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

INTRODUCTION

The genus *Polymyxa* represents one of ten genera in the family *Plasmodiophoraceae* (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*) roots in Canada, and *P. betae* (Kesklin, 1964), described from roots of sugar beets (*Beta vulgaris*) in Europe. *P. graminis* infests only monocotyledonous species in the *Gramineae* (Barr, 1979; Kanuuka et al., 2003), whereas *P. betae* occupies dicotyledonous species in the *Chenopodiaceae* and the related plant families *Amaranthaceae*, *Portulacaceae*, and *Caryophyllaceae* (Barr, Asher, 1992; Legrèves 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.

Legrèves et al. (2002) proposed classifying *P. graminis* into five different formae specialises, including *P. graminis* f. sp. *temperata* (Pg-I), f. sp. *tepida* (Pg-II), f. sp. *tropicalis* (Pg-III₀ or III₁), f. sp. *subtropicalis* (Pg-IVᵢ or IVᵢ), 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; Vainopoulos 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, Faquett, 1980; Langenberg, 1984; Bastin et al., 1989; Skipp, Christensen, 1989; Ratna et al., 1991; Legrèves 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, Agropyron 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 (Legrèves et al., 2002). Ward et al. (2005) confirmed that P. graminis f. sp. tepida predominantly infects wheat, whereas P. graminis f. sp. temperata is more often found on barley. Subsequently, these preferences were affirmed by Vainopoulos 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 (Maraité, 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 testing 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 (Plumberg 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 (Kettat 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

<table>
<thead>
<tr>
<th>No.</th>
<th>Region</th>
<th>Locality</th>
<th>Type of stand/crop</th>
<th>P. graminis</th>
<th>No.</th>
<th>Region</th>
<th>Locality</th>
<th>Type of stand/crop</th>
<th>P. graminis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Středočeský</td>
<td>Čáslav</td>
<td>field/oilseed rape</td>
<td>+</td>
<td>30</td>
<td>Ústecký</td>
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<td>field/wheat</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Hořešovice</td>
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<td>–</td>
<td>–</td>
<td>31</td>
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<td>field/wheat</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>Polepy 1</td>
<td>field/barley</td>
<td>–</td>
<td>–</td>
<td>32</td>
<td>Březno 4</td>
<td>field/wheat</td>
<td>–</td>
<td>–</td>
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<tr>
<td>4</td>
<td>Libice 1*</td>
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<td>–</td>
<td>+</td>
<td>33</td>
<td>Sobotka</td>
<td>field/oilseed rape</td>
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<td>+</td>
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<tr>
<td>5</td>
<td>Libice 2</td>
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<td>–</td>
<td>–</td>
<td>34</td>
<td>Holín</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Libice 3</td>
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<td>–</td>
<td>–</td>
<td>35</td>
<td>Hradec Králové</td>
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<td>–</td>
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<td>Byšice</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>9</td>
<td>Kolin</td>
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<td>–</td>
<td>38</td>
<td>Výrávová</td>
<td>meadow</td>
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</tr>
<tr>
<td>10</td>
<td>Starý Kolín</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>+</td>
<td>39</td>
<td>Milovice u Hořic 1</td>
<td>meadow</td>
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<td>–</td>
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<tr>
<td>11</td>
<td>Milosín</td>
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<td>–</td>
<td>–</td>
<td>40</td>
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<td>–</td>
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<tr>
<td>12</td>
<td>Osečany</td>
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<td>–</td>
<td>41</td>
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<td>–</td>
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<tr>
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<td>Plaňany</td>
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<td>–</td>
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<td>Šárovcová Lhota 1</td>
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<td>–</td>
</tr>
<tr>
<td>14</td>
<td>Čáslav</td>
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<td>+</td>
<td>–</td>
<td>43</td>
<td>Šárovcová Lhota 2</td>
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<td>–</td>
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<tr>
<td>15</td>
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<td>+</td>
<td>44</td>
<td>Holousový</td>
<td>forest</td>
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<td>–</td>
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<tr>
<td>16</td>
<td>Zahradnice</td>
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<td>–</td>
<td>–</td>
<td>45</td>
<td>Stračový</td>
<td>meadow</td>
<td>+</td>
<td>–</td>
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<tr>
<td>17</td>
<td>Zdice, Hředle</td>
<td>field/barley</td>
<td>–</td>
<td>–</td>
<td>46</td>
<td>Třebnoušovec</td>
<td>meadow</td>
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<td>–</td>
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<tr>
<td>18</td>
<td>Slaný, Lotouš</td>
<td>field/wheat</td>
<td>–</td>
<td>–</td>
<td>47</td>
<td>Záměl</td>
<td>meadow</td>
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<td>19</td>
<td>Hostouň</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>–</td>
<td>48</td>
<td>U letiště*</td>
<td>field/oilseed rape</td>
<td>+</td>
<td>–</td>
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<tr>
<td>20</td>
<td>Sukorady</td>
<td>meadow</td>
<td>+</td>
<td>–</td>
<td>49</td>
<td>Havlíčkův Brod*</td>
<td>field/wheat</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td>Praha CULS</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>–</td>
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<td>Vysočina</td>
<td>field/wheat</td>
<td>+</td>
<td>–</td>
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<tr>
<td>22</td>
<td>Chlumčany*</td>
<td>field/oilseed rape</td>
<td>+</td>
<td>–</td>
<td>51</td>
<td>Olšínky*</td>
<td>field/wheat</td>
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<td>–</td>
</tr>
<tr>
<td>23</td>
<td>Dřemčice*</td>
<td>field/wheat</td>
<td>+</td>
<td>+</td>
<td>52</td>
<td>Věž</td>
<td>field/oilseed rape</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>24</td>
<td>Kochovice 1</td>
<td>field/wheat</td>
<td>–</td>
<td>+</td>
<td>53</td>
<td>Plzeňský</td>
<td>field/wheat</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>25</td>
<td>Kochovice 2</td>
<td>field/wheat</td>
<td>–</td>
<td>–</td>
<td>54</td>
<td>Svojkovice 1</td>
<td>field/wheat</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>26</td>
<td>Počepice</td>
<td>field/barley</td>
<td>–</td>
<td>+</td>
<td>55</td>
<td>Svojkovice 2</td>
<td>field/wheat</td>
<td>–</td>
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<tr>
<td>27</td>
<td>Citoliby</td>
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<td>–</td>
<td>56</td>
<td>Černovice</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>28</td>
<td>Staňkovice</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>–</td>
<td>57</td>
<td>Černovice</td>
<td>field/oilseed rape</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>29</td>
<td>Březno 1</td>
<td>field/wheat</td>
<td>+</td>
<td>–</td>
<td>58</td>
<td>Olomoucký</td>
<td>Pavlov</td>
<td>meadow</td>
<td>+</td>
</tr>
</tbody>
</table>

*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 (Legrèv 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 (War 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 (Ward 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 (Ward et al., 2005), which amplifies a 430-
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× DreamTag™ Buffer, 0.1 mM of each deoxynucleoside triphosphate, 0.2 μM of each primer, and 1 U of DreamTag™ 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 Pgfw2d/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)**

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 (Legrèv 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 (Vianopoulos et al., 2007) (Fig. 2).

**Sequence analysis for determination of *P. graminis* forms**

PCR amplification was performed with Platinum® Tag DNA Polymerase High Fidelity (Invitrogen, Carlsbad, USA) using the primers Psp1/Psp2rev (Legrèv et al., 2003) or Pgfw2d/Pxrev7 (Ward, Adams, 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 (Altschul 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 (Legrèv et al., 2003), Pgfw2d/Pxrev7 (Ward, Adams, 1998), Pg.F1/Pg.R1 and Pg.F2/Pg.R2 (Ward 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/Pg.R1 and Pg.F2/Pg.R2 were used during PCR amplification, no sequence analysis was

![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 *tepida*.](image)
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 soil samples.

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; Legrèве et al., 2002; Legrève et al., 2003; Vaiopoulos 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...
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, Pgfw2/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 Vaiannopoulos 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, Faquet, 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.

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