



The occurrence of bacteria from different species of *Pectobacteriaceae* on seed potato plantations in Poland

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Abstract Bacteria from the genera *Dickeya* and *Pectobacterium*, the causative agents of soft rot and blackleg, trigger significant economic losses in potato production worldwide. Efficient struggle with these phytopathogens is highly challenging taking into consideration the lack of available control procedures. As only preventive measures are accessible, we decided to provide insight into the soft rot *Pectobacteriaceae* (SRP) present in Poland. During the growing seasons of 2013 and 2014, altogether 531 potato plants were collected from 138 seed potato fields and 23 storage facilities. Plant origin of the isolated bacteria, frequencies of coinfections with different species, the affected potato cultivars in addition to seasonal variation in the occurrence of SRP were studied. It was shown that bacteria from the *Pectobacterium* genus were abundant and outnumbered the ones classified to *Dickeya* spp. The vast majority of strains was isolated from the plant samples collected in July 2013 or in June–July 2014. The presence of all taxa of interest: *Pectobacterium*

atrosepticum, *Pectobacterium carotovorum*, *Pectobacterium parmentieri*, *Dickeya dianthicola* and *Dickeya solani* were confirmed in July each year. We were able to isolate bacteria from the genus *Dickeya* and *Pectobacterium* from 35 out of 58 potato cultivars tested. The majority of SRP was isolated from potato stems, not from potato tubers. In four cases, coinfections of potato samples with even three diverse species of SRP, i.e. *P. atrosepticum*, *P. carotovorum* and *P. parmentieri*, were noted. It seems that since the first documented appearance of *Dickeya solani* in Poland in 2005, this pathogen has not played a dominating role in our country. The reported data describing the appearance and distribution of SRP in Poland might allow for prediction of the risks associated with infections initiated by these bacteria.

Keywords Blackleg · Soft rot · *Dickeya* spp. · *Pectobacterium* spp. · Pectinolytic erwinias · Soft rot *Enterobacteriaceae*

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Introduction

Dickeya and *Pectobacterium* spp. are the causative agents of blackleg of potato and soft rot on various plant species. These phytopathogenic bacteria underwent numerous taxonomic reclassifications and were designated to miscellaneous species, genera and families (Gardan et al. 2003; Samson et al. 2005). Previously, they had been referred to as soft-rot *Enterobacteriaceae* or pectinolytic erwinias (Perombelon and Kelman 1980).

However, due to recent revision in their phylogeny based on the sequences of 1548 core proteins and 16S rDNA, *Dickeya* and *Pectobacterium* spp. belong now to a newly established *Pectobacteriaceae* family (Adeolu et al. 2016). Therefore, the term soft-rot *Pectobacteriaceae* (SRP) seems suitable with reference to these disease-causing bacteria (Motyka et al. 2017).

SRP exhibit a rather broad host range as they are capable of infecting at least 35% of angiosperm plant orders (Ma et al. 2007) including crops, vegetables and ornamentals e.g. potato, tomato, maize, cabbage, beetroot, chicory, pepper, carrot, chrysanthemum or saintpaulia (African violet). Potato plants gained special attention as they belong to the top five crops (after sugar cane, maize, rice and wheat) with a rising production trend from 327,600 Kt in 2000 to 368,096 Kt in 2013 (Food and Agriculture Organization of the United Nations (FAO), 2015). A large scale potato cultivation started in the eighteenth century and up to 1970 Poland produced 50 million tonnes of potatoes a year, being the 2nd largest producer in the world, following the Soviet Union (Zarzecka 2009). Currently, Poland is the 7th producer of potato worldwide behind China, India, Russia, Ukraine, USA and Germany (FAO 2012).

A high significance of *Dickeya* and *Pectobacterium* spp. was underlined by Mansfield et al. (2012) as they were listed on positions 9th and 10th, respectively, among the top ten plant pathogenic bacteria. It is challenging, however, to assess the worldwide economic impact of SRP on potato production, especially taking into consideration the common occurrence of these pathogens in diverse geographical zones, variable crop pricing and lack of uniform seed certification policies in different countries (Motyka et al. 2017; Toth et al. 2011). Additional obstacles are associated with the fact that *Dickeya* and *Pectobacterium* cause losses in the fields, during harvest, storage and marketing of the crop (Perombelon 2002). Over 40 years ago, Perombelon and Kelman (1980) estimated the total economic damage associated with SRP to be $50\text{--}100 \times 10^6$ US dollars annually on a worldwide basis. More recent data originate from the Netherlands, where direct losses connected with rejections and downgrading of seed tubers amount to 30 million euros a year (Toth et al. 2011).

Blackleg and soft rot are considered seed-borne diseases. However, *Dickeya* and *Pectobacterium* spp., aside from spreading from the affected mother tuber (latently infected seed potato), are capable of penetrating plant or tuber tissue through natural openings (stomata

and lenticels) or wounds. Also, the contaminated surface or irrigation waters, soil, plant remains, insects, nematodes or agricultural machines not meeting hygiene standards can be a source of infection (Cappaert et al. 1988; Nykyri et al. 2014; Rossmann et al. 2018; van der Wolf and Kastelein 2014). Once virulent strains reach the vulnerable host under disease-promoting environmental conditions (Barrett et al. 2009), typical SRP-triggered syndromes develop (Perombelon and Kelman 1980). Soft rot disease involves water-soaked, slimy maceration of the inner potato tuber tissue, while blackleg is associated with decay of the stem base followed by chlorosis, wilting leaves and lack of progeny tubers (Perombelon 2002). These outcomes result mainly from the action of plant cell wall degrading enzymes i.e. pectinases, cellulases and proteases (Hugouvieux-Cotte-Pattat et al. 1996).

Currently, no control methods have been successfully implemented in order to eradicate *Dickeya* and *Pectobacterium* spp. after infecting susceptible crops (Toth et al. 2011). Therefore, the only preventive measures routinely applied to limit the spread of SRP are regular controls of seed plantations and removal of the infected plants. Our group has been monitoring the presence of SRP on seed potato fields of Poland since 1996. At that time, *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* species turned out to be widespread (Sledz et al. 2000; Waleron et al. 2002). Our studies that followed indicated also common presence of *Pectobacterium parmentieri* (earlier *Pectobacterium wasabiae*) on potato plantation (Waleron et al. 2013; Motyka et al. 2017; Zoledowska et al. 2018b). The first appearance of *Dickeya solani* in Poland was noted during the routine testing of seed potato plantation in 2005 (Slawiak et al. 2009). According to our data, solely *D. solani* and *Dickeya dianthicola* have been isolated from potato plant/tuber samples in Poland, while *Dickeya zaeae* and *Dickeya chrysanthemi* were detected in the investigated surface water samples (Potrykus et al. 2016). Finally, our special attention was driven to the recently established species, namely *D. solani* (van der Wolf et al. 2014) and *P. parmentieri* (Khayati et al. 2016). In spite of significant genomic homogeneity among the studied *D. solani* isolates, we showed big differences in their virulence (Potrykus et al. 2016; Golanowska et al. 2017, 2018). On the contrary, a high divergence in the virulence factors production, correlating with variations in the genomic structure, has been revealed in *P. parmentieri* strains (Zoledowska et al. 2018a,

2018b). It is worth noting that *P. parmentieri* strains formed a significant group within the SRP population in Poland already in 1996, though, being misclassified to *Pectobacterium carotovorum* subsp. *carotovorum*, they remained unnoticed (Waleron et al. 2013).

As our former studies mainly focused on phenotypic and genotypic characterisation of solely *D. solani* and *P. parmentieri* (Golanowska et al. 2017, 2018; Potrykus et al. 2016; Zoledowska et al. 2018a, b), in this work we analysed the prevalence of various groups of *Dickeya* and *Pectobacterium* spp. on seed potato plantations in Poland in the years 2013 and 2014. In more detail, the sources of SRP, seasonal variation in their occurrence, and the type of fertilizing practices applied to the seed potato fields yielding *Dickeya* or *Pectobacterium* isolates were investigated. The collected data may contribute to the description of environmental factors favouring infections of plants with different SRP.

Materials and methods

Plant material

Altogether, 531 samples of potato plants with the symptoms of blackleg and/or soft rot, soft rot-affected potato tubers and asymptomatic weeds accompanying the diseased potato plants were collected in Poland from 138 seed potato plantations (from May to September) or from 23 storage facilities (from October till December), mainly by the inspectors of Regional Inspectorates of Plant Health and Seed Inspection Service in Poland (RIPH & SIS) in the years 2013 and 2014. Also, several of these samples were acquired from the collaborating institutions: Institute of Plant Breeding and Acclimatization - Jadwisin and Mlochow Research Centers, Potato Breeding Zamarte, Plant Breeding in Szyldak and Pomeranian-Mazurian Potato Breeding in Strzekecino. Together with the plant tissue provided in separate string bags to the Laboratory of Plant Protection and Biotechnology of the Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk (IFB UG & MUG), the inspectors or cooperating growers attached detailed surveys on the geographical origin and collection date of the samples, the planted potato variety and the fertilizing methods applied to the corresponding field as described by Potrykus et al. (2016).

Altogether, 248 plant samples from 2013 (124 potato stems, 74 potato tubers, and 50 accompanying weeds; Table 1) and 283 from 2014 (153 potato stems, 87 potato tubers, and 43 weeds; Table 1) were subjected to analysis. Material from 58 diverse potato varieties was sampled. The plant samples were collected from 13 voivodeships in 2013 and 12 in 2014 (out of 16 in Poland; Supp. Figure 1).

Isolation and to-species identification of SRP

SRP were isolated as described previously by Potrykus et al. (2016) and Zoledowska et al. (2018b). Briefly, 1 g of the sampled plant material was suspended in 10 ml of 50 mM phosphate buffer pH 7.2 and subsequently homogenized with a hand homogenizer in a universal 12 × 15 cm extraction bag (Bioreba, Switzerland). The resultant plant homogenate was serially diluted to 10^{-6} in sterile 0.85% NaCl and then 100 μ l of the 10^{-5} and 10^{-6} dilutions were plated on two Crystal Violet Pectate (CVP) plates (Hélias et al. 2012). Subsequently, the CVP plates were incubated for 48 h at 28 °C. If no bacterial growth was observed, the plating procedure was repeated with less diluted plant homogenates. Bacterial colonies that formed characteristic cavities were collected and replated in a reductive manner on CVP and TSA media until the axenic culture state was reached. All isolates were subjected to multiplex PCR-based identification (Potrykus et al. 2014). At first, bacterial lysates were prepared by freezing the biomass of one colony per strain in 200 μ l of distilled water at -20 °C for 20 min. Then, the multiplex PCR-reaction was performed as described previously by Potrykus et al. (2014). *P. atrosepticum* strains were assigned to the species level basing on the acquisition of a specific amplicon of 439 bp with the Y₄₅ and Y₄₆ primers (Frechon et al. 1998; Potrykus et al. 2014). *Dickeya* spp. strains were identified by amplification of a specific DNA fragment of 133 bp with the use of Df and Dr starters (Laurila et al. 2008; Potrykus et al. 2014). In order to identify *Dickeya* strains to the species level we analysed the *dnaX* gene sequence and performed the rep-PCR according to Potrykus et al. The strains yielding PCR amplicons with the ExpccF and ExpccR primers (550 bp; Kang et al. 2003; Potrykus et al. 2014) in the multiplex PCR reaction were further tested with a *P. parmentieri*-specific single PCR reaction accordingly to De Boer et al. (2012). The strains that gave specific amplicons with PhF and PhR primers (De Boer et al. 2012) were attributed to the *P. parmentieri* species, while the ones that yielded specific amplicons just with

Table 1 Plant origin, months of collection, fertilizing methods and the number of the isolated SRP in the years 2013 and 2014. The numbers of the plant samples fulfilling the depicted requirement are listed

Plant origin	2013							2014						
	No. of obtained plants			No. of isolated strains				No. of obtained plants			No. of isolated strains			
	SRP ^a	Pba	Pc	Pba	Pc	Ppa	Dsp	SRP ^a	Pba	Pc	Ppa	Dsp		
Seasonal collection														
Fields	124	18	21	18	5	153	90	33	28	21	8			
Blackleg-affected potato stems														
Soft rotten potato tubers	74	7	13	11	2	87	25	9	10	4	2			
Accompanying weeds	50	1	2	3	0	43	9	5	1	3	0			
Total plants	248	101	26	36	32	283	124	47	39	28	10			
May	4	0	0	0	0	0	0	0	0	0	0			
June	7	2	0	1	1	0	83	16	18	16	9			
July	193	66	23	23	19	1	158	59	31	17	10			
August	24	17	0	6	5	6	18	2	0	1	1			
September	3	4	1	1	2	0	12	2	0	2	0			
October	15	9	0	5	4	0	8	0	0	0	0			
November	0	0	0	0	0	0	4	2	0	1	1			
December	2	3	2	0	1	0	0	0	0	0	0			
Fields + storage	248	101	26	36	32	7	283	124	47	39	28			
Fertilizing methods														
NPK fertilizers	100	32	3	11	13	5	75	36	10	11	12			
NPK + other minerals	38	19	8	6	4	1	37	12	5	1	3			
NPK + organic	40	29	5	13	11	0	41	23	10	8	3			
Organic	45	14	8	2	3	1	56	25	15	8	1			
No data	9	1	1	0	0	0	49	21	5	7	8			
NPK + minerals + organics	16	6	1	4	1	0	25	7	2	4	1			
All types of fertilizing	248	101	26	36	32	7	283	124	47	39	28			

SRP - soft rot *Pectobacteriaceae*, Pba - *P. atrosepticum*, Pc - *P. carotovorum*, Ppa - *P. parmentieri* and Dsp - *Dickeya* spp.

^a infections with diverse SRP within one plant sample have been counted separately

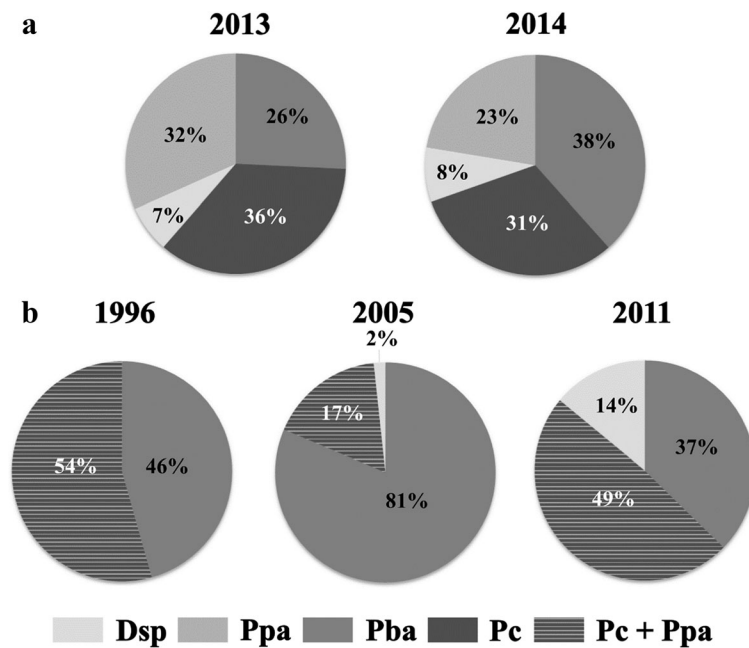


Fig. 1 The most frequently isolated groups of soft rot *Pectobacteriaceae* (SRP) during long-term monitoring of seed potato plantations in Poland **a** Prevalence of distinct *Pectobacteriaceae* revealed within the herein described study of 2013 and 2014. **b** The dominating species from the *Pectobacteriaceae* family according to the previous surveys from

years 1996, 2005 and 2011. From the lightest tint: – *Dickeya* spp. (Dsp), – *P. parmentieri* (Ppa), – *P. atrosepticum* (Pba), – *P. carotovorum* (Pc). In the years 1996, 2005 and 2011, Pc was not distinguished from Ppa, therefore the occurrence of these two species is depicted together in a striped pattern -

ExpccF and ExpccR starters and negative in the *P. parmentieri*-specific test were assigned to *P. carotovorum* species. Electrophoretic separation (100 V, 40 min) of the PCR amplicons was conducted in 1.5% agarose gel in 0.5 x Tris Borate EDTA (TBE) buffer. 0.5 mg l⁻¹ ethidium bromide was applied for DNA staining prior to washing and visualization under UV light with a ChemiDoc XRS system (Bio-Rad, USA). In the performed PCR assays, the following reference strains originating from international bacterial collections were used: *D. solani* IFB0099, *P. atrosepticum* SCRI 1086, *P. carotovorum* subsp. *carotovorum* SCRI 136, *P. parmentieri* SCC3193. Full genomic sequences of 14 SRP strains (12 of *P. parmentieri* and two of *D. solani*) isolated in this study are available in the Genbank database under the following accession numbers: GCA_003992745.1, GCA_003628655.1, GCA_003628675.1, GCA_003628575.1, GCA_003628635.1, GCA_003628735.1, GCA_003628595.1, GCA_003628695.1, GCA_003628615.1, GCA_003628715.1, GCA_003628015.1, GCA_003628025.1,

JABAOP000000000 and JBAOQ000000000 (Zoledowska et al. 2018a; Motyka-Pomagruk et al. 2020).

Data analysis and visualization

Data from the surveys describing the collected plant samples together with the frequencies of distinct SRP isolations were summarized, analysed and plotted with the use of Microsoft Excel 365 software (Microsoft Corporation, USA). The final high resolution figures in addition to Venn diagrams were prepared and assembled with the use of Inkscape software (version 0.92, GNU General Public License).

Results

Abundance of SRP on the territory of Poland

In the years 2013 and 2014, 101 and 124 strains belonging to SRP (Table 1) were isolated from the investigated plant samples, respectively. Therefore, excluding the

noted coinfection events, 73 out of 248 (29%) in addition to 106 out of 283 (37%) of the plant samples collected in 2013 and 2014 harboured SRP. Notably, SRP strains were detected on the majority of Polish territory, namely in 11 regions out of 13 or 12 included in the 2013 or 2014 study, respectively (Supp. Figure 1). The *Pectobacterium* spp. isolation events (94 in 2013 and 114 in 2014; Table 1) turned out to outnumber the ones yielding *Dickeya* spp. strains (seven in 2013 and 10 in 2014; Table 1). In both years a similar number of *D. solani* (4 and 5) and *D. dianthicola* (3 and 5) strains was isolated. *Pectobacterium* spp. were widespread on the territory of Poland (their occurrence was noted in at least 11 Polish regions; Supp. Figure 1), while the presence of *Dickeya* spp. was restricted solely to four regions in each year of the study (Supp. Figure 1).

The most frequently isolated SRP species in 2013 was *P. carotovorum* (36% of the total number of collected strains), followed by *P. parmentieri* (32%), *P. atrosepticum* (26%) and *Dickeya* spp. (7%) (Fig. 1a). In 2014, the dominating trends were similar, however this time *P. atrosepticum* was isolated more commonly (38%) in comparison to *P. carotovorum* (31%), *P. parmentieri* (23%) and *Dickeya* spp. (8%) (Fig. 1a). In Fig. 1b, the outcomes of the former studies conducted by our group in 1996, 2005 and 2011 are depicted. It is worth noting that *P. carotovorum* is presented together with *P. parmentieri* as the identification method utilized at that time did not differentiate these two species. Notably, both in 1996 and 2011, *P. carotovorum* together with *P. parmentieri* constituted the most abundant SRP group, while *P. atrosepticum* dominated in 2005 (Fig. 1b). Considering all the data, *Pectobacterium* spp. strains were the leading cause of potato soft rot and blackleg in Poland from 1996 to 2014 contrary to *Dickeya* spp., whose population was minor and rather stable (Fig. 1) since the first report on the occurrence of *Dickeya* spp. in our country in 2005 (Slawiak et al. 2009).

Seasonal variation in the occurrence of SRP

Figure 2a shows the percentage distribution of plant samples collected by the RIPH & SIS inspectors or specialists from the collaborating institutions from seed potato fields in Poland during each month of the study. The majority of the tested plants were sampled in July. Interestingly, plant samples collected in August and September of 2013

yielded higher percentages of SRP isolates (19% and 4%; Fig. 2b) than it was expected from their abundance (10% and 1%, respectively; Fig. 2a). We made similar observations in case of the samples picked up from the fields in June 2014 (31% of total plants investigated that year), which were proven to harbour 48% of SRP (Fig. 2a, b).

Focusing on distinct SRP, a higher interdependence between the plant collection period and the year of study rather than the SRP group involved was noted (Fig. 2ac). The monthly distribution of *P. carotovorum* isolates in 2013 and 2014 reflected the observed partitioning among the total number of SRP stated for the corresponding year (Fig. 2bc). A similar remark regarded *P. parmentieri* isolates in 2013, however in 2014 the highest percentage of strains belonging to this species (59%) originated from the plants collected in June, which did not represent the highest fraction of the total samples tested (31%) (Fig. 2ac). Of the SRP species investigated, the monthly distribution of *P. atrosepticum* isolates was standing out from the frequencies of plant samples collections and the total SRP isolations. *P. atrosepticum* was not only a dominating species in potato fields in 2014, but also most of these strains (96% in 2013 and 100% in 2014) were isolated at the beginning of summer i.e. June or July (Fig. 2c). As shown in Fig. 2ac, months in which *Dickeya* spp. were isolated did not relate with the abundance of the received plant samples, but the low number of the obtained *Dickeya* spp. strains (Table 1) needs to be considered. Interestingly, during both years of study, July turned out to be the only month in which the presence of bacteria from all the analysed taxons, namely *P. atrosepticum*, *P. carotovorum*, *P. parmentieri* and *Dickeya* spp., was confirmed on seed potato fields in Poland from 2013 to 2014 (Table 1, Fig. 2c).

Beside the diseased potato stems and tubers in addition to the weed samples collected from the fields, symptomatic potato tubers (17 from four regions in 2013 and 12 samples from five voivodeships in 2014; Table 1) originating from storage facilities (collected from October until December) were also tested for the presence of SRP with in the frames of this research. Interestingly, no *Dickeya* spp. were isolated from potato tubers of such origin in contrast to *P. atrosepticum*, *P. carotovorum* or *P. parmentieri*, which were acquired from some of these samples (2, 6 and 6, respectively; Table 1) during the 2-year study.

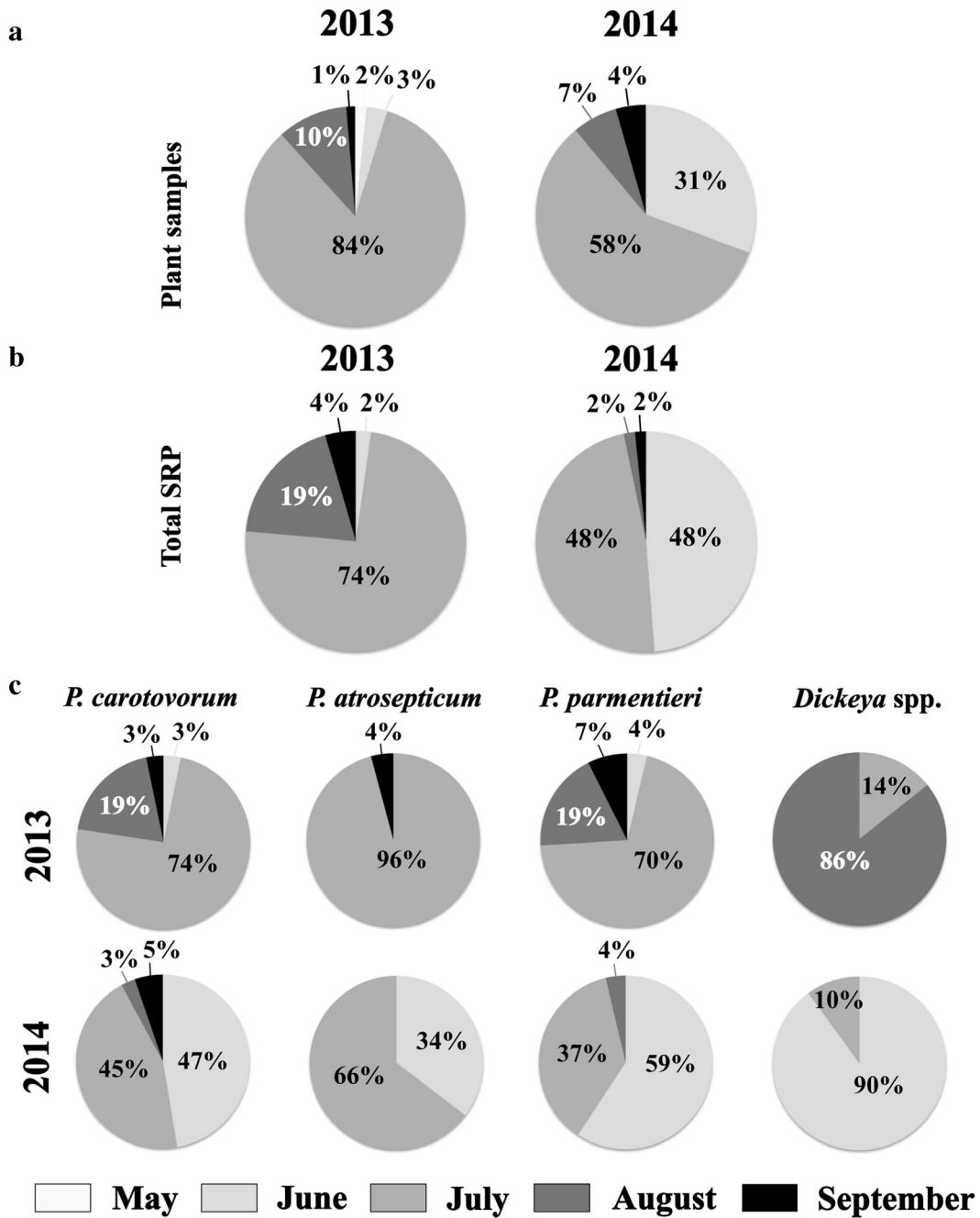


Fig. 2 Months in which the strains of SRP were isolated from seed potato fields in Poland during 2013 and 2014 growing seasons. **a** Months of plant samples collection. **b** Months of SRP isolation. **c** Months of isolation of the distinct *Dickeya* and *Pectobacterium* spp. strains. The included months starting from

the lightest tint: May – , June – , July – , August – , September – . Solely samples that originated from potato fields (collected from May until September), not the ones picked from storage facilities, are shown

SRP coinfection events

From 26 plant samples collected in 2013, more than one SRP species was isolated (Fig. 3a). Coinfection of *P. carotovorum* with *P. parmentieri* occurred most often in 2013 (13 cases; Fig. 3a). Notably, two cases were recorded in which three diverse pathogens, namely *P. parmentieri*, together with *P. carotovorum* and *P. atrosepticum*, were isolated from the same plant sample (Fig. 3a). In 2014, 16 coinfection events were detected (Fig. 3b). Similarly to the year 2013, most common coinfections in 2014 involved also *P. parmentieri* and *P. carotovorum* (four cases; Fig. 3b). Analogously to 2013, there were two plant samples collected in 2014 that yielded three diverse SRP species (Fig. 3b). It is worth noting that the SRP species co-occurrence pattern is exactly the same throughout the years of study, in other words, each year the same species either were detected together or were not present in such a combination (Fig. 3). Noteworthy, no *P. atrosepticum* strain coinfects any of the tested plants together with *Dickeya* spp. It also seems interesting that in each year of study, there was one weed sample that was noted to harbour multiple pathogens (either *P. carotovorum* and *P. atrosepticum* in 2013 or *P. parmentieri* and *P. atrosepticum* in 2014).

Plant origin of SRP strains

In 2013 and 2014, the RIPH & SIS Inspectors and other specialists from the collaborating institutions collected the highest number of blackleg- and soft rot-affected samples of potato stems (124 and 153, respectively),

followed by the diseased potato tubers (74 and 87) and the asymptomatic accompanying weeds (50 and 43) (Table 1). In this view, it does not seem surprising that in 2013 most SRP strains were isolated from potato stems (61%), a significantly lower number from potato tubers (33%), while solely several strains from the accompanying weeds (6%) (Fig. 4a, b). A similar observation concerned the year 2014, namely 73% of SRP isolates originated from potato stems, 20% from potato tubers, whereas 7% were acquired from the weeds (Fig. 4b). Interestingly, in both years of study, SRP strains were isolated from potato stems more frequently (61% and 73%) than it was expected basing on the number of the obtained samples of such material (50% and 54%, respectively; Fig. 4a, b). The accompanying weeds were revealed to be the environmental source of SRP significantly less often (6% and 7%) than it was predicted (20% and 15%, respectively; Fig. 4a, b). The abundance of diverse SRP groups in different types of the studied plant material is shown in Fig. 5. We observed that the distribution of the studied species in potato stems and tubers (Fig. 5) resembles the pattern of the general prevalence of distinct SRP in all the studied plant material in the corresponding years 2013 and 2014 (Fig. 1a). Focusing on the analysed accompanying weed samples, we isolated most frequently either *P. parmentieri* or *P. atrosepticum* (6 isolates of each in 2013–2014) from such material (Table 1). Curiously, no *Dickeya* spp. were acquired from the weed samples tested (Table 1; Fig. 5).

Altogether, SRP strains were isolated from 35 potato cultivars out of 58 investigated (Fig. 6). The SRP-affected potato cultivars represented miscellaneous cooking qualities: AB (Denar, Lord and Vineta), B

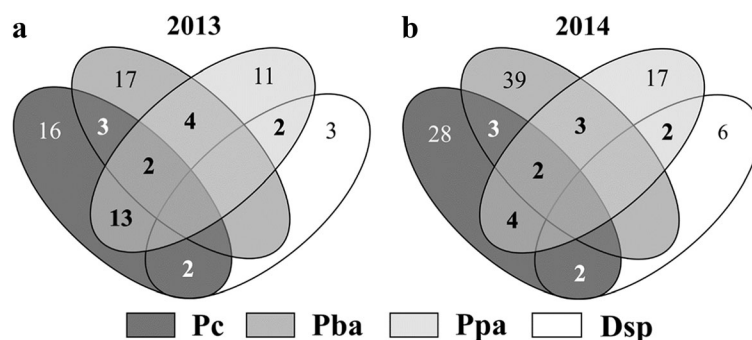
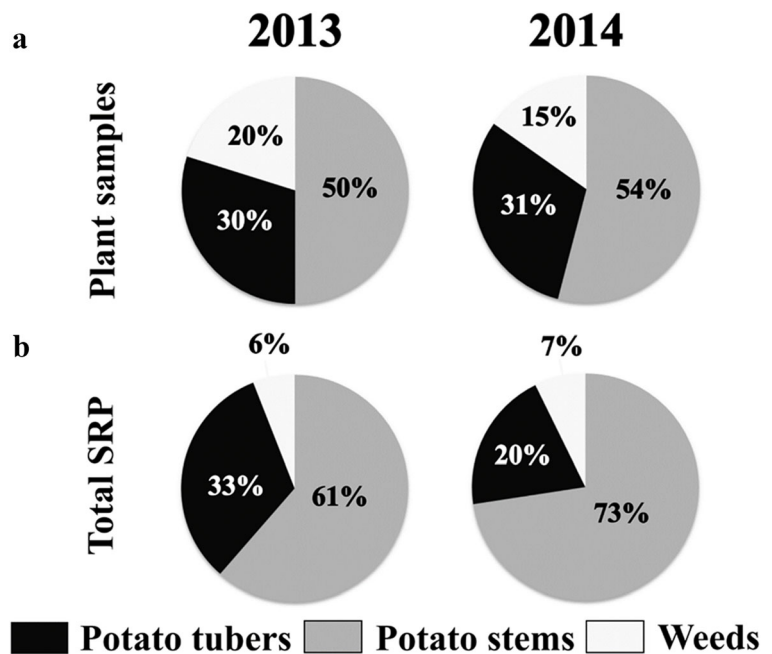


Fig. 3 Coinfection events with *Dickeya* and *Pectobacterium* spp. isolates in 2013 (a) and 2014 (b). Numbers referring to the number of isolates classified to certain species of *Pectobacteriaceae* are enclosed within the ellipses colored in **■** for *P. carotovorum* (Pc), in **■** for *P. atrosepticum* (Pba), in **■** for *P. parmentieri*

(Ppa) and in **□** for *Dickeya* spp. (Dsp). Numbers presented in bold within the common regions of the ellipses correspond to the number of coinfection events with the strains belonging to the *Pectobacteriaceae* species marked accordingly to the above-described coloration

Fig. 4 Plant or plant organ origins of SRP isolated in 2013 and 2014. Distribution of the plant samples tested in 2013 and 2014 (a) was juxtaposed to the percentages of different sample types yielding the *Pectobacteriaceae* isolates (b). ■ corresponds to potato tubers, ■ refers to potato stems, while □ shows weed samples







(Irga, Satina, Bellarosa and Jelly) or B-BC (Tajfun and Owacja), both starch and table potatoes (Fig. 6) in addition to early season (Denar, Milek, Lord) or late-season (Jelly, Bryza, Gustaw) varieties (Nowacki 2012). 21 diverse potato cultivars were affected by *P. carotovorum* strains (Fig. 6). A lower number (19) of the analysed cultivars yielded either *P. atrosepticum* or *P. parmentieri* isolates (Fig. 6). The less frequently acquired *Dickeya* spp. strains were obtained just from 10 of the investigated potato cultivars (Fig. 6). Interestingly, all the analysed *Dickeya* and *Pectobacterium* spp. were isolated from Irga, Satina, Vineta and Bellarosa cultivars (Fig. 6), while Jelly, Denar, Harpun, Lord, Owacja, Kuba, Cyprian and Tajfun cultivars were the environmental source of different *Pectobacterium* species of interest (Fig. 6).

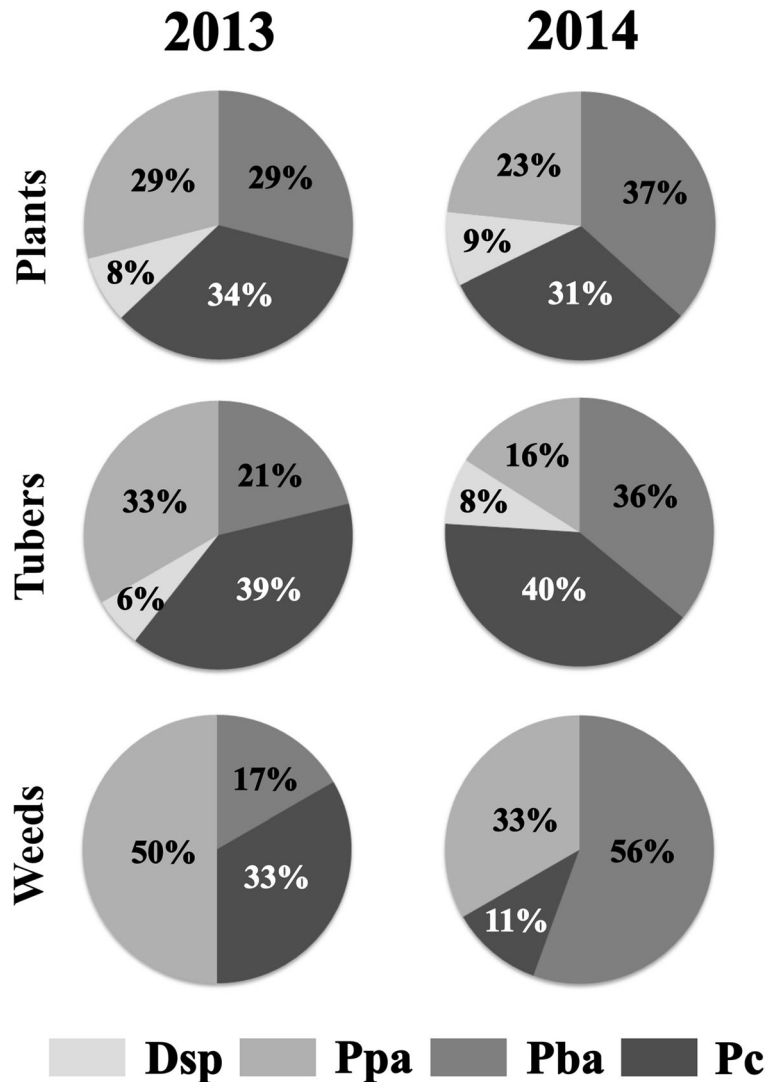
Fertilizing methods applied to plants infected with SRP strains

We also analysed the distribution of the applied fertilizing practices to potato fields from which the investigated plant samples had been collected (Table 1). In 2013, 100 of the plants included in this study (Table 1) were fertilized with commercial mixtures of nitrogen, phosphorous, and potassium complexes, i.e. inorganic NPK fertilizers. In addition, 45 of the plants were subjected to treatment with solely organic fertilizers such as

manure, compost, slurry, after-crop, natural urea or any other referred to as ‘ecological’ (Table 1). Besides, NPK fertilizers with organic additives concerned 40 (Table 1) of the tested plants. Also, the NPK mixture with mineral supplements, in this case usually secondary minerals (Ca, S or Mg) applied for pH adjustment in liming materials or micronutrients (e.g. Zn, Cl, B, Mo, Cu, Fe, Mn, Co or Ni), was commonly implemented (38 plants). Last but not least, most complex mixtures of NPK fertilizers supplemented with both organic and mineral additives concerned 16 of the fields studied in 2013 (Table 1). Similarly in 2014, implementation of just NPK fertilizers was most common (75 of the obtained plants), followed by organics (56 plants), NPK supplemented with organics (41 plants), NPK with additional minerals (37 plants) and NPK enriched with both organic and mineral supplements (25 plants) (Table 1).

The detailed information concerning samples from which a distinct SRP species was acquired in a stated year is shown in Table 1. It is worth noticing that during both years of study approx. 50% of plant samples yielding *P. atrosepticum* isolates were treated together with organic fertilizers or NPK supplemented with organics (Table 1). Concerning *P. carotovorum* strains, they were predominantly isolated in 2013 from the fields subjected to rich fertilization with NPK supplemented with organics (36%), which diverged from the overall

Fig. 5 Plant source of diverse *Pectobacteriaceae* groups isolated in 2013 and 2014. The colors mark from the lightest tint:  – *Dickeya* spp. (Dsp),  – *P. parmentieri* (Ppa),  – *P. atrosepticum* (Pba),  – *P. carotovorum* (Pc)







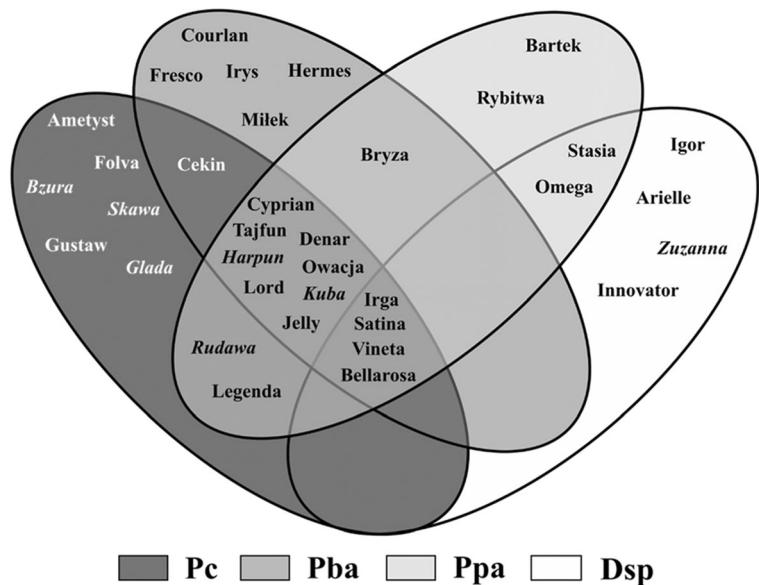
fertilizing pattern applied to potato fields included in 2013 (Table 1). Also in 2014, this fertilization method was overrepresented (21% vs. 14%; Table 1) among *P. carotovorum*-yielding plant samples. In the case of *Dickeya* spp. and *P. parmentieri*, the majority of plant samples from which these isolates were acquired underwent NPK fertilizing, exceeding the frequency of this treatment utilization each year (Table 1).

Discussion

The first scientific reports on blackleg and soft rot diseases affecting potato production in Europe date back

to 1972 (Naumann and Zielke 1977). Despite significant improvements allowing for instance the detection of distinct SRP species responsible for a given disease outbreak, still significant economic losses result from the presence of bacteria from the genera *Pectobacterium* and *Dickeya* in potato fields, especially taking into account a high percentage of latent infections amounting even to 2–30% under intensive potato production (Toth et al. 2011). Therefore in this work, we investigated the prevalence of *Pectobacterium* and *Dickeya* spp. on seed potato fields in Poland with a focus on the dominating SRP species in 2013 and 2014 growing seasons. This knowledge supported our long-term monitoring studies, which revealed that *Pectobacterium* spp. in contrast to

Fig. 6 Potato cultivars collected in 2013 and 2014 that harbored the isolated *Pectobacteriaceae* strains. Potato cultivars from which the *Pectobacteriaceae* isolates were obtained are enclosed within the ellipses colored, starting from the left and from the darkest tint: in  for *P. carotovorum* (Pc), in  for *P. atrosepticum* (Pba), in  for *Pectobacterium parmentieri* (Ppa) and in  for *Dickeya* spp. (Dsp). More than one *Pectobacteriaceae* strain was acquired from the cultivars presented in the common regions of the ellipses. Starch potatoes are italicized, while the table potatoes are depicted in the normal font



Dickeya spp. was the leading cause of blackleg and soft rot diseases on seed potato fields in Poland from 1996 to 2014 (Dees et al. 2017; Golanowska et al. 2017; Potrykus et al. 2016; Slawiak et al. 2013; Sledz et al. 2000; Waleron et al. 2002; Zoledowska et al. 2018b).

As herein reported, *P. atrosepticum* turned out to be one of the most frequently isolated SRP species in Poland. Isolates classified to this species were not only most prevalent in 2014 (38%), but also formed a significant fraction of SRP strains in 2013 (26%). High importance of *P. atrosepticum* isolates in our country was already indicated by Sledz et al. (2000). The predominance of *P. atrosepticum* in temperate climate regions was stated over 35 years ago by Perombelon and Kelman (1980). This species is widely present in many potato growing regions, for instance in Scotland (Toth et al. 2011), Finland (Laurila et al. 2008), Turkey (Ozturk et al. 2018), USA (especially Colorado, Charkowski 2018) or Canada (De Boer et al. 2012).

Our research showed that the dominating SRP species in Poland changed throughout the years of study. *P. carotovorum* dominated in the screened regions in 2013 (36%), which is in line with the previously reported outcomes on notable contribution of this species to potato decay under temperate climate conditions (de Haan et al. 2008; Toth et al. 2011). For example in the Netherlands, all the investigated blackleg incidences in 1987, 1989, 1993, 2001 and 2005 were associated with the occurrence of *P. carotovorum* (van Beckhoven et al. 2001). Similarly in France, solely *P. carotovorum* was

identified in approx. 20% of blackleg-diseased plants (after de Haan et al. 2008). Even in cooler geographical regions like Finland, a high abundance of *P. carotovorum* strains was noted (Pasanen et al. 2013). In Norway, both *P. atrosepticum* and *P. carotovorum* were frequently detected on potato fields, however the other commonly isolated SRP species in Poland (32% and 23% in 2013 and 2014, respectively) *P. parmentieri*, was acquired in that country just in a single case (Dees et al. 2017).

It is worth considering that there was a specific group of *P. carotovorum* subsp. *carotovorum* strains, which included highly virulent *P. c.* subsp. *carotovorum* isolated in the Netherlands (vPcc; de Haan et al. 2008) and virulent *P. carotovorum* subsp. *carotovorum* isolated in Poland (Slawiak et al. 2013). These strains adhered earlier to the *recA* PCR-RFLP group 3 Pcc (Waleron et al. 2002). The reclassification of *P. carotovorum* SCC3193 to *P. wasabiae* SCC3193 (Nykyri et al. 2012) initiated changes in the taxonomy of other *P. carotovorum* isolates. A large group of *P. carotovorum* strains isolated in Poland were reclassified at first to *P. wasabiae* (Slawiak et al. 2013; Waleron et al. 2013) and then to *P. parmentieri* (Khayri et al. 2016). To this day, *P. parmentieri* has been reported to contribute to the SRP populations of Canada, Germany, Ireland, Finland, France, Poland, Scotland, USA, Turkey, Spain and the Netherlands among others (De Boer et al. 2012; Ge et al. 2018; Nabhan et al. 2012; Nykyri et al. 2012; Ozturk et al. 2016; Suárez et al.

2017; Waleron et al. 2013). With reference to the recent detection of *Pectobacterium brasiliense* in Poland (Babinska and Waleron, personal communication), our study will be enriched in the future by monitoring of the presence of this species on seed potato fields in our country. This effort is motivated by the current data indicating the growing impact of this species on European potato production (Nunes Leite et al. 2014; van der Wolf et al. 2017).

In the herein presented work we reported low abundance of *D. dianthicola* and *D. solani* isolates in Poland. A few years ago, *D. solani* was believed to be highly prevalent in Europe (Cahill et al. 2010; Degefu et al. 2013; Helias 2006; Laurila et al. 2008; Palacio-Bielsa et al. 2006; Toth et al. 2011; van der Wolf et al. 2014). The studies of the Norsk Institutt for Bioøkonomi also support the current minor significance of this species in the development of soft rot or blackleg disease outbreaks in comparison to the other members of SRP (Dees et al. 2017).

The encountered weather conditions influence to a high extent blackleg- or soft rot- related disease severity (Perombelon and Kelman 1980). The average air temperature and insolation in Poland in 2014 was higher than in 2013, however, lower precipitation was noted (Institute of Meteorology and Water Management - National Research Institute, Poland). As suggested by Perombelon (2002) and Toth et al. (2011) the total incidence of blackleg (pre- and post- emergence) tends to increase together with an elevated soil temperature and moisture if the pathogen inoculum is low. As throughout each year of study only one of the above-listed disease-promoting factors occurred, this might be the reason explaining the collection of similar numbers of blackleg- and soft rot-affected plants or the comparable quantities of the isolated SRP in 2013 and 2014.

Higher air temperatures in 2014 were reflected in the percentage distribution throughout the months in which the diseased plant samples were collected, meaning that a greater number of blackleg- or soft rot-affected plants was acquired from the fields earlier (already in June) in 2014 than in 2013. Nearly all *P. atrosepticum* strains were obtained in early summer i.e. in June or July, which is the most favourable period for blackleg symptoms development, as reported before (de Haan et al. 2008). Considering better adaptation of *P. atrosepticum* to lower temperatures, the corresponding disease symptoms might be the outcome of earlier infections in comparison to these caused by the other SRP species. Also in Japan, a seasonal variation in the occurrence of

P. atrosepticum in potato fields was noted – these strains were abundant in early summer and fall, contrary to the hot season of midsummer (Tsuyama and Sakamoto 1952). Interestingly, in the current research the members of all SRP species were detected on potato fields in Poland in July during both years of study. This observation correlates with the earlier reports of Powelson (1980), who noted the highest seasonal blackleg and soft rot incidence throughout July, while testing potato fields in Columbia Basin of Oregon.

In this work, more SRP strains were obtained from potato stems than tubers. It is worth noting that the RIPH & SIS inspectors provided us with a higher number of stem samples than these of tubers, however normalization of the number of successful SRP isolations to the number of samples tested would lead also to the above-presented conclusion. Likewise, De Boer et al. (2012) provided proofs for obtaining a greater number of SRP from potato stems than tubers.

In spite of the plants and tubers collected from the fields, we searched for SRP in potato tubers acquired from October to December from the storage facilities. All species of the interest from the genus *Pectobacterium* were isolated from this material, however, no strains belonging to the *Dickeya* genus were acquired from these samples. There were previous reports on the isolation of *Pectobacterium* spp. strains from potatoes of the warehouse origin (Perombelon 2002; Toth et al. 2011).

In our research, the isolates of all the included *Pectobacterium* species, contrary to *Dickeya* spp., were acquired from the accompanying weeds tested. By now, little is known about the alternative plant hosts of *Dickeya* spp. For instance, this pathogen was isolated in Sweden from *Solanum dulcamara* (Olsson 1985), perennial herbaceous weed growing in lowlands in proximity to potato fields (Toth et al. 2011). In Poland, solely *P. c.* subsp. *carotovorum* was isolated from the naturally occurring *S. dulcamara* (Fikowicz-Krosko et al. 2017). The fact of successful isolations of *P. carotovorum* from the investigated asymptomatic weed samples might be linked with a wide host range of this pathogen. For instance, *P. carotovorum* strains were obtained earlier from *Tagetes patula*, *Arctium minus*, *Cichorium intybus*, *Brassica oleracea*, *Zantedeschia aethiopica*, *Ornithogalum dubium*, *Capsicum annuum*, *Solanum esculentum*, *Cucumis melo*, *Lilium* spp., *Hippeastrum* spp., *Schismatoglottis* spp. and *Ornithogalum saundersiae* (Ma et al. 2007;

Yishay et al. 2008). It is interesting to notice that contrary to Ma et al. (2007), who described the host range of *P. atrosepticum* as mostly limited to potato, we did isolate 6 *P. atrosepticum* strains from the accompanying weeds. Also six *P. parmentieri* strains were isolated in this research from such material. Interestingly, other groups acquired *P. parmentieri* strains from e.g. sweet pepper (Waleron et al. 2013), *Solanum melongena*, *Solanum lycopersicum*, *Ipomoea batatas* or *Brassica rapa* (Golkhandan et al. 2013). Nonetheless, due to the recent separation of *P. parmentieri* from *P. wasabiae* species (Khayati et al. 2016), there is still an ongoing discussion on the alternative hosts reported for each of these taxa.

Focusing on potato, currently there are 114 diverse potato cultivars (64 domestic and 50 foreign) catalogued in the Polish registry of potato varieties (Stypa 2015). We obtained soft rot bacteria from the samples of 35 cultivars from 58 collected in this study. The most commonly grown table potato cultivar in Poland with 9.5% of the total acreage, namely Vineta (Stypa 2015), was the environmental source of all four taxa of SRP. Second in line are Denar, Lord and Tajfun from which we collected bacterial isolates from all three *Pectobacterium* spp. of interest. It is worth mentioning that from the cultivars Arielle, Igor, Inovator and Zuzanna only strains of *D. dianthicola* and *D. solani* but not those belonging to the genus *Pectobacterium* were isolated. Concerning the most common cultivar intended for industrial processing, namely Innovator (9.1% of market share; Stypa 2015), it yielded solely *Dickeya* spp. isolates. As regards the starch variety, named Kuba, of the greatest market share (1.7%; Stypa 2015), all the studied *Pectobacterium* spp. were acquired from the analysed samples. Former studies on the appearance of SRP on different potato cultivars in Poland are scarce. However, Kapsa and Gawinska-Urbanowicz (2013) reported a high (25%) disease incidence caused by *Pectobacterium* spp. on potato cv. Owacja in the Pomeranian region.

In addition, we noted that coinfections with diverse SRP occurred quite often i.e. out of 101 or 124 strains collected in 2013 and 2014, respectively, either 54 or 34 isolates originated from plant samples harbouring more than a single SRP species. It indicated that in 2013 about 50% of strains were isolated from potato plants coinfecting with at least 2 bacterial species. In contrast, in 2014 only about 27% of strains were acquired from coinfecting plants. Both in 2013 and 2014 the most

common co-existence was observed among *P. carotovorum* and *P. parmentieri*. However in 2014, this most frequent type of coinfection took place 3 three times less often than in 2013, even if the number of *P. carotovorum* and *P. parmentieri* strains isolated both years was similar. Previously, Gross et al. (1991) and Sledz et al. (2000) showed that *P. carotovorum* subsp. *carotovorum* strains were present in the same potato field as the isolates of *P. atrosepticum*. Kim et al. (2009) revealed that not only multiple *Pectobacterium* species were encountered in one field, but also sporadically, *P. carotovorum* and *P. wasabiae* (currently *P. parmentieri*) were detected in the same symptomatic tuber. Besides, we noticed the lack of *Dickeya* spp. and *P. atrosepticum* coinfections, which might be associated with diverse temperature optima of these bacteria (Perombelon and Kelman 1980). Nonetheless, according to the herein reported study, coinfections with diverse SRP tend to be more common than it was previously thought.

The appropriate provision of plants with micro and macro elements is essential for their growth, development and resistance to environmental factors including phytopathogens. Also the impact of natural microflora, which is influenced to a higher extent by cropping and management practices than the soil type, was pointed as important for the suppression of plant pathogens (Huber and Watson 1970). Therefore, we studied the fertilizing methods applied on potato fields in relation to the frequency of SRP isolations. Unfortunately, no obvious patterns were found. However, it seems interesting that over 50% of plant samples yielding *P. atrosepticum* each year were subjected to organic fertilizers or NPK supplemented with organics, which mostly included animal manures. It is in agreement with the fact that *P. atrosepticum* can efficiently utilize nutrients available in swine, cattle or sheep manures (Sledz et al. 2017).

In conclusion, the herein described study devoted to the monitoring of SRP on seed potato fields in Poland in 2013 and 2014 revealed that *P. atrosepticum*, *P. carotovorum* and *P. parmentieri* were widespread in our country and outnumbered the *D. dianthicola* and *D. solani* isolates. Basing on the results of our former research, we state that since its appearance in 2005, *Dickeya* spp. never played a dominating role in the SRP population in Poland. During both years of study, the largest number of SRP was isolated in July and it turned out to be the only month in which the presence of bacteria from all the analysed species was confirmed on

seed potato fields in Poland. During the performed studies we confirmed co-infections of potato plants by bacteria from 2 or 3 different species. The most common coinfections were these of *P. parmentieri* and *P. carotovorum*. Besides, our data indicated that SRP strains were isolated from majority of the tested potato cultivars. The conducted studies on the environmental sources and occurrence patterns of soft rot pathogens might contribute to more efficient evaluation of the risks associated with infections caused by distinct SRP species.

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Compliance with ethical standards

Conflict of interest No conflict of interest.

Human and/or animals rights Not applied.

Informed consent Not applied.

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