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Bacterial canker pathogens present in the materials of *Prunus armeniaca* propagation

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Abstract

The aim of this study was to find out whether causal agents of bacterial canker and the premature death of apricot trees are present in the internal tissues of propagating material of various origins. In total, 33 samples of apricot (*Prunus armeniaca* L.) scion materials from eight and six samples of rootstocks from four European localities were analysed for the presence of the pathogenic *Pseudomonas* species. Significant differences were found in the diversity of the culturable bacterial population and the incidence of *Pseudomonas* bacteria in internal tissues of scion and rootstock buds. In most of the rootstocks, no *Pseudomonas* bacteria were detected. A total of 148 *Pseudomonas*-like strains isolated from scion samples were collected mainly from localities F1–F5 (in France) and G1–G2 (in Germany). These strains were clustered into 11 groups by means of fatty acid methyl ester (FAME) analysis; 91.9% of them were able to induce a hypersensitive reaction on tobacco leaves and 37.8% were positive in ice nucleation activity. A set of 89 *Pseudomonas* strains was characterised by means of *Psy*-PCR targeting of the *Pseudomonas syringae* (*Ps*) complex, complex phylogroup (PG), *syrB*-PCR targeting of the syringomycin synthesis, pathogenicity on detached apricot twigs, and the ability to survive within epiphytic microflora. *Pseudomonas* strains attributed to PG01, PG04, and PG07–08 were slightly pathogenic to detached apricot twigs. Altogether, 5% of *Pseudomonas* strains highly pathogenic to detached apricot twigs were isolated from apricot scion samples originated in localities F2, F5, and G1. These strains were attributed to PG02 and PG03, and *rpoD* sequencing confirmed a similarity to strains of *P. syringae* pv. *syringae* and *P. amygdali* pv. *morsprunorum* known to be pathogenic to apricot, respectively. The occurrence of these pathogens in buds of scion materials increases the risk of bacterial canker and the premature death of young apricot trees.

Keywords: apricot, scion, rootstock, *Pseudomonas amygdali* pv. *morsprunorum*, *Pseudomonas syringae* pv. *syringae*.

Introduction

Bacterial canker and the premature dying of young stone fruit trees caused by different members of the *Pseudomonas syringae* (*Ps*) complex affects all commercially grown *Prunus* species, predominantly apricot (*Prunus armeniaca* L.), cherry, peach, and plum (Lamichhane et al., 2015). Losses can result from a direct reduction in yield due to a cold-induced injuries or the death of buds and flowers (Gross et al., 1984), or from tree decline and death due to the development of cankers in branches and major scaffold limbs (Scortichini, 2010). All over the world, apricot tree losses of up to 80% due to the disease have been reported (Kennelly et al., 2007). In the past two decades, severe disease outbreaks have been reported in Mediterranean European countries characterised by different climates (Giovanardi et al., 2018).

The apricot fruit crop is one of the few clearly profitable commodities in orchards in Central Europe (Kocsis, Major, 2018). In this area, one of the biggest obstacles to the successful production of apricots is the premature death of young 3–5-year-old trees. The most harmful pathogens of apricot are *P. syringae* pathovars *syringae* and *morsprunorum* causing significant damage in nurseries and orchards and reducing the fruit quality and yield (Giovanardi et al., 2018; Hulin et al., 2018; Parisi et al., 2019). A different mechanism of infection can be involved in the development of the disease, e.g., through leaf scars, infected propagating material, pruning wounds or frost injuries (Marcelletti, Scortichini, 2019). Much less common than bacterial canker is Eutypa dieback, which causes a sudden wilting in apricots in late spring or summer. Diseased limbs can be pruned out after crop.

For production of apricot trees, only certified rootstocks and scions of apricot cultivars should be used (OEPP/EPPO, 2001). Propagating material is compulsorily tested for the presence of viruses, virus-like diseases, and phytoplasmas. The presence of bacterial pathogens such as *P. syringae* pv. *syringae* and *Xanthomonas arboricola* pv. *pruni*, which can be transmitted by propagating material, is tested rarely, if at all (Roos et al., 2019). The causal agents are readily

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perpetuated, albeit unwittingly, in the apricot progeny, and the disease develops under favourable conditions in orchards within a few months to several years depending on the virulence of infecting strains (Dosba, 2003). The pressure to rapidly change the range of planted apricot cultivars based on customer preferences does not enable a longer-term monitoring of their health status. The scions of apricot cultivars and rootstocks for the production of apricot trees come from different localities with different climates. In the place of origin, the external symptoms of bacterial infection may not be expressed. Once a commercial orchard is established, no economically viable curative control measures can be applied to the trees. The most effective way to control the disease is to remove infected symptomatic trees. The breeding of resistant apricot cultivars is complicated because of the diversity of bacteria able to cause the canker on apricot trees (Donmez et al., 2010). Infected nursery planting stocks may also serve as a source of inoculum that can be spread in orchards secondarily by agriculture practices, wind and rain or natural vectors (Rowhani et al., 2005). Clarifying the role of all potential reservoirs of pathogenic bacteria is important for implementing disease control measures.

Primers *Psy* F/R can be used for a rapid, specific, and sensitive PCR identification of taxa within the entire *P. syringae* group (Guilbaud et al., 2016). Currently, an accurate and effective means for classifying strains in the *Ps* complex is to compare the sequences of their *cts* and *rpoD* genes (Berge et al., 2014). Classification of bacterial strains based on multilocus sequence analysis (MLSA) of four housekeeping genes (Mulet et al., 2010) and whole-genome sequence data comparisons (Gomila et al., 2017) led to the proposal of 13 phylogroups (PG) and 23 clades within the *Ps* complex (Berge et al., 2014). Known causal agents of the bacterial canker on *Prunus* species fall into four phylogroups (Berge et al., 2014). Strains of *P. syringae* pv. *syringae* described in cultivated and wild apricot are attributed to phylogroup PG02, sub-phylogroups PG02b and PG02d, *Pseudomonas amygdali* pv. *morsprunorum* race 1 (*Pa* pv. *morsprunorum* R1) – to phylogroup PG03, race 2 (*Pa* pv. *morsprunorum* R2) – to phylogroup PG01b (Bultreys, Kaluzna, 2010), epiphytic *Pseudomonas* species *P. savastanoi* – to phylogroup PG04 and *P. viridiflava* – to phylogroup PG07–08. According to Borschinger et al. (2016), the epiphytic strains occurring in apricot buds of apricot also belong to phylogroups PG10 and PG13 of *Ps* complex.

Agents that cause bacterial canker in *Prunus* may differ in lifestyle and aggressiveness on different host plants and plant tissues. According to the results of pathogenicity tests on apricot or peach seedlings in greenhouses, it is not possible to predict the pathogenicity of causal agents of the bacterial canker to apricot in orchards (Little et al., 1998). To determine the fundamental ability of putative causal agents to cause bacterial canker, a whole-tree wound inoculation test on apricot cultivars should be carried out (Parisi et al., 2019).

The objective of the present study was to screen samples of apricot propagating material from different localities for the presence of pathogenic *Pseudomonas* bacteria, especially causal agents of cork necrosis and the premature death of apricot trees. The isolated *Pseudomonas*-like strains were classified into FAME groups (FGs) and phylogroups (PGs) of *Ps* complex. Their pathogenicity on tobacco leaves and detached apricot twigs, ice nucleation activity, and the ability to survive on the surface of apricot leaves within epiphytic microflora was assessed.

Materials and methods

Isolation of bacterial strains. Altogether, 33 scion materials of apricot (*Prunus armeniaca* L.) from eight European localities: five localities in France (F1–F5), two localities in Germany (G1–G2) and one locality in the Czech Republic (C1), were sampled in 2019 and 2020 (Table 1). For analysis of the bacterial population in the internal tissues of buds, one sample from each scion material was prepared. One sample consisted of 10 randomly selected twigs (length 40–50 cm, diameter 0.5–1.5 cm, growth stage 89–91) of one apricot cultivar used as scion material (Table 1). Twigs were superficially disinfected by 90% ethyl alcohol and transported at 4–6°C temperature to the laboratory and processed immediately.

Altogether six rootstock materials (Table 1) originating from (1) *in vitro* cultures ‘Wawit’ (*Prunus domestica* L.) ‘Torinel’ (*Prunus domestica* L.), and ‘Adesoto’ (*Prunus insititia* L.) were obtained from one locality in Italy (I), (2) seedlings ‘Myrobalan’ (*Prunus cerasifera* Ehrh.) – from one locality in Poland (P), and (3) rooted mother twigs ‘St. Julien’ (*Prunus insititia* L.) – from localities in the Netherlands (N) and the Czech Republic (C1). Rootstocks were transported to the Czech Republic and outplanted in the nursery. For analysis of the bacterial population in the internal tissues of buds, twigs (length 40–50 cm, diameter 0.5–1.0 cm, growth stage 89–91) were cut before grafting. One sample was prepared from each rootstock material. It consisted of 10 detached twigs from 10 trees of one given rootstock. Twigs were superficially disinfected with 90% ethyl alcohol, transported to the laboratory under the same conditions as scion samples and processed immediately.

A total of 30 buds were cut from one sample (from 10 twigs) of each propagating material. Thereafter, 200 mg of internal phloem tissues of each bud were separately homogenized in 1 ml of sterile water, 20 µl of homogenates were streaked onto one plate of King’s B medium and incubated for 72 h at 25°C temperature. According to the fraction of a plate covered with bacterial colonies, the culturable bacterial population in the internal tissues of each bud was estimated (Miliute et al., 2015). Subsequently, the average culturable bacterial population in the sample of given propagating material was calculated. Based on the colony morphology (size, colour, and pigment production), different *Pseudomonas*-like colonies from each sample were subcultured in new Petri dishes containing King’s B medium at 25°C temperature for 48–72 h before characterisation (Bultreys, Kaluzna, 2010).

Fatty acid methyl ester (FAME) analysis. A total of 148 *Pseudomonas*-like strains from samples of scion and rootstock materials were determined by the rapid FAME method using the database Sherlock MIS RTSBA6 (MIDI Inc., USA), as described by Kunitsky et al. (2006). In addition, cluster analysis was carried out using the unweighted pair group method algorithm based on known FAME profiles of *Pseudomonas* strains (Gardan et al., 2000). According to the results of FAME analysis, FAME groups (FGs) were determined.

Hypersensitive reaction (HR) on tobacco leaves and ice nucleation activity (INA). The suspensions of 148 *Pseudomonas*-like strains in sterilised water were adjusted to $OD_{600} = 0.1$ and used for a HR test on tobacco (*Nicotiana tabacum* L.) leaves and determination of

INA. Sterile distilled water and *Pseudomonas* pv. *syringae* CPABB 41 (Collection of Phytopathogenic and Agriculturally Beneficial Bacteria, the Czech Republic) were used as a negative and positive control for both methods, respectively. The induction of HR on three tobacco leaves was tested according to Klement and Goodman (1967). A strain was considered HR-positive, when a necrotic lesion on tobacco leaves developed within 24 hours. INA analysis was performed according to Lindow et al. (1982). The freezing of 5 ml of bacterial suspensions in test tubes was scored at 0.5°C temperature intervals from 0°C to −6°C providing three replicates. The *Pseudomonas* strain was considered INA-positive, when the suspension froze at a temperature above −6°C. All results were reported as the average of three replicates in the experiment.

Real-time polymerase chain reaction (PCR). Genomic DNA was isolated from a subset of 89 strains determined by FAME as belonging to the genus *Pseudomonas* with a similarity index SimIndex ≥ 0.5 and/or strains with positive HR and INA from 0°C to −6°C. DNA was isolated according to the manufacturer's protocol for the GenElute™ Bacterial Genomic DNA Kit (Merck and Co. Inc., USA). Reaction mixes for individual real-time PCR analysis were composed of 12 µl of RotorGene SYBR® Green II PCR Kit (QIAGEN GmbH, Germany), 0.5 µl of 10 mmol L^{-1} forward (Fw) and reverse (Rv) primers, 1–2 µl of concentrated (20–25 ng) and diluted DNA (1:1) used in duplicate. The real-time PCR amplifications were performed using a Rotor-Gene Q 5plex HRM (QIAGEN GmbH). *Pseudomonas* strains were tested by primer pair *Psy* F/R targeting the whole *P. syringae* group (Guilbaud et al., 2016) and by primers *syrB* B1/B2 that helped determine the syringomycin synthesis genes in *P. syringae* strains (Sorensen et al., 1998). The distribution of *Pseudomonas* strains across the phylogroups PG01–03 and epiphytic PG04 was determined according to Borschinger et al. (2016) and Parisi et al. (2019), and across PG07–08 according to Bartoli et al. (2014) and Parisi et al. (2019).

Phylogenetic characterisation. A subset of 26 *Pseudomonas* strains representing all phylogroups mentioned above was selected for phylogenetic characterisation based on partial sequences of the housekeeping *rpoD* gene (Berge et al., 2014). For amplification, primers *rpoD*-FP and *rpoD*-RP were used. PCR products were purified using Monarch® PCR & DNA Cleanup Kit (New England Biolabs, USA). For Sanger sequencing, primer *rpoD*-Fs was used (Eurofins Genomics Germany GmbH, Germany). The alignment of sequences was made by using software *MEGAX*, and sequence similarity searching was performed against the GenBank database of NCBI (http://www.ncbi.nlm.nih.gov/). The cut-off for species was 95–96% and for pathovars ≥99%.

Pathogenicity on detached apricot twigs and survival ability assay. For the same subset of 89 *Pseudomonas* strains, the positive control CPABB 41 suspension in sterile distilled water was prepared by adjusting the concentration (optical density (OD)) of bacterial cells to OD_{600} = 0.1. In addition, 0.1 ml of polysorbate Tween 20 was added to 100 ml of each bacterial suspension. Sterile water served as a negative control. Twigs for pathogenicity tests were collected from apricot trees of the cultivar 'Bergarouge' in an untreated orchard. Twigs with a length of 40–50 cm, a diameter of 0.5–1.5 cm, and growth stage 79–81 were used. Suspension of 10 ml of each *Pseudomonas* strain for positive and negative control were sprayed on leaves of three test twigs, placed in an individual container with sterile distilled water, and covered with a plastic bag for 24 h. All containers were transferred to the growth chamber and incubated at the temperature regime 20/15°C (day/night), relative humidity of 80–90%, and a photoperiod regime of 12/12 h (day/night), respectively. The development of withering symptoms and superficial necroses on apricot leaves was evaluated daily for 10 days. The development of symptoms was assessed using the following scale: (i) symptomless twigs (marked as −); (ii) withered twigs or up to 5 small superficial necroses on leaves (≤0.5 cm in diameter; +); (iii) 5–10 superficial necroses (≤0.5 cm in diameter; ++), and (iv) more than 10 necrosis (≥0.5 cm in diameter; +++).

A total of three leaves from each container (one leaf from each test twig), altogether 2 ± 0.2 g of leaf tissue, was shaken at room temperature for 2 h in 5 ml of sterile distilled water, and 10 µl of suspension was streaked on two Petri dishes containing King's B medium. Competitive interactions between *Pseudomonas* strains and the biofilm-proficient microorganisms associated with untreated apricot leaves were evaluated after four days of incubation at 25°C temperature according to the fraction of a plate covered with *Pseudomonas* colonies. The survival ability of *Pseudomonas* strains within epiphytic microflora was assessed using the following scale: (i) negative survival ability (–), when *Pseudomonas* colonies made up to 5% of all bacterial colonies; (ii) weak survival ability (+), up to 30%; (iii) medium survival ability (++), up to 70%, and (iv) strong survival ability (+++), >70%.

The data obtained from different localities and different samples of propagating material were subjected to analysis of variance (ANOVA). Values of $p \leq 0.05$ were considered as statistically significant. For the statistical analysis, software *STATISTICA*, version 13.3.721.1 (StatSoft Inc., USA) was used.

Results

The culturable bacterial population and the occurrence of colonies of Pseudomonas-like bacteria in buds of apricot propagating material. In Table 1, the results of the screening of apricot propagating material (33 scion and 6 rootstock samples) for the culturable bacterial population, the occurrence of colonies of *Pseudomonas*-like bacteria in internal tissues of buds, and the incidence of HR- and INA-positive *Pseudomonas* strains isolated from the screened samples are summarised.

The average culturable bacterial population in the buds of scion and rootstock samples ranged 0.1–59.2% and 0.3–1.6%, respectively. Significantly larger ($p \leq 0.05$) culturable bacterial population were isolated from the samples collected in localities F1–F2, F5 and G1–G2 (Table 2). The percentage of buds, from which *Pseudomonas*-like bacteria were isolated, varied 0–73.3% in scion samples and up to 10% in rootstock samples. No *Pseudomonas*-like bacteria were isolated from scion samples of the cultivars 'Adriana', 'Kompakta', 'Vegama' and 'Darina' from the C1 locality. In most of the rootstock samples, no *Pseudomonas*-like bacteria were found.

Table 1. Estimation of culturable bacterial population and incidence of *Pseudomonas*-like bacteria, hypersensitive reaction (HR) positive and ice nucleation activity (INA) positive *Pseudomonas* strains isolated from samples of apricot propagating material

Origin	Locality	Year of isolation	Propagating material	Cultivar	Culturable bacterial population[1] %	Incidence %		
						Pseudomonas-like strains[2]	HR-positive *Pseudomonas* strains[3]	INA-positive *Pseudomonas* strains[4]
Czech Republic	C1	2019	scion	Bhart	1.1 ± 0.6	10.0	3.0	0.0
	C1	2019	scion	Harlayne	2.7 ± 1.8	6.7	6.7	0.0
	C1	2019	scion	Harogem	0.7 ± 0.4	16.7	6.7	3.3
	C1	2019	scion	Adriana	2.6 ± 1.8	0.0	0.0	0.0
	C1	2020	scion	Velita	0.6 ± 0.4	3.3	0.0	0.0
	C1	2020	scion	Kompakta	1.1 ± 0.4	0.0	0.0	0.0
	C1	2020	scion	Leskora	1.0 ± 0.3	10.0	3.3	3.3
	C1	2020	scion	Vegama	1.1 ± 0,2	0.0	0.0	0.0
	C1	2020	scion	Darina	0.1 ± 0.1	0.0	0.0	0.0
France	F1	2019	scion	Congat	55.0 ± 11.4	43.3	23.3	10.0
	F1	2019	scion	Bergarouge	58.7 ± 10.5	20.0	16.7	6.7
	F2	2019	scion	Anegat	54.7 ± 10.6	70.0	40.0	24.5
	F2	2019	scion	Bergeval	48.0 ± 13.8	73.0	36.7	6.7
	F2	2019	scion	Sefora	54.0 ± 12.0	73.3	40.0	6.7
	F1	2019	scion	Elgat	41.7 ± 18.7	63.3	23.3	0.0
	F1	2019	scion	Vertige	53.3 ± 13.9	30.0	23.3	6.7
	F1	2019	scion	Koolgat	29.1 ± 16.3	43.3	20.0	3.3
	F3	2019	scion	Kioto	7.3 ± 3.1	36.7	16.7	13.3
	F4	2019	scion	Kuresia	12.8 ± 10.2	36.7	16.7	0.0
	F2	2019	scion	Digat	42.1 ± 9.9	40.0	20.0	10.0
	F3	2020	scion	Tsunami	13.3 ± 9.1	25.2	3.3	3.3
	F3	2020	scion	Pinkcot	15.8 ± 9.3	20.3	3.3	3.3
	F3	2020	scion	Spring Blush	6.7 ± 2.3	18.2	3.3	0.0
	F5	2020	scion	Vertige	53.0 ± 12.4	21.7	16.7	16.7
	F5	2020	scion	Bergarouge	59.0 ± 14.2	55.0	6.7	6.7
	F5	2020	scion	Congat	57.5 ± 10.7	30.0	10.0	6.7
	F5	2020	scion	Koolgat	54.0 ± 9.7	36.7	20.0	20.0
Germany	G1	2019	scion	Ungarishe Beste	14.4 ± 10.3	30.0	20.0	10.0
	G1	2019	scion	Harlane	28.7 ± 10.2	36.7	16.7	3.3
	G1	2019	scion	Bergeron	43.2 ± 9.0	20.0	13.3	3.3
	G1	2019	scion	Goldrich	15.9 ± 9.5	40.0	16.7	3.3
	G2	2020	scion	Ungarishe Beste	57.2 ± 10.7	30.7	10.0	10.0
	G2	2020	scion	Closter Neuburger	25.5 ± 9.8	29.3	6.7	6.7
Italy	I1	2019	rootstock	Wavit	0.5 ± 0.4	0.0	0.0	0.0
	I1	2020	rootstock	Torinel	0.3 ± 0.4	0.0	0.0	0.0
	I1	2020	rootstock	Adesoto	1.6 ± 0.5	10.0	3.3	0.0
Poland	P1	2020	rootstock	Myrobalan	0.5 ± 0.4	0.0	0.0	0.0
Netherlands	N1	2019	rootstock	St. Julien	0.3 ± 0.4	0.0	0.0	0.0
Czech Republic	C1	2020	rootstock	St. Julien	0.3 ± 0.4	0.0	0.0	0.0

Note. [1] – fraction of Petri plate surface covered by bacterial colonies calculated for each propagating material (mean ± standard deviation); in each propagating material: [2] – percentage of apricot buds, from which *Pseudomonas*-like bacteria were isolated; [3] – average percentage of HR-positive *Pseudomonas* strains; [4] – average percentage of INA-positive *Pseudomonas* strains.

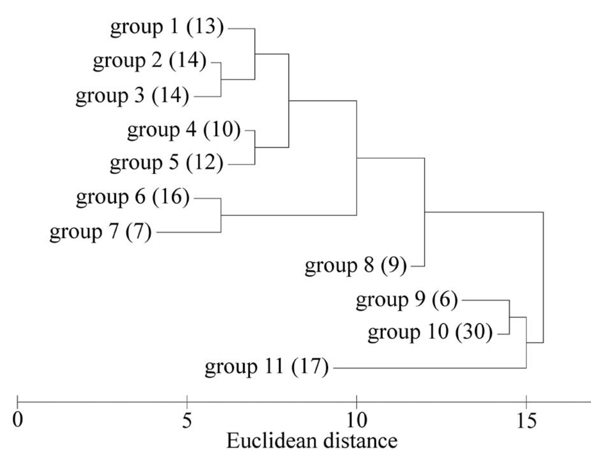
Table 2. Effect of locality on culturable bacterial population, on incidence of *Pseudomonas*-like bacteria, *Pseudomonas* strains positive in ice nucleation activity (INA) and hypersensitive reaction (HR) on tobacco leaves and pathogenic to detached apricot twigs according to ANOVA

Source of variation	Locality		
	MS	*F*	*p*
Culturable bacterial population	2665.141	15.458	0.002
Incidence of *Pseudomonas*-like bacteria	2795.766	15.870	0.001
Incidence of INA-positive *Pseudomonas* strains	69.306	4.432	0.054
Incidence of HR-positive *Pseudomonas* strains	438.903	8.419	0.012
Incidence of *Pseudomonas*-like strains	36.125	6.196	0.024

MS – mean square, F – variance distribution, $p \leq 0.05$ significant probability value

Several *Pseudomonas*-like colonies were isolated from the buds of the sample of rootstock 'Adesoto'. From 148 *Pseudomonas*-like strains tested for their ability to induce HR on tobacco leaves and INA, 91.9% and 37.8% were positive, respectively. Significantly more ($p \leq 0.05$) *Pseudomonas*-like strains, which induced HR and showed INA, were isolated from the samples of various cultivars collected in localities F1–F5 and G1–G2. Just one *Pseudomonas*-like strain isolated from the buds of the sample of rootstock 'Adesoto' induced HR.

FAME analysis. The subset of the 148 *Pseudomonas*-like strains was classified by the FAME method. Cluster analysis divided the strains into 11 FAME groups within a Euclidean distance of 3–16 (Figure 1).



Note. Numbers in brackets indicate the number of tested strains.

Figure 1. Cluster analysis of the fatty acid profiles of 148 *Pseudomonas*-like strains isolated from buds of apricot scion and rootstock samples

Seven FAME groups (FG) were in a Euclidean distance of 3–8. FG1 included *P. corrugata* and 3 pathovars of *P. syringae.* FG2–7 included 8 different *P. syringae* pathovars only, while FG8 and FG11 were composed of the mixture of strains identified as *P. syringae* and *P. viridiflava.* FG9 was the most diverse group with 7 different *Pseudomonas* species: *P. fluorescens*, *P. viridiflava*, *P. savastanoi*, *P. putida* and *P. viridiflava*, clustered into it. The strains which formed the largest group FG10 were identified as *P. putida. Pseudomonas* species diversity was similar in all localities and samples of scion materials.

Characterisation of strains from Ps complex. Phenotypic characterisation, pathogenicity, and phylogenetic distribution of a subset of 89 strains isolated from bud internal tissues of samples of propagating material in the *Ps* complex are shown in Table 3.

According to the results of *Psy*-PCR analysis, 93.3% of isolated *Pseudomonas* strains belonged to the *Ps* complex. Altogether, four *Psy*-PCR-negative strains

Table 3. Phenotypic characterisation, pathogenicity, survival ability and distribution in five phylogroups (PG) of the *Pseudomonas syringae* (*Ps*) complex of 89 *Pseudomonas* strains isolated from buds of apricot propagating material

Cultivar	Strain name	FAME identification	FAME group	HR[1]	INA[2] °C	*Psy*[3]	PG[4]	*syrB*[5]	Pathogenicity to apricot[6]	Survival Assay[7]
1	2	3	4	5	6	7	8	9	10	11
Bhart	1/20B	*Ps*	2	−	<−6.0	+	PG02	+	+	−
Harlayne	2/2	*P. viridiflava*	11	+	<−6.0	+	PG07–08	/	−	−
	2/24L	*Ps* pv. *atrofaciens*	5	+	<−6.0	+	PG02	+	+	−
Harogem	3/2	*Ps* pv. *solidagae*	11	+	−3.0	+	PG04	/	−	−
	3/12	*Ps* pv. *solidagae*	5	+	<−6.0	+	PG01	/	+	−
Leskora	7/14	*Ps* pv. *aptata*	5	+	−3.0	+	PG02	+	−	−
Congat	10/6/2	*Ps* pv. *syringae*	4	+	−4.0	+	PG02	+	++	−
	10/11/3	*Ps* pv. *maculicola*	7	+	−2.5	+	PG02	+	+	−
	10/12	*Ps*	11	+	<−6.0	+	PG07–08	/	−	−
	10/30/1	*Ps* pv. *syringae*	2	+	−3.5	+	PG02	+	+	+
Bergarouge	11/8/2	*Ps* pv. *syringae*	7	−	−3.0	+	PG02	−	+	−
	11/21/1	*Ps* pv. *maculicola*	8	+	−4.5	+	PG03	/	−	−
Anegat	12/5	*Ps* pv. *syringae*	2	+	−3.5	+	PG02	+	+	+++
	12/9/2	*Ps* pv. *syringae*	4	−	−2.5	+	PG04	/	+	−
	12/11	*P. viridiflava*	11	−	−6.0	+	PG07–08	/	−	−
	12/12	*Ps* pv. *atrofaciens*	4	+	−3.5	+	PG02	+	−	−
	12/13	*P. savastanoi*	4	+	−3.0	+	PG02	+	−	−
	12/14/1	*Ps* pv. *syringae*	7	−	−4.0	+	PG02	+	+	−
	12/17/2	*Ps* pv. *syringae*	2	+	−3.5	+	PG02	+	++	−
	12/24	*Ps* pv. *syringae*	1	+	−4.5	+	PG02	+	−	−
Bergeval	13/12/4	*Ps*	8	+	<−6.0	−	PG01/PG03	/	+++	+++
	13/15	*P. viridiflava*	11	+	<−6.0	+	PG07–08	/	−	−
	13/27/2	*Ps* pv. *syringae*	3	+	−5.0	+	PG02	+	+	−
Sefora	14/2/1	*Ps* pv. *syringae*	7	+	−2.5	+	PG02	+	+	−
	14/3/1	*Ps*	1	+	<−6.0	+	PG01	/	+	−
	14/8	*Ps* pv. *berberis*	1	+	<−6.0	+	−	−	+	+++
	14/10/1	*P. corrugata*	1	−	<−6.0	+	PG03	/	++	++
	14/23/2	unidentified	1	+	<−6.0	+	PG01	/	−	−
Elgat	15/11/1	*Ps* pv. *syringae*	3	+	−5.0	+	PG02	−	+++	+++
	15/22/2	*Ps* pv. *syringae*	6	+	<−6.0	+	PG04	/	−	−
	15/24	*Ps* pv. *syringae*	2	+	<−6.0	+	PG01	/	+	−
	15/30	*P. viridiflava*	11	+	<−6.0	+	PG07–08	/	−	−
Vertige	16/2/2	*P. corrugata*	1	+	<−6.0	−	PG01	/	+	−
	16/18/1	*Ps* pv. *syringae*	7	+	<−6.0	+	PG01/PG03	/	+++	++
	16/21/4	*P.* sp.	2	−	<−6.0	−	PG01	/	+++	−
Koolgat	17/2	*Ps* pv. *syringae*	6	−	−3.0	+	PG02	+	+	++
	17/17	*Ps* pv. *syringae*	6	+	<−6.0	+	PG03	/	+++	++
	17/21/2	*Ps*	6	+	<−6.0	+	PG07–08	/	+	−
	17/22/2	*Ps* pv. *syringae*	6	+	<−6.0	−	PG01	/	−	++
	17/25	*P. savastanoi*	5	+	<−6.0	+	PG03	/	+++	+++
	17/30/1	*Ps* pv. *syringae*	1	+	<−6.0	+	PG03	/	−	++

Table 3 continued

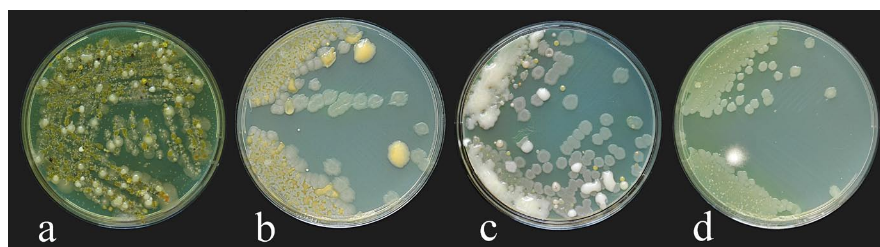
1	2	3	4	5	6	7	8	9	10	11
Kioto	18/6	*P. savastanoi*	5	+	<−6.0	+	PG03	/	+++	++
	18/11	*Ps* pv. *syringae*	4	+	−4.5	+	PG02	+	+	−
	18/12	*P. savastanoi*	3	+	−3.5	+	PG02	+	−	−
	18/14	*Ps* pv. *syringae*	4	+	−3.0	+	PG02	+	−	−
	18/26	*Ps* pv. *viburni*	3	+	<−6.0	−	PG01	/	+	−
	18/30	*Ps* pv. *syringae*	5	+	−2.5	+	PG02	+	+	−
Kuresia	19/7/2	*P. savastanoi*	5	+	<−6.0	+	PG04	/	−	−
	19/10/1	*Ps* pv. *syringae*	1	+	<−6.0	+	PG01	/	+	−
Digat	20/6	*Ps* pv. *syringae*	5	+	−4.0	+	PG02	+	+++	−
	20/7	*Ps*	1	+	<−6.0	+	PG01	/	++	+
	20/10/2	*Ps* pv. *atrofaciens*	8	−	−3.5	+	PG02	+	+++	+++
	20/30/1	*Ps* pv. *syringae*	2	+	−4.0	+	PG02	+	+	−
Ungarishe Beste	21/7	*Ps* pv. *syringae*	6	+	−5.0	+	PG02	+	+	−
	21/8	*Ps* pv. *aptata*	8	+	−5.0	+	PG02	+	−	+++
	21/15/2	*Ps* pv. *syringae*	4	+	<−6.0	+	PG02	+	+	−
	21/18/1	*Ps* pv. *syringae*	2	+	−3.0	+	PG04	/	−	−
Harlane	22/9/2	*P. savastanoi*	5	+	<−6.0	+	PG01	/	+	−
	22/22	*P. viridiflava*	11	+	<−6.0	−	PG07–08	/	+	−
	22/27	*Ps* pv. *syringae*	6	+	−3.5	+	PG02	+	+	−
Bergeron	23/5	*Ps* pv. *syringae*	6	+	−2.5	+	PG01	/	+	−
Goldrich	24/4	*Ps* pv. solidagae	1	+	<−6.0	+	PG01/PG03	/	+++	+++
	24/25	*Ps*	5	+	<−6.0	+	PG01	/	++	−
	24/26/1	*Ps* pv. *syringae*	8	−	−3.0	+	PG02	+	+	−
Vertige	25/1	*Ps* pv. *syringae*	6	+	−2.5	+	PG04	/	−	−
	25/3	*Ps* pv. solidagae	3	+	−3.0	+	PG02	+	+++	−
	25/4	*Ps* pv. *atrofaciens*	8	+	−3.0	+	PG01	/	+	−
	25/6	*Ps* pv. *syringae*	6	+	−4.0	+	PG02	+	+	−
	25/7B	*Ps* pv. *syringae*	7	+	−2.5	+	PG02	+	++	−
Bergarouge	26/8	*Ps* pv. *syringae*	7	+	−2.5	+	PG02	+	+++	−
	26/15B	*Ps* pv. *atrofaciens*	8	+	−3.0	+	PG04	/	++	−
Congat	27/2	*Ps* pv. *atrofaciens*	8	+	−3.0	+	PG01	/	++	−
	27/4	*Ps* pv. *syringae*	2	+	<−6.0	+	PG04	/	+	−
	27/8	*Ps* pv. *syringae*	6	+	−3.0	+	PG02	+	++	−
Koolgat	28/2	*Ps* pv. *syringae*	2	+	−3.5	+	PG02	+	+++	−
	28/4	*Ps* pv. *syringae*	4	+	−4.5	+	PG02	+	++	−
	28/7	*Ps* pv. *atrofaciens*	8	+	−3.5	+	PG02	+	++	−
	28/9	*Ps* pv. *glycineae*	6	+	−3.0	+	PG02	+	++	−
	28/12	*Ps*	3	+	−2.5	+	PG01	/	+	−
	28/18	*Ps* pv. *glycineae*	3	+	−4.0	+	PG04	/	+	−
Ungarishe Beste	29/2	*Ps*	3	+	−2.0	+	PG03	/	+++	−
	29/7	*Ps* pv. *glycineae*	3	+	−3.0	+	PG02	+	++	−
	29/9	*P. fluorescens*	3	+	−3.0	+	PG01	/	+	−
Closter	30/4	*Ps* pv. *glycineae*	5	+	−2.5	+	PG01	/	+	−
Neuburger	30/11	*Ps*	5	+	−3.0	+	PG01	/	+	−
Tsunami	31/1	*Ps* pv. *tabaci*	6	+	<−6.0	+	PG07–08	/	+	−
Pinkcot	32/3	*Ps* pv. *syringae*	4	+	−5.5	+	PG02	+	++	−
Spring Blush	33/2B	*Ps* pv. *glycineae*	4	+	<−6.0	+	PG04	/	++	−
Adesoto	34/1	*Ps*	2	+	<−6.0	+	−	−	+	+

[1] – hypersensitive reaction to tobacco leaves: + – positive, − – negative; [2] – ice nucleation activity: + – positive 0–−6.0°C, − – negative <−6.0°C; [3] – *Ps* complex classification (Berge et al., 2014): + – *Psy*-PCR product present, − – no *Psy*-PCR product; [4] – *P. syringae* phylogroups: PG01–04 (Borschinger et al., 2016) and PG07–08 (Bartoli et al., 2014); [5] – syringomycin synthesis (Sorensen et al., 1998): + – *syrB*-PCR product present; − – no *syrB*-PCR product; [6] – no symptoms on apricot twigs; + – withered twigs or superficial necrosis on apricot leaves; ++ – necrosis ≤0.5 cm on apricot leaves; +++ – necrosis ≥0.5 cm on apricot leaves; [7] – survival ability level (Figure 2): − – negative survival ability; + – weak survival ability; ++ – medium survival ability; +++ – strong survival ability; / – not tested

belonged to PG01, and one to PG01–03 and PG07–08 each. These strains caused superficial necrosis on apricot leaves of detached apricot twigs and exhibited different ability to survive within epiphytic microflora on Petri dishes (Figure 2).

The only strain 34/1 isolated from buds of rootstock ‘Adesoto’ and strain 14/8 isolated from buds of a scion sample of the apricot cultivar ‘Sefora’ were not attributed to any typical apricot phylogroup. *Pseudomonas* strain 14/8 was pathogenic to apricot and showed a strong ability to survive within epiphytic microflora in the survival assay, and *Pseudomonas* strain 34/1 had a weak ability to survive on the surface of apricot leaves (Table 3). They showed a rather low (about 95%) sequence similarity to *P. syringae* pv. *avii* and *P. syringae* pv. *cerasicola*, respectively (Table 4).

Nearly one half (44.9%) of the subset of 89 *Pseudomonas* strains presented in Table 3 were attributed to PG02 of the *Ps* complex. PG02 strains were isolated from the vast majority (87.9%) of scion samples. The *syrB* gene was detected in 95% of PG02 strains, 82.5% of PG02 strains showed pathogenicity towards apricot, 17.5% of PG02 strains showed a certain ability to survive within epiphytic microflora in the survival assay, and



Note. The levels of survival ability: negative (a), weak (b), medium (c) and strong (d) (see Materials and methods section).

Figure 2. Survival ability assay of four *Pseudomonas* strains isolated from buds of apricot propagating material on the leaf surface of the apricot cultivar 'Bergarouge' within epiphytic microflora

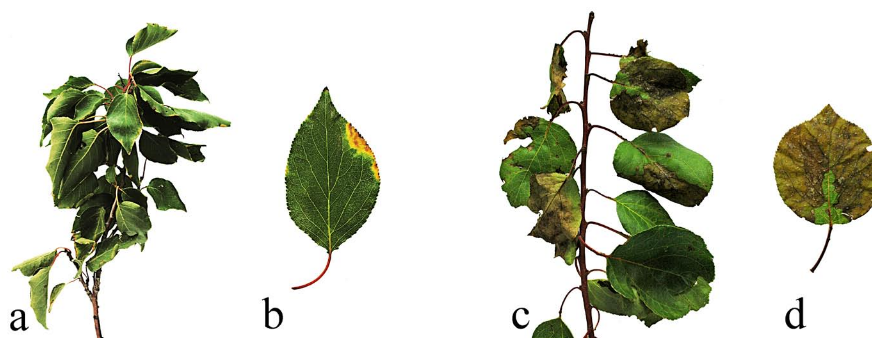
Table 4. Identification of a subset of 26 strains from *Pseudomonas syringae* (*Ps*) complex isolated from buds of apricot propagating material, based on partial *rpoD* gene sequences

Strain name	Phylogroup (PG)	Average nucleotide identity based on Basic Local Alignment Search Tool (BLAST)	Sequence similarity %
14/8	/	*Ps* pv. *avii* strain ICMP 14479	95.20
14/23/2	PG01	*Ps* pv. *syringae* strain SM	97.24
17/25	PG03	*Pa* pv. *tabaci* strain ATCC 11528^T	97.90
18/6	PG03	*P. savastanoi* pv. *phaseolicola* strain 1449B	99.79
24/25	PG01	*Ps* pv. *apii* strain ICMP 11947	96.88
3/12	PG01	*Ps* pv. *avii* strain ICMP 14479	92.47
13/12/4	PG01–03	*Pa pv. morsprunorum* strain FTRS_U7805	99.77
13/15	PG07–08	*P. viridiflava* isolate p6D10	99.38
3/2	PG04	*Ps* CC94	99.38
12/11	PG07–08	*P. viridiflava* isolate p26.C9	99.18
15/30	PG07–08	*P. viridiflava* strain ICMP 11296	91.84
13/27/2	PG02	*Pseudomonas syringae* strain DSM 10604	98.92
16/21/4	PG01	*Pseudomonas avellanae* strain BPIC 631^T	95.02
19/7/2	PG04	*Ps* pv. *solidagae* strain ICMP 16926	99.29
19/10/1	PG01	*Ps* pv. *tomato* strain DC3000	95.02
22/22	PG07–08	*P. viridiflava* isolate p2D10	99.34
22/30/1	PG02	*Ps* strain DSM 10604	95.60
20/6	PG02	*Ps* pv. *syringae* strain ICMP 3688	99.36
12/5/2	PG02	*Ps* strain P108	99.60
16/18/1	PG01–03	*Pa* pv. *morsprunorum* strain ICMP 4983	99.01
17/21/2	PG07–08	*P. viridiflava* strain ICMP 3272	95.46
17/17	PG03	*Pa* pv. *morsprunorum* strain ICMP 4983	99.18
10/12	PG07–08	*P. viridiflava* strain ICMP 11296	95.60
21/8	PG02	*Ps* strain DSM 10604	98.90
17/22/2	PG01	*Ps* pv. *avii* strain ICMP 14479	95.30
34/1	/	*Ps* pv. *cerasicola* strain CFBP 6110	95.03

/ – not identified

87.5% of PG02 strains were INA-positive. The *rpoD* sequences of 5 pathogenic and INA-positive PG02 strains showed a high similarity to sequences of *P. syringae* pv. *syringae* (Table 4).

The second most numerous phylogroup, PG01, included 22.5% of the subset of 89 *Pseudomonas* strains. PG01 strains were isolated from 50% of scion samples. Most of PG01 strains were pathogenic to apricot (80%), 30% were INA-positive, and 15% showed the ability to survive within epiphytic microflora (Table 3). Their *rpoD* sequences were close to sequences of different *P. syringae* (92.47–97.24%) and *P. avellanae* (95.02%) pathovars. Altogether 11.2% of *P. syringae* strains from 10 different scion samples (30%) were attributed to PG04. Almost half of PG04 strains (45.5%) were slightly pathogenic to apricot (Figure 3b) and INA-positive (54.5%), and all of them showed little or negative ability to survive within leaf epiphytic microflora (Figure 2a; Table 3). The *rpoD* sequences of strain 19/7/2 showed high similarity (99.29%) to sequences of *P. syringae* pv. *solidagae* (Table 4).



Note. Comparison of pathogenic response on apricot detached twigs: "apricot epiphytic" PG04 (a, b) and "apricot pathogenic" PG03 (c, d).

Figure 3. Pathogenicity test of *Pseudomonas syringae* strains on detached apricot twigs

Seven *Pseudomonas* strains were attributed to PG03. These strains were isolated from 5 scion samples (15.1%). Half of PG03 strains were highly pathogenic to detached apricot twigs (Figure 3c, d) and strongly positive in the survival assay (Figure 2d; Table 3). Two strains from this phylogroup were INA-positive (Table 3). Partial sequences of the *rpoD* gene showed a close similarity to sequences of *Pa* pv. *morsprunorum*, pv. *tabaci* and *P. savastanoi* pv. *phaseolicola* (Table 4).

According to the results of PCR used for the distribution of *P. syringae* strains across the different phylogroups, three *Pseudomonas* strains were placed in PG01 and PG03 simultaneously (Table 3). These strains were highly pathogenic to apricot twig, strongly positive in the survival assay, were INA-negative, and their *rpoD* sequences were close to sequences of the PG03 reference strains *Pa* pv. *morsprunorum* FTRS_U7805 and ICMP4983 (Table 4).

Eight *Pseudomonas* strains attributed to PG07–08 were negative in the survival assay and non-pathogenic or slightly pathogenic to apricot (Table 3). Only one strain was INA-positive. The *rpoD* sequences of PG07–08 strains showed a high similarity to sequences of *P. viridiflava* (Table 4).

Discussion

Introduction of new apricot cultivars highly sensitive to *P. syringae* bacteria was considered to be one of the main reasons for the apricot dieback outbreaks over the last 10 years. The sudden death of 3–5-year-old almost symptomless apricot saplings usually takes place in spring. Isolated minor injuries are not sufficient for the penetration of a large population size of *Pseudomonas* pathogen inoculum, which would be able to cause the sudden death of apricot saplings. Given the young tree age and the absence of external symptoms of the disease, the possibility of systemic infection in the production of apricot trees was considered. Large scale dissemination can occur, when apparently healthy but latently infected propagating material is introduced. The pathogen can be transmitted by bud grafting; it overwinters in dormant buds and colonises vascular tissues of young trees and the surface of twigs and leaves in spring (Scortichini, 2010; Havenga et al., 2019).

Despite the different origin of the rootstocks, a lower count of culturable bacterial populations in the internal tissues of all tested buds was determined. Contrary to this, the culturable bacterial population in the buds of scions varied in a wide percentage range from 0.1% in the sample of the cultivar ‘Darina’ from the Czech locality C1 to 59.2% from the French locality F5. As the total culturable bacterial population in scion buds increased, a higher portion of *Pseudomonas*-like colonies was observed. *Pseudomonas*-like strains were isolated from 85% of scion samples originated in all localities involved in this study. According to the results of ANOVA, there was a significant variation ($p \leq 0.05$) of the incidence of *Pseudomonas* strains pathogenic to detached apricot twigs and positive in INA and HR between localities. This is probably due to the average ages of apricot scion mother orchards. Older apricot orchards have been pruned for longer accumulating more wounds on the trees, which have been exposed to aerial inoculum for longer (Havenga et al., 2019). In warmer climate, the colonisation via leaf scars was found to be less effective than wounds. Consequently, mild winter and exceptional spring frost do not dramatically favour the penetration and/or the spreading and expressing of causal agents of bacterial canker (Schortichini, 2010).

FAME analysis has been previously reported as a reliable method for the identification of *Pseudomonas* spp. at the species and the pathovar level (Donmez et al., 2010). Almost one-quarter of the subset of 148 *Pseudomonas*-like strains was included in FAME groups FG9 and FG10 comprised of HR- and INA-negative *P.fluorescens* and *P. putida* complex strains, respectively. These strains constituted an important part of the endophytic bacterial community of the internal tissues of buds. From their multifunctionality, plant-growth-promoting and antagonistic abilities, their role in nutrient fixation and solubilization has been described thus far (Visnovsky et al., 2019). Altogether, FG1–8 and FG11 encompassed 89 strains of HR- and/or INA-positive *Pseudomonas* spp. and *P. syringae*, whose pathogenicity to apricot and their phylogenetic context in the *Ps* complex was further determined (Guilbaud et al., 2016).

Based on recent studies (Borschinger et al., 2016; Parisi et al., 2019), *Pseudomonas* strains were tested with apricot relevant phylogroup’s primer pairs of PG01–03 and PG07–08. Due to the quantification and identification of *Pseudomonas* species from different environmental sources that may contaminate buds (Morris et al., 2019), the determination of PG04 has been included in this study. The pathogenicity test on the detached apricot twigs and the survival assay showed the competence of *Pseudomonas* strains isolated from internal tissues for adaptation to life on the leaf surface and the ability to establish an epiphytic population after a single spray inoculation. Additionally, a small subset of 26 *Pseudomonas* strains representing strains without phylogroup determination, strains co-assigned into two phylogroups, and strains from individual phylogroup with different levels of pathogenicity to the apricot was sequenced and similarity searching was performed on the GenBank database.

The most numerous groups of strains isolated from internal tissues of scion samples were attributed to PG02. The *rpoD* sequences of PG02 representatives showed a high similarity to sequences of collection strains *P. syringae* pv. *syringae* DSM10604 and ICMP3688, the main causal agent of cork necrosis and the premature death of apricots in commercial orchards (Lamichhane et al., 2014). PG02 strains were pathogenic to apricot and INA-positive but showed a weak ability to survive on leaves within epiphytic microflora (Hirano, Upper, 2000). When living as an endophyte, *P. syringae* can systematically spread within the twigs, also when frost injuries are not present. During the epiphytic life, it can grow and survive on healthy leaves and in autumn colonise and penetrate into the tree through the leaf scars (Scortichini, 2010). The *rpoD* sequences of strains attributed to PG01 did not show a significant similarity to sequences of any *P. syringae* pathovar determined to be pathogenic to apricot. PG01 strains caused just small superficial necrosis on detached apricot twigs, and their ability to survive within epiphytic microflora was weak.

In total, one-tenth of all isolated strains isolated from scion samples were attributed to the “environmental” group PG04. Their *rpoD* sequences showed a similarity to the broad spectrum of *P. syringae* pathovars reported from diverse cropped and wild plants (Berge et al., 2014). Thus, knowledge of various reservoirs of *Pseudomonas* bacteria and their traits relative to the aptitude to survive and spread in apricot orchards is particularly pertinent

for implementing preventive measures (Morris et al., 2019). In agreement with the description by Bartoli et al. (2014) and Lamichhane et al. (2015), strains attributed to PG07–08 included apricot non-pathogenic, INA-negative strains with *rpoD* sequences highly similar to sequences of *P. viridiflava*. According to Bartoli et al. (2014), *P. viridiflava* maintains a high level of adaptability, both as a saprophyte and as a pathogen.

The pathogenicity test on detached apricot twigs showed that the most pathogenic *Pseudomonas* strains were attributed to PG03. *P. syringae* strains were isolated from seven scion samples and four different localities in France and Germany. They caused severe symptoms to apricot and showed the ability to survive on the surface of apricot leaves within epiphytic microflora. Their *rpoD* sequences showed a high similarity to *P. savastanoi* pv. *phaseolicola* strain 1449B and *Pa* pv. *morsprunorum* strain ICMP 4983. According to recent studies, PG03 is attributed with a causal agent of premature death of apricot, *Pa* pv. *morsprunorum* (Parisi et al., 2019), and other pathogens from woody plants (Berge et al., 2014). Three *Pseudomonas* strains highly pathogenic to detached apricot twigs with a strong ability to survive on the surface of apricot leaves within epiphytic microflora were isolated from localities F2, F5, and G1 and co-assigned to PG01 and PG03. Their *rpoD* sequences showed a high similarity to the reference strain FTRS_U7805 *Pa* pv. *morsprunorum* rase 1 in PG03 (Gomila et al., 2017). This case of misallocation of the phylogroups by PCR can be explained by the extreme genetic variability within the *Ps* complex (Berge et al., 2014; Borschinger et al., 2015).

Conclusions

1. Regardless of the origin of the apricot rootstocks, no pathogenic bacteria from the genus *Pseudomonas* were isolated from the internal tissues of the bud.

2. The results demonstrated a broad spectrum of *Pseudomonas* bacteria isolated from the buds of most apricot scion samples. Altogether, 80% of isolated strains included into 5 phylogroups (PG) of *Pseudomonas syringae* (*Ps*) complex were pathogenic to detached apricot twigs and ice nucleation activity (INA)-positive.

3. Altogether, 5% of highly pathogenic *Pseudomonas* strains isolated from the buds of different apricot scion samples originated in localities F2, F5 (in France) and G1 (in Germany) were attributed to PG02 and PG03. The *rpoD* sequencing confirmed a similarity to strains of *P. syringae* pv. *syringae* and *P. amygdali* pv. *morsprunorum* known to be pathogenic to apricot, respectively. The occurrence of these pathogens in buds of scion materials increases the risk of bacterial canker and the premature death of young apricot trees.

4. The results of this experiment displayed the necessity to screen propagating material of apricot for the causal agent of bacterial canker and to improve management practices in scion mother orchards.

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Bakterinio vėžio sukėlėjai *Prunus armeniaca* dauginamojoje medžiagoje

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Santrauka

Tyrimo metu siekta išsiaiškinti, ar įvairios kilmės dauginamosios medžiagos vidiniuose audiniuose yra bakterinio vėžio ir ankstyvos abrikosų žūties sukėlėjų. Dėl patogeninių *Pseudomonas* rūšių buvo ištirti 33 paprastojo abrikoso (*Prunus armeniaca* L.) atžalų mėginiai iš aštuonių ir šeši šaknų atžalų mėginiai iš keturių Europos vietovių. Nustatyti reikšmingi kultivuojamų bakterijų populiacijos įvairovės ir *Pseudomonas* bakterijų paplitimo poskiepių ir šaknų atžalų pumpurų vidiniuose audiniuose skirtumai. Daugumoje šaknų atžalų *Pseudomonas* bakterijų neaptikta. Iš poskiepių mėginių išskirti 148 *Pseudomonas* kamienai daugiausia buvo surinkti iš F1–F5 (Prancūzijoje) ir G1 bei G2 (Vokietijoje) vietovių. Atlikus riebalų rūgščių metilo esterių (FAME) analizę, jos buvo suskirstytos į 11 grupių; 91,9 % iš jų galėjo sukelti didesio jautrumo reakciją ant tabako lapų, o 37,8 % turėjo teigiamą ledo kristalų susidarymo audiniuose aktyvumą. 89 *Pseudomonas* kamienų rinkinys buvo apibūdintas pagal *Psy*-PGR, nukreiptą į *Pseudomonas syringae* (*Ps*) kompleksą, kompleksinę filogrupę (PG), *syr*B-PGR, nukreiptą į siringomicino sintezę, patogeniškumą ant atskirtų abrikosų šakelių ir gebėjimą išgyventi epifitinėje mikrofloroje. PG01, PG04 ir PG07-08 filogrupėms priskirti *Pseudomonas* kamienai buvo šiek tiek patogeniški atsiskyrusioms abrikosų šakelėms. 5 % *Pseudomonas* kamienų, itin patogeniškų atskirtoms abrikosų šakelėms, buvo išskirti iš abrikosų poskiepių mėginių, paimtų F2, F5 ir G1 vietovėse. Šie kamienai buvo priskirti PG02 ir PG03 filogrupėms, o *rpoD* sekos nustatymas patvirtino panašumą į *P. syringae* pv. *syringae* ir *P. amygdali* pv. *morsprunorum* kamienus, kurie, kaip žinoma, yra patogeniški abrikosams. Šių patogenų atsiradimas poskiepių pumpuruose padidina bakterinio vėžio riziką ir ankstyvą jaunų abrikosų medelių žūtį.

Reikšminiai žodžiai: abrikosas, poskiepis, šaknų atžala, *Pseudomonas amygdali* pv. *morsprunorum*, *Pseudomonas syringae* pv. *syringae*.