, Switzerland, 40–43.

Maraite H (1991): Transmission of viruses by soil fungi. In: Beemster ABR, Bollen GJ, Gerlagh M, Ruissen MA, Schippers B, Tempel A (eds): Developments in agricultural and managed-forest ecology: biotic interactions and soil-borne diseases. In: Proc. 1st Conference of the European Foundation for Plant Pathology, Wageningen, the Netherlands, 67–82.

Mutasa-Gottgens ES, Chwarszczynska DM, Halsey K, Asher JC (2000): Specific polyclonal antibodies for the obligate plant parasite *Polymyxa* – a targeted recombinant DNA approach. Plant Pathology, 49, 276–287. doi: 10.5424/sjar/2014123-4385.

Plumb RT, Lennon EA, Gutteridge RA (1986): The effects of infection by *Barley yellow mosaic virus* on the yield and components of yield of barley. Plant Pathology, 35, 314–318. doi: 10.1111/j.1365-3059.1986.tb02020.x.

Ratna AS, Rao AS, Reddy AS, Nolt BL, Reddy DVR, Vijayalakshmi M, McDonald D (1991): Studies on transmission of *Indian peanut clump virus* – disease by *Polymyxa graminis*. Annals of Applied Biology, 118, 71–78. doi: 10.1111/j.1744-7348.1991.tb06086.x.

Robe P, Nalin R, Capellano C, Vogel TM, Simonet P (2003): Extraction of DNA from soil. European Journal of Soil Biology, 39, 183–190. doi: 10.1016/S1164-5563(03)00033-5.

Rush CM (2003): Ecology and epidemiology of Benyviruses and plasmodiophorid vectors. Annual Review of Phytopathology, 41, 567–592. doi: 10.1146/annurev.phyto.41.052002.095705.

Simpson AGB, Roger AJ (2004): The real 'kingdoms' of eukaryotes. Current Biology, 14, 693–696.

Skipp RA, Christensen MJ (1989): Host specificity of root-invading fungi from New Zealand pasture soils. Australasian Plant Pathology, 18, 101–103. doi: 10.1071/APP9890101.

Smith MJ, Adams MJ, Ward E (2011): Evidence that *Polymyxa* species may infect *Arabidopsis thaliana*. FEMS Microbiology Letter, 318, 35–40. doi: 10.1111/j.1574-6968.2011.02236.x.

Smith MJ, Adams MJ, Ward E (2013): Ribosomal DNA analyses reveal greater sequence variation in *Polymyxa* species than previously thought and indicate the possibility of new ribotype–host–virus associations. Environmental Microbiology Reports, 5, 143–150. doi: 10.1111/1758-2229.12026.

Thouvenel JC, Fauquet C (1980): *Polymyxa graminis* on new Sorghum species in Africa. Plant Disease, 64, 957–958.

Vaianopoulos C, Bragard C, Moreau V, Maraite H, Legrève A (2007): Identification and quantification of *Polymyxa graminis* f. sp. *temperata* and *P. graminis* f. sp. *tepida* on barley and wheat. Plant Diseases, 91, 857–864. doi: 10.1094/PDIS-91-7-0857.

Ward E, Adams MJ (1998): Analysis of ribosomal DNA sequences of *Polymyxa* species and related fungi and the development of genus and species-specific PCR primers. Mycology Research, 102, 965–974. doi: 10.1017/S0953756297005881.

Ward E, Kanyuka K, Motteram J, Kornyukhin D, Adams MJ (2005): The use of conventional and quantitative real-time PCR assays for *Polymyxa graminis* to examine host plant resistance, inoculum levels and intraspecific varia-

tion. New Phytologist, 165, 875–885. doi: 10.1111/j.1469-8137.2004.01291.x.

Wiese MV (1977): Primitive root fungi. In: Compendium of wheat diseases. The American Phytopathological Society, St. Paul, USA, 54–55.

Ziegler A, Fomitcheva V, Zakri AM, Kastirr U (2015): Occurrence of *Polymyxa graminis* ribotypes in Germany and their association with different host plants and viruses. Cereal Research Communication, 44, 251–262. doi: 10.1556/0806.43.2015.051.

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