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The presence of pathogenic bacteria *Pseudomonas syringae* in cereals in Lithuania

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Abstract

Pseudomonas syringae pathovars cause bacterial diseases of cereals in nearly all temperate and subtropical cereal-growing regions. As being of low importance compared to fungal diseases, *P. syringae* has not been studied in cereal crops in Lithuania. However, leaf blights and glume discolorations, uncharacteristic of fungal diseases, were found relatively frequently in the fields. Therefore, the study aimed to identify the occurrence of *P. syringae* in cereals grown in Lithuania.

In this study, cereal crops were tested for the presence of plant pathogen *P. syringae*. In total, 452 symptomatic plant samples of winter wheat, winter triticale, spring wheat and spring barley were collected in 2013–2015. Symptoms of leaf blight and basal glume blotch on grain were more frequently detected in spring and winter wheat crops than in winter triticale and spring barley. Symptomatic leaves and grain yielded 113 *P. syringae* strains (51 from leaves and 62 from grain), but only 13 strains (10 from wheat, 2 from triticale and 1 from barley) were pathogenic to host plants when tested by spray method (SM). These strains were isolated from grain (10 strains) and leaves (3 strains) of all sampled plant species originating from different districts of the country. Based on 16S rRNA gene sequence analysis, leaf injection method (LIM) and origin of pathogenic strain, it can be assumed that *P. syringae* pv. *atrofaciens* is the dominant causal agent of bacterial diseases of cereals, particularly basal glume blotch of wheat.

Key words: wheat, pathogenic bacteria, basal glume blotch, leaf blight.

Introduction

Cereal plants play an essential role in the agriculture of Lithuania and worldwide. Plant pathogenic bacteria may reduce grain yield, especially when environmental conditions are favourable for epidemic development, and host plants are susceptible to the pathogen.

Pseudomonas syringae van Hall 1902 is a heterogeneous widespread bacterial phytopathogen, subdivided into more than 60 pathovars based on the pathogenic characteristics, 9 genomospecies defined by DNA-DNA hybridisation and 13 phylogenetic groups defined by multilocus sequence analysis (Baltrus et al., 2017). Leaf blight and basal glume blotch of cereals are caused by *P. syringae* pv. *syringae* and *P. syringae* pv. *atrofaciens* (Matveeva et al., 2008; Kazempour et al., 2010; Valencia-Botín, Cisneros-López, 2012; Glinushkin et al., 2016). *P. syringae* pv. *syringae* can infect an extensive host range of monocotyledonous and dicotyledonous plants (Ilić et al., 2016; Choi et al., 2017). *P. syringae* pv. *atrofaciens* mainly infects

the monocotyledonous plants and is considered as one of the primary bacterial pathogens of cereals in Europe (Pasicznyk, Butsenko, 2018); however, it can also cause leaf spots of dicotyledonous weeds such as *Sonchus arvensis* and *Papaver argemone* (Butsenko et al., 2021).

Leaf symptoms incited by *P. syringae* pv. *syringae* appear at booting to early heading. Numerous tiny, water-soaked spots are formed on the flag leaf and the two leaves below this leaf. Within two to three days, spots expand and often coalesce into large, greyish-green and desiccated areas, which within a week become necrotic and bleach to a light tan or white (Valencia-Botín, Cisneros-López, 2012). Leaves affected by *P. syringae* pv. *atrofaciens* show small, dark and water-soaked spots, which tend to enlarge and turn yellow and finally brown, when they become necrotic. Wheat glumes affected by *P. syringae* pv. *atrofaciens* have a dull, brownish-black area (blotches or streaks) at the base of each glume covering the kernel. Blotches appear near the base of glumes, while streaks may extend more than halfway

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up glumes (Kazempour et al., 2010). Diseased seeds at the basal end have dark brown lesions and are the most important pathogen reservoir. Symptoms may also occur on the upper part of the stem, significantly just above the nodes (Duveiller et al., 1997).

Diagnosis of bacterial diseases based on expressed symptoms is difficult, because they can be confused with those caused by some fungi or other biotic and abiotic factors (Morris et al., 2013; Xin et al., 2018). Therefore, it is likely that these diseases could be more widespread and important than is commonly thought. In general, compared to the other plant pathogenic bacteria, *P. syringae* pathovars are noted as the most common and well-studied ones (Tripathi, 2017; Xin et al., 2018). However, those causing leaf blight and basal glume blotch of wheat and other cereal pathogens are limited, and only scarce information is available, especially on yield losses and disease epidemiology (Valencia-Botín, Cisneros-López, 2012; Butsenko et al., 2021). *P. syringae* pv. *atrofaciens* has been more extensively studied since the eighties of the 20th century in Europe and was noted as important pathogen of wheat basal glume blotch in Russia, Ukraine, Bulgaria, Italy, Germany, New Zealand and Iran (Matveeva et al., 2008; Kazempour et al., 2010; Butsenko et al., 2021). In Germany, this pathogen has been regularly isolated since 1986, and epiphytic strains of *P. syringae* pv. *atrofaciens* were frequently detected on seeds and plants of wheat and barley (Kietzel, Rudopph, 1997). Epiphytic populations are the primary inoculum for plant infection and can cause significant plant damage in favourable weather conditions (Xin et al., 2018; Butsenko et al., 2021). At such conditions, yield losses may reach up to 50% (Valencia-Botín, Cisneros-López, 2012).

In Lithuania and other Baltic countries, fungal diseases of cereals received much attention, and the studies on bacterial diseases caused by *P. syringae* covered only some leguminous plants, stone fruits and nuts (Vasinauskienė, 1997; Vasinauskienė et al., 2008; Konavko et al., 2014). As a cereal pathogen, *P. syringae* has not been studied not only in Lithuania but also in neighbouring countries. Supposedly bacterial diseases in cereals have not been investigated thoroughly, because they occur sporadically and only under extremely humid conditions in spring or summer (Glinushkin et al., 2016; Xin et al., 2018). Recently, the atypical for fungal injuries, leaf blights and glume discolorations have been frequently detected on Lithuania-grown cereal plants suggesting the *P. syringae* infections.

Therefore, this study was aimed to isolate the causal agents from symptomatic plants, identify the presence of *P. syringae* on cereals and test its pathogenicity.

Materials and methods

The experiment was carried out during 2013–2020 at the Laboratory of Microbiology, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, and Nature Research Centre.

Sample collection and isolation of bacteria. Symptomatic leaves and seeds of winter and spring wheat (*Triticum aestivum* L.), winter triticale (\times *Triticosecale* Wittm. ex A. Camus) and spring barley (*Hordeum vulgare* L.) were collected from various regions of Lithuania in 2013–2015. The samples of leaves were collected during the cereal growing season (BBCH 13–77) and those of seeds after harvest (BBCH 89). Seeds and segments (approximately 1.0 cm in length) of leaves were dipped in a 1% NaClO (sodium hypochlorite) solution for 3 minutes, rinsed three times in sterile

distilled water and placed to dry for 1 h on sterile filter paper in a laminar. Then the seeds or leaves were placed on a modified King's B (KBC) medium, supplemented with 75 mg of cycloheximide, 75 mg of penicillin and 45 mg novobiocin (Schaad et al., 2001) and incubated at 26–27°C temperature for 1–2 days. Bacteria were purified on KBC medium, and those producing blue-green fluorescent pigment under UV light (312 nm) were subjected to physiological tests.

Physiological tests. Purified fluorescent bacteria were analysed for LOPAT tests: levan production from sucrose, oxidase reaction, pectolytic activity on potato, the presence of arginine dihydrolase activity and hypersensitivity to tobacco leaves (Schaad et al., 2001). After the performance of LOPAT tests, strains positive for levan production and tobacco leaves hypersensitivity but negative for oxidase reaction, pectolytic activity on potato and arginine dihydrolase activity were considered as *Pseudomonas syringae* and were subjected to the pathogenicity tests performed on host plants.

Pathogenicity tests. For the evaluation of the *P. syringae* strain pathogenicity to host plants, three different methods (SM, LIM and DLA) were performed.

Spray method (SM). The test was carried out in controlled growth chamber Climacell 707 (MMM Medcenter Einrichtungen GmbH, Germany) conditions. Spring wheat (*Triticum aestivum* L.) cultivar 'Hamlet', spring barley (*Hordeum vulgare* L.) cultivar 'Luokė', winter wheat (*Triticum aestivum* L.) cultivar 'Širvinta 1' and winter triticale (\times *Triticosecale* Wittm. ex A. Camus) cultivar 'Borowik' were grown at 20 ± 0.5°C temperature, 16 h day light period, 70–80% humidity and were watered according to the need (approximately twice per week). Five plants per pot (90 mm wide and 110 mm high) were grown in compost produced by UAB 'Agrochema' (pH 5.5–7.0, N 50–150 mg L⁻¹, K₂O 100–200 mg L⁻¹, P₂O₅ 30–70 mg L⁻¹, MgO 20–50 mg L⁻¹). When plants reached BBCH 12–13 growth stage, they were spray inoculated (~1 ml per plant) with bacterial suspension (10⁷ cells ml⁻¹). Host plant seedlings were inoculated with 13 strains of selected *P. syringae* and two reference bacteria: *P. syringae* pv. *syringae* (PSS), strain No. CFBP 4108, Van Hall 1902, collection No. ICMP 6386, geographic origin Japan, host plant *Hordeum vulgare*, and *P. syringae* pv. *atrofaciens* (PSA), strain No. CFBP 3587, Mac Culloch 1920, Young, Dye & Wilkie 1978, collection No. IMV 2399, geographic origin Ukraine, Kiev, host plant *Triticum durum* L. For negative control, seedlings were inoculated with sterile distilled water (SDW). Pots were randomized with four replications of each treatment. Inoculated plants were covered with plastic bags for the initial 72 h to keep plant surfaces wet and incubated at the above mentioned chamber conditions. After incubation (3–7 days), strains causing small, water-soaked, greyish-green lesions on seedlings' leaves were considered pathogenic to host plants. When disease symptoms appeared, re-isolations of pathogenic bacteria were performed: to ensure the presence of *P. syringae*, parts of the symptomatic tissues were plated on KBC medium and incubated for 48 h at 25°C temperature. The severity of leaf blight was assessed according to the scale from 1% to 75% proposed by Duveiller et al. (1997). Experiment was repeated three times.

Leaf injection method (LIM). Seedlings of host plants (spring wheat, spring barley, winter triticale and winter wheat) were grown on wet filter paper in the Petri dishes (90 × 20 mm) at room temperature (21 ± 2°C) until they reached 2–4 cm in length (BBCH 09). The upper part of the leaves was inoculated by inserting a sterile needle filled with fresh bacterial cell suspension (10⁷ cells ml⁻¹). *P. syringae* strains were tested on four seedlings,

and the test was repeated three times. Reference bacteria *P. syringae* pv. *syringae* and *P. syringae* pv. *atrofaciens* and SDW were also included in the test. Leaves were examined after 2–4 days, strains causing necrotic lesions surrounded by chlorotic area were considered pathogenic to the host plant.

Detached leaf assay (DLA). For this test, seedling leaves (BBCH 13–14) were sampled from the fields, and surface was sterilized in a 3% NaClO solution for 3 minutes. Five-cm long leaves were cut off, and the centre of the top of each leaf was wounded using a sterile pipet tip. Then leaf segments were placed in 100 mm Petri dishes (four leaves per plate) on the surface of 0.5% water agar containing 10 mg L⁻¹ kinetin as senescence retardant and inoculated with a 5 µl droplet of bacterial suspension (10⁷ CFU ml⁻¹) containing 0.08% of Tween 20 (G-Biosciences, USA). Control leaves were inoculated with SDW containing Tween 20. For this test, reference strains of both pathogens were also used. Petri dishes were kept at room temperature (21 ± 2°C) for 5 days. Lesion size in mm, based on its average length and width, was measured daily.

DNA extraction. For the DNA extraction, bacteria were grown on KBC medium at 27°C temperature for 1 day. DNA extraction was performed using ZR Fungal/Bacteria DNA MiniPrep (Zymo Research, USA) according to the manufacturer's instructions. DNA concentration was measured using Biophotometer (Eppendorf, Germany).

Polymerase chain reaction (PCR). Bacterial ribosomal RNA gene was amplified by PCR using universal bacterial primers 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GTT ACC TTG TTA CGA CTT-3') (Barghouthi, 2011). PCR reaction was performed in a 50 µl of the reaction mixture

containing 5 µl of 10 × PGR reaction buffer (included in polymerase kit) and 1 µl of dNTP Mix, 10 mM each (Thermo Fisher Scientific Baltics, Lithuania), 1 µl of each primer (20 pmol µl⁻¹), 0.25 µl of 5U µl⁻¹ AmpliTaq Gold polymerase (Applied Biosystems, USA), 1 µl of sample DNR (100 ng µl⁻¹) and 40.75 µl of nuclease-free water. PCR amplification was performed in a Veriti 96-Well Thermal Cycler (Applied Biosystems) using the following conditions: 95°C for 10 min, 40 cycles (95°C for 1 min, 54°C for 1 min, 72°C for 1 min) and 72°C for 10 min.

Electrophoresis. PCR products were visualized under UV-light in 1% agarose gel using GeneRuler 100bp plus DNA Ladder (Thermo Fisher Scientific Baltics) as a marker.

PCR product excision from gel. PCR products were cut out from agarose gel and purified using GeneJET Gel Extraction Kit (Thermo Fisher Scientific Baltics) according to the manufacturer's protocol.

Nucleotide sequence analysis. PCR products were sequenced at BaseClear (The Netherlands). Identified nucleotide sequences were processed and analysed using the program package DNASTAR and the database NCBI BLAST.

Statistical analysis. All experiments were repeated three times. The experimental data were processed by the analysis of variance (ANOVA). The least significant difference (LSD) was calculated using the ANOVA.

Results

Isolation of bacteria. A total of 452 grain (273) and leaf (179) samples showing symptoms of bacterial leaf blight and grain infection were collected during 2013–2015 (Table 1).

Table 1. Sampling information

Coding of strains	Host plant	Plant part	Number of samples	Number of bacteria isolations	Fluorescent bacteria on KBC medium
SWG	spring wheat	grain	142	129	119
SWL	spring wheat	leaves	88	95	61
WWG	winter wheat	grain	110	117	74
WWL	winter wheat	leaves	74	118	102
TG	winter triticale	grain	12	25	17
TL	winter triticale	leaves	11	33	14
BG	spring barley	grain	9	10	6
BL	spring barley	leaves	6	15	11
Total			452	542	387

Bacteria isolated from necrotic leaf segments and seeds (whose base end was dark brown) on KBC medium yielded 542 bacterial isolates. The fluorescent blue-green pigment on this medium was produced by 387 whitish, translucent bacterial colonies after 48 h of incubation. During the performance of LOPAT tests, 113 strains (51 isolated from leaves and 62 from grain) gave positive reaction for levan production but negative for oxidase reaction and arginine dihydrolase activity and did not cause the soft rot on potato tubers; however, 45 of these strains induced a clear hypersensitive reaction to tobacco leaves, and the rest (69) did not (Table 2).

Using the spray method (SM), all 113 strains were evaluated for their pathogenicity to host plants, and only 13 strains (10 from grain and 3 from leaves) showed the positive reaction and, therefore, were selected for further studies. These strains were isolated from grain and leaves of all sampled plant species and originated from different districts of Lithuania (Table 3).

Pathogenicity tests. Leaf blight symptoms on host plant seedlings sprayed with the test bacteria mainly were very weak (disease severity 0.1–0.5%)

(Table 3). No symptoms on host plants were observed with SDW. Leaf blight severity caused by *P. syringae* pv. *atrofaciens* (7.5%) was higher than by *P. syringae* pv. *syringae* (3.5%). Leaf injection method (LIM) showed that 4 strains isolated from winter wheat grain (WWG74, WWG10, WWG2 and WWG11), 1 from winter wheat leaves (WWL4), 2 from spring wheat grain (SWG32, SWG11) and 1 from winter triticale leaf (TL1) as well as *P. syringae* pv. *atrofaciens* were able to cause the typical for *P. syringae* pv. *atrofaciens* dark margins around the inoculation points (Toben et al., 1989). Other strains from spring wheat grain (SWG41 and SWG53), spring wheat leaf (SWL6), winter triticale grain (TG1) and spring barley grain (BG8) were tested, and like SDW did not cause any discoloration. *P. syringae* pv. *syringae* induced water-soaked spots on leaves, which became necrotic and turned from grey-green to tan-white (Figure 1). The entire leaves later became necrotic. *P. syringae* pv. *atrofaciens* induced small dark green to brown, water-soaked irregular lesions, 2–5 mm in length (Figure 2). These elongated up to 5 mm lesions became yellow and then brown, when the tissue finally dried.

Table 2. *Pseudomonas syringae* strain reaction to the LOPAT tests and pathogenicity to host plants (spay method)

Preliminary species name	L	O	P	A	T	HP	Coding of strains	Total No. of isolates
<i>P. syringae</i>	+	-	-	-	+	+	SWG32, SWG41, SWG53, WWG74, TG1, BG8, SWL6, SWG11, WWL4, WWG2, TL1, WWG10, WWG11	13
<i>P. syringae</i>	+	-	-	-	+	-	SWG70, SWG72, SWG84, SWG89, SWL21, SWL17, SWL27, SWL38, SWL47, SWL53, SWL57, SWL64, SWL2, SWL11, SWL19, SWL39, SWL62, WWL1, WWL8, WWL18, WWL19, WWL20, WWL26, WWL33, WWL35, WWL42, TL2, TL13, TL21, TL27, TL32, TL33	32
<i>P. syringae</i>	+	-	-	-	±	±	SWG58, SWG59, SWG60, SWG61, SWG62, SWG64, SWG94, SWL1, SWG81, SWG91, SWG97, SWG98, TG3, TG5, TG14, TG17, TG23	17
<i>P. syringae</i>	+	-	-	-	±	-	SWG7, SWG25, SWG28, SWG47, SWG48, SWG55, SWG82, SWG83, SWG79, SWG87, SWG90, SWG92, SWG96, SWG99, SWL3, SWL29, SWL30, SWL36, WWG37, WWG45, WWG54, WWG61, WWG70, WWG21, WWG29, WWG46, WWG71, WWG86, WWG98, WWG99, WG110, WWL2, WWL5, WWL14, WWL25, WWL29, WWL34, WWL36, WWL37, WWL40, WWL50, WWL57, WWL59, TG2, TG18, TG19, TG20, TG24, TG25, TL4, TL14, TL30	51

L – levan production, O – oxidase reaction, P – pectolytic activity on potato, A – arginine dihydrolase activity, T – tobacco leaves hypersensitivity, HP – host plant sensitivity

Table 3. Pathogenicity of strains of *Pseudomonas syringae* to cereals using seedling spray (SM), leaf injection (LIM) and detached leaf assay (DLA) methods (2013–2020)

Strain	Host plant	Plant part	Area of origin	SM:	LIM:	DLA:
				SLB HP %	HP	WW lesion size mm
SWG11	spring wheat	grain	Pakruojis	0.2 a	+	4.9 bcdef
SWG32	spring wheat	grain	Kėdainiai	0.3 a	+	4.6 ab
SWG41	spring wheat	grain	Jurbarkas	0.3 a	-	4.5 a
SWG53	spring wheat	grain	Kretinga	0.4 a	-	4.7 abc
SWL6	spring wheat	leaves	Kaunas	0.5 a	-	4.9 cdef
WWG2	winter wheat	grain	Pasvalys	0.1 a	+	4.8 bcdef
WWG10	winter wheat	grain	Kaunas	0.3 a	+	4.8 bcdef
WWG11	winter wheat	grain	Kėdainiai	0.1 a	+	4.6 abc
WWG74	winter wheat	grain	Biržai	0.3 a	+	5.0 def
WWL4	winter wheat	leaves	Kaunas	0.1 a	+	4.8 bcdef
TG1	winter triticale	grain	Telšiai	0.3 a	-	4.9 cdef
TL1	winter triticale	leaves	Kaunas	0.1 a	+	4.9 bcdef
BG8	spring barley	grain	Ukmergė	0.4 a	-	4.8 abcd
PSA	<i>T. durum</i>	-	Ukraine	7.5 c	+	5.1 f
PSS	<i>H. vulgare</i>	-	Japan	3.5 b	+	5.1 def

Note. PSA – reference bacteria of *P. syringae* pv. *atrofaciens*, PSS – reference bacteria of *P. syringae* pv. *syringae*; SLB HP – severity of leaf blight on the host plant (HP); WW – winter wheat; means followed by the same letters are not significantly different.



Figure 1. Symptoms using leaf spray method (SM) of *Pseudomonas syringae* pv. *syringae*



Figure 2. Symptoms using leaf spray method (SM) of *Pseudomonas syringae* pv. *atrofaciens*

Using detached leaf assay (DLA), disease symptoms on host plant leaf were clearly expressed in all tested bacterial strains. Symptoms on a leaf at the point of inoculation initially appeared as a small water drop. After two days, the water-soaked spots subsequently became oval-shaped necrotic lesions surrounded by yellow chlorotic areas. Leaf segments inoculated with SDW displayed no visible lesions. Inoculation with bacterial strains produced 4.5–5.1 mm size lesions on the leaf (Table 3).

Nucleotide sequence analysis. For 16S rRNA gene sequence, *P. syringae* 6 strains: SWG11,

SWG32, WWG11, WWG2, WWG74 and TL1 that were pathogenic to host plants during all three (SM, LIM and DLA) methods, were selected. Amplification of the 16S rRNA gene resulted in 1411 bp length fragments. Based on nucleotide sequence analysis, all selected strains exhibited greater than 99.8% (pairwise nucleotide identity) similarity to *P. syringae* pv. *atrofaciens* strain No. LMG 5095, host plant *Triticum aestivum* (GenBank ID CP028490). It is known that the 16S rRNA gene does not provide sufficient resolution to separate the closely related strains (pathovars) within the same species. Therefore, it just can be assumed that strains isolated

from symptomatic cereal grain and leaves during 2013–2015 belong to *P. syringae* species.

Discussion

Researchers around the world (Sundin et al., 2016; Tripathi, 2017; Xin et al., 2018) have noticed an increasing risk of phytopathogenic bacteria for plant growth including cereals, especially wheat, one of the most valuable and important essential grain crops that ranks first in the world's sown area (Valencia-Botín, Cisneros-López, 2012; Suszanowicz et al., 2019; Butsenko et al., 2021). Considering that bacterial diseases have not been studied in cereal crops of Lithuania and other Baltic countries before and that the atypical for fungal diseases leaf spots and glume discolorations are found rather frequently on cereals in the fields, this study was aimed to identify the occurrence of *P. syringae* in symptomatic leaves and grain of wheat, barley and triticale.

In the current study, symptoms of leaf blight and basal glume blotch caused by *P. syringae* have been examined in Lithuania grown cereal crops for the first time. Symptoms of above mentioned diseases were more frequently detected in spring and winter wheat crops than in winter triticale and spring barley suggesting that the latter may be more resistant to *P. syringae* infections. The other reason is that the spread of bacterial diseases may be also influenced by the environmental conditions. It was shown previously that the symptoms of the basal glume blotch caused by *P. syringae* pv. *atrofaciens* varied depending on farming practices, the growth stage and the environmental factors (Patyka, 2016). Increased amounts of nitrogen, phosphate and potassium fertilisers increased the potential of wheat infection with this bacteria.

Symptomatic leaf and grain samples collected during the experiment yielded 113 strains (51 isolated from leaves and 62 from grain), which were identified based on the LOPAT tests as *P. syringae*. However, most of the strains (88.5%) did not induce the disease symptoms on host plants when tested by spray method in the controlled conditions. It is known from previous studies (Kietzel, Rudopph, 1997; Valencia-Botín, Cisneros-López, 2012; Butsenko et al., 2021) that epiphytic *P. syringae* is present on the surfaces of wheat plants and other hosts. High humidity favours the abundance of epiphytic population of *P. syringae*, and the size of such population correlates quantitatively with the incidence of disease outbreaks (Xin et al., 2018). Pathogenic and epiphytic *P. syringae* have been identified in cereals in Lithuania for the first time. *P. syringae* pv. *atrofaciens* has been the primary causative agent of bacterial diseases of wheat, particularly basal glume rot of wheat, for many years in other countries (Matveeva et al., 2008; Kazempour et al., 2010; Butsenko et al., 2021).

However, spray method (SM) showed that the isolates of *P. syringae* were weak cereal pathogens on their own hosts. The SM and DLA methods showed that they have the capacity to cause disease. Using DLA, the isolates were almost as effective as a positive control of reference strains (Table 3). Both latter methods involved physical wounds on plants, while SM did not. In addition, host-pathogen interaction is complicated and multi-layered. Also, an important role might have the exact spraying time (morning or evening) and method. *P. syringae* is abundant in the environment and it participates in snow formation in the atmosphere, is present in rain, streams and other water sources (Morris et al., 2013). If it could invade plants freely, the devastation would be immeasurable. Instead, it is an opportunistic pathogen relying on other factors like fungal infections or feeding insects to make wounds in plants that help it infiltrate hosts. However, its

share abundance ensures that *P. syringae* is always present and ready to take advantage of favourable conditions that might result in a spontaneous epidemic. According to Patyka (2016) and Butsenko et al. (2021), the disease did not cause a lot of losses, but there need to be favourable weather conditions for the disease to spread out. It can be stated that, if Lithuanian summers are warm and rainy, the disease will spread out quite fast.

Analysing the results of 16S rRNA gene sequence analysis, it cannot be proved that the obtained strains belong to *P. syringae* pv. *atrofaciens*. However, based on the sequence data supported by typical for *P. syringae* pv. *atrofaciens* lesions caused on the leaf injection points and origin of pathogenic strain (most of strain obtained from symptomatic grain), it can be assumed that *P. syringae* pv. *atrofaciens* is the dominant causative agent of bacterial diseases of cereals, particularly basal glume blotch of wheat. This assumption is supported by the studies of other researches (Kazempour et al., 2010; Suszanowicz et al., 2019; Butsenko et al., 2021).

Conclusions

1. Research results showed that symptoms of bacterial blight on leaves and dark brown lesions at the basal end of grain were around 10 times more frequently detected in spring and winter wheat than in winter triticale and spring barley.

2. Symptomatic leaves and grain (total 452) yielded 113 *Pseudomonas syringae* strains (45% from leaves and 55% from grain), most of which (88.5%) were non pathogenic to host plants when tested by spray method (SM) in the controlled growth chamber conditions.

3. Host pathogenic *P. syringae* strains (total 13) were isolated from grain (10 strains) and leaves (3 strains) of all sampled plant species (spring wheat, winter wheat, winter triticale and spring barley) originating from different parts of the country, confirming that diseases caused by *P. syringae* are present in Lithuania grown cereals.

4. Based on 16S rRNA gene sequence analysis, leaf injection method (LIM) and origin of pathogenic strain (most of the strains obtained from symptomatic grain), it can be assumed that *P. syringae* pv. *atrofaciens* is the dominating causative agent of bacterial diseases of cereals, particularly basal glume blotch of wheat.

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Pseudomonas syringae patogeninės bakterijos Lietuvoje auginamuose javuose

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Santrauka

Šiltėjant klimatui ir kintant meteorologinėms sąlygoms, plinta ir naujos augalų ligos. Beveik visose vidutinio ir subtropinio klimato zonose javų bakterinės ligas sukelia *Pseudomonas syringae* patogenai. Lietuvoje auginamuose migliniuose javuose *P. syringae* iki šiol nebuvo tirti, nes lyginant su grybinėmis ligomis, nedarė didelės žalos. Tačiau pastaruoju metu pasėliuose gana dažnai pastebima grybinėms ir fiziologinėms ligoms nebūdingų lapų bei varpažvynių pažeidimų. Tyrimo tikslas – nustatyti *P. syringae* paplitimą Lietuvoje auginamuose javuose.

Tirti javų pasėliai, ar juose nėra paplitęs šis augalų patogenas. Per trejus tyrimo metus (2013–2015) iš viso buvo surinkti 452 simptominiai žieminių kviečių, žieminių kvietrugių, vasarinių kviečių ir vasarinių miežių ėminiai. Lapų dėmėtligės ir bazalinės bakteriozės pažeidimai gerokai dažniau buvo aptikti vasarinių ir žieminių kviečių nei žieminių kvietrugių ir vasarinių miežių pasėliuose. Iš simptominių lapų ir grūdų buvo išskirta 113 *P. syringae* kamienų (51 iš lapų ir 62 iš grūdų), tačiau testuojant purškimo metodu augalams šeiminkams buvo patogeniški tik 13 iš jų: 10 iš kviečių, 2 iš kvietrugių ir 1 iš miežių. Šie kamieniai buvo išskirti iš grūdų (10 kamienų) ir lapų (3 kamieniai), surinktų įvairiuose šalies rajonuose. Atlikus 16S rRNR genų sekos analizę, lapų injekcijos testą ir nustatius patogeninių kamienų kilmę darytina prielaida, kad Lietuvoje dominuojantis javų bakterinių ligų sukėlėjas yra *P. syringae* pv. *atrofaciens*, ypač kviečių bazinės bakteriozės.

Tyrimo duomenys patvirtina, kad Lietuvoje auginamuose migliniuose javuose plinta *P. syringae* sukeliama liga, į kurias reikia atkreipti dėmesį.

Reikšminiai žodžiai: kviečiai, patogeninės bakterijos, bazalinė bakteriozė, lapų dėmėtligė.