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Pectobacterium carotovorum subsp. *carotovorum* can cause potato blackleg in temperate climates

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Abstract It is well established that the pectinolytic bacteria *Pectobacterium atrosepticum* (Pca) and *Dickeya* spp. are causal organisms of blackleg in potato. In temperate climates, the role of *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) in potato blackleg, however, is unclear. In different western and central European countries plants are frequently found with blackleg from which only Pcc can be isolated, but not Pca or *Dickeya* spp. Nevertheless, tubers vacuum-infiltrated with Pcc strains have so far never yielded blackleg-diseased plants in field experiments in temperate climates. In this study, it is shown that potato tubers, vacuum-infiltrated with a subgroup of Pcc strains isolated in Europe, and planted in two different soil types, can result in up to 50% blackleg diseased plants.

Keywords *Erwinia carotovora* subsp. *carotovora* · Pathogenicity · PCR-amplification · Symptom expression · Vacuum infiltration

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Abbreviations

Pca *Pectobacterium atrosepticum*
Pcc *Pectobacterium carotovorum* subsp. *carotovorum*

Introduction

Pectobacterium atrosepticum (Dye 1969; Gardan et al. 2003) (former name *Erwinia carotovora* subsp. *atroseptica*) (Pca), *Pectobacterium carotovorum* subsp. *carotovorum* (Dye 1969; Gardan et al. 2003) (former name *Erwinia carotovora* subsp. *carotovora*) (Pcc) and *Dickeya* spp. (Burkholder 1953; Samson et al. 2005) (former name *Erwinia chrysanthemi*) are economically important diseases in (seed) potatoes. All three pathogens can cause tuber rot during storage, whereas infections of seed potatoes with Pca and *Dickeya* spp. can also result in the occurrence of various field symptoms, including reduced emergence, chlorosis, wilting, tuber and stem rot, blackleg, haulm desiccation and plant death (Pérombelon and Kelman 1980; Pérombelon 1992; Fraaije et al. 1996; Helias et al. 2000). These symptoms can be caused by Pca as well as by *Dickeya* spp., making identification of the causal organism by visual observation in the field unreliable. Regulatory field inspections in seed potatoes in many countries are therefore based on recognition of this ‘blackleg complex’ without discriminating between the various blackleg-causing organisms.

In 1987, 1989, 1993, 2001 and 2005, surveys were conducted in the Netherlands to identify the blackleg-causing organisms in plants showing typical Erwinia symptoms, using serological and molecular methods (Van Beckhoven et al. 2001). During these years, only Pcc could be isolated on average from 20% of plants, but not Pca or *Dickeya* spp.

The ability of Pca and *Dickeya* spp. to cause blackleg is undisputed (Lumb et al. 1986; Helias et al. 2000; Toth et al. 2003) but the role of Pcc in the blackleg syndrome is controversial. Pcc is widely distributed in the world and has a broad host range (De Boer et al. 1987; Smith and Bartz 1990; Kang et al. 2003). Studies under high temperature conditions in North America (Molina and Harrison 1977, 1980) and Australia (Peltzer and Sivasithamparam 1985) showed that Pcc isolates, inoculated into tubers or stems, could cause typical blackleg symptoms in the field. Also Powelson and Apple (1984) suggested that soil-borne strains of Pcc can cause disease symptoms under irrigated field conditions in the Pacific Northwest of America. Under temperate climate conditions, such as in Scotland and Canada, Pcc is considered, however, a secondary invader, only causing symptoms after earlier infection by other pathogens, in particular Pca or *Dickeya* spp. (Pérombelon and Kelman 1980; De Boer 2002). For plants with typical, early (primary) blackleg symptoms, from which only Pcc can be isolated, this theory implies that Pcc has overgrown or displaced the primary pathogen. The fact, however, that frequently only Pcc could be detected in blackleg-diseased plants, and no other pathogens or specific antigens or DNA from Pca and *Dickeya* spp., raised questions on the possible role of Pcc as a primary causative agent of blackleg.

In 2003, a first field trial was conducted in the Netherlands with a selection of European Pcc strains (Van der Wolf et al. 2003). Potatoes were vacuum-infiltrated with a broad panel of 36 Dutch strains and 10 strains from other European countries. Strains originated from plants with blackleg symptoms that yielded Pcc only. Ten of the Dutch and five of the foreign Pcc isolates showed a high percentage of diseased plants in the field (≤37%), although plants showed wilting symptoms and not the typical blackening of the stem base.

The Pcc strains used in the field studies were characterised by REP-PCR (Van der Wolf et al. 2003). Strains were highly diverse, but the virulent strains could be classified into two groups. Thirteen out of the 15 virulent isolates shared 500 bp fragments which were only present in three of the isolates not causing symptoms in the field. PCR assays were developed, using primer pairs (contig1F/1R and contig3F/3R) designed on the basis of two of these 500 bp fragments (unpublished results). These PCR assays were used to select and isolate Pcc strains from blackleg-diseased plants for new field experiments.

This study describes a second field trial conducted to show that Pcc isolated from blackleg-diseased plants can cause true blackleg symptoms in a temperate climate.

Materials and methods

Bacterial isolates

Bacterial isolates used in this study are listed in Table 1. Thirteen Pcc isolates were isolated from plants, collected during field surveys in 2001 and 2005 in the Netherlands, with early blackleg symptoms, in which no *Dickeya* spp. and Pca could be detected using an enrichment-ELISA. In previous field experiments in 2003, the Dutch strains of 2001 and the strains from Switzerland, Germany and the UK included in this study had caused wilting symptoms. All strains were PCR-positive with Pcc primers EXPCCF and EXPCCR (Kang et al. 2003) and primer pairs contig1F/1R and contig3F/3R (see enrichment PCR). Two Pca strains (Eca 1007, Eca HV/1987) and two *Dickeya* spp. strains (nrs 1651 and 1741) were included as positive controls.

Biochemical tests

To confirm the identity of Pcc and differentiate them from Pca and *Dickeya* spp., all strains used in this study were tested for cavity formation on CVP-medium (27°C, 48 h), growth in 5% (w/v) NaCl, production of reducing substances from sucrose, production of phosphatase and acid production from lactose and α-methyl glucoside according to the protocols described by Hyman et al. (1998). All strains were tested in duplicate.

Vacuum-infiltration of tubers

Inoculum was produced from cultures grown in LB (Luria Broth Base, Miller; Duchefa, Haarlem, The

Table 1 *Pectobacterium* and *Dickeya* strains used in this study

Organism	PRI collection nr.	Host	Origin	Year of isolation
Pectobacterium atrosepticum	1007	Potato	The Netherlands	1991
Pectobacterium atrosepticum	1987	Potato	The Netherlands	1979
Dickeya spp.	1651	Saintpaulia	The Netherlands	1996
Dickeya spp.	1741	Potato	The Netherlands	1992
P. carotovorum subsp. *carotovorum*	5014	Potato	United Kingdom	Unknown
P. carotovorum subsp. *carotovorum*	91/84	Potato	Switzerland	Unknown
P. carotovorum subsp. *carotovorum*	1250	Potato	Germany	Unknown
P. carotovorum subsp. *carotovorum*	40b	Potato, var. Spunta	The Netherlands	2001
P. carotovorum subsp. *carotovorum*	50a	Potato, var. Spunta	The Netherlands	2001
P. carotovorum subsp. *carotovorum*	119a	Potato, var. Kondor	The Netherlands	2001
P. carotovorum subsp. *carotovorum*	151	Potato, var. Asterix	The Netherlands	2001
P. carotovorum subsp. *carotovorum*	204d	Potato, var. Exquisa	The Netherlands	2001
P. carotovorum subsp. *carotovorum*	S31.1	Potato, var. Kondor	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S60.3	Potato, var. Victoria	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S83.1	Potato, var. Mercator	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S109.1	Potato, var. Voyager	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S110.1	Potato, var. Hermes	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S121.1	Potato, var. Spunta	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S188.3	Potato, var. Bildstar	The Netherlands	2005
P. carotovorum subsp. *carotovorum*	S189.1	Potato, var. Spunta	The Netherlands	2005

Netherlands) at 28°C for 40 h, with agitation. Bacterial suspensions were diluted in tap water to a concentration of approximately 10^6 CFU ml^{-1}.

A high grade seed stock (class S) of cv. Kondor (35–45 mm fraction) was chosen. During seed production no symptoms of blackleg were observed in the field. Before vacuum-infiltration 200 tubers were tested with enrichment ELISA and no Pca or *Dickeya* spp. were detected. The same seed lot was also tested for Pcc with PCR using primer set EXPCCF and EXPCCR (Kang et al. 2003), and primers set contig 1F/1R and contig 3F/3R (unpublished results). Only with the primer set EXPCCF and EXPCCR, was Pcc detected in a low percentage of the tubers (<1%).

Two samples of 100 tubers each were vacuum-infiltrated in netted bags inside a milk can, adapted to apply a vacuum. Seed tubers were completely submerged with 15 l of bacterial suspension. A vacuum of 0.6 bar was applied for 15 min. Tubers were left in the suspension for another 5 min. Tubers were spread in plastic crates without washing and air-dried overnight at room temperature before being stored at 5°C for 7–9 days until planting. Control tubers were untreated or vacuum-infiltrated with tap water only.

Field experiments

The experiments were carried out at two locations. Tubers were planted 25 April 2006 at the NAK experimental farm at Tollebeek in a light clay soil and 27 April 2006 at an experimental field in a cleared peat land at Valthermond. Per treatment, 96 tubers were planted per location with six replicates of 16 tubers randomized over the field. Each subplot consisted of four rows of four plants and was separated from the other subplots by an unplanted row.

Symptom assessment

Disease development was assessed seven times from June to August 2006. Plants with typical bacterial symptoms such as blackleg, aerial stem rot, wilting, chlorosis, haulm desiccation in combination with die-back of stems or plant, or a combination of symptoms, were scored.

Sampling and enrichment of blackleg-diseased plants

From all treatments that resulted in blackleg-diseased plants, one to three plants with symptoms were

analysed to characterize the blackleg causal organism. From the stems, a section of 3 cm was taken containing symptomatic and asymptomatic tissue. Stems were washed in running tap water, surface-sterilized in 1% sodium hypochlorite and rinsed in sterile distilled water (SDW). Infected stem sections were crushed with a homogeniser (BioReba, Reinach, Swiss) in a filter bag with 3 ml of SDW. The samples were enriched for *Pectobacterium* and *Dickeya* by adding 100 µl of the sample to 1 ml of polypectate enrichment medium (De Boer and Kelman 2001). Samples were incubated at 20°C for 3 days under anaerobic conditions.

Enzyme-linked immunosorbent assay (ELISA)

Enriched samples were tested in a double antibody sandwich ELISA with polyclonal antibodies 9022bc specific for serogroup I of Pca, and 8276/502 specific for serogroup O1Ha of *Dickeya* spp. (Primediagnostics, Wageningen, The Netherlands) essentially as described by Clark and Adams (1977). After enrichment, 50 µl of sample and 50 µl of PBS (0.8% NaCl, 0.1% KH_2PO_4, 1.45% $Na_2HPO_4.12H_2O$, 0.05% NaN_3, pH 7.4) were loaded in antibody-coated polystyrene plates and incubated overnight at 4°C. After washing, 100 µl alkaline phosphatase-conjugated antibodies per well were loaded and plates incubated for 2 h at 37°C. After a second wash step, wells were loaded with 100 µl *p*-nitrophenylphosphate substrate (0.5 mg ml^{-1}) and plates incubated for 1 h at 20°C in the dark. Each plate contained a positive and a negative control. The sample was considered positive when the absorbance at 405 nm, blanked against healthy potato extract, was >0.20.

DNA extraction and PCR amplification

After enrichment, 500 µl of the culture medium was used for extraction of genomic DNA, using the Puregene DNA purification kit according the manufacturer's protocol for Gram-negative bacterial cultures (Gentra).

Characterization of Pca strains by PCR amplification and analysis of enriched samples with PCR was performed using primers ECA1f/ECA1r as described by De Boer and Ward (1995). Similarly, PCR amplification of *Dickeya* spp. was done using primers ADE1/ADE2 as described by Nassar et al. (1996). PCR amplification of Pcc was done using primers EXPCCF/EXPCCR as described by Kang et al. (2003) and using primer pairs contig1F/1R and contig 3F/3R. The contig primers were based on two 500 bp DNA fragments, specific for a subgroup of Pcc strains, of which most were able to cause wilting symptoms in a field experiment in 2003 (Van der Wolf et al. 2003).

The PCR conditions for the contig primers were identical. The PCR mix consisted of 2 µl of DNA, 25 pmol of each primer set (contig 1F CCTGCTG GCGTGGGGTATCG, contig 1R TTGCGGAAGAT GTCGTGAGTGCG or contig 3F GCATTGACCAGT TTCGCCAGTTAC, contig 3R CTTTTTGAGCAGC GCGGGTTGTG), 200 µM of each dNTP, 2.5 µl of PCR buffer 10× including $MgCl_2$ (Roche) and 0.5 U of Taq DNA Polymerase (Roche) and made up to 25 µl with SDW. After denaturation at 94°C for 5 min, amplification was done in 25 cycles of 94°C for 30 s and 72°C at 90 s. The reaction was terminated with an incubation of at 72°C for 5 min. Fragment lengths were 258 and 132 bp, respectively, for contig 1F/1R and contig 3F/3R. Reactions were run on a 1.5% agarose gel in 1× TBE at 100 V.

Statistical analysis

Results on symptom expression were analysed per treatment and per location using a generalized linear model after a logit transformation (McCullagh and Nelder 1989) with the help of the statistical package GenStat (2007). For each treatment, the pair-wise difference was calculated ($P<0.05$). In addition, the difference in symptom expression per location was calculated for all treatments together, using the same model.

Results

Characterization of strains

The identity of the bacterial strains used in this study was checked by PCR-amplification assays using different primer and biochemical assays. In addition the identity of Pca and *Dickeya* spp. was confirmed by DAS-ELISA. In DAS-ELISA, Pca and *Dickeya* spp. strains were positive with their homologous antibodies. In PCR, primers ECA1f and ECA1r specifically amplified Pca strains, and primers ADE1

Table 2 Physiological and biochemical tests of several *P. atrosepticum* (Pca), *Dickeya* spp. and *P. carotovorum* subsp. *carotovorum* (Pcc)

Bacterium	Strain	Origin	CVP	5% NaCl	Phosphatase	Sucrose	Lactose	α-Methyl glucoside
Pca	1007	PRI	+	+	−	+	+	+
Pca	1987	PRI	+	+	−	+	+	+
Dickeya spp.	1651	PRI	+	+	+	−	−	−
Dickeya spp.	1741	PRI	+	−	+	−	−	d
Pcc	5014	CSL	+	+	−	−	+	−
Pcc	91/84	Swiss	+	+	−	−	+	d
Pcc	1250	Germany	+	+	−	−	+	−
Pcc	40b	NAK2001	+	+	−	−	+	−
Pcc	50a	NAK2001	+	+	−	−	+	−
Pcc	119a	NAK2001	+	+	−	−	+	−
Pcc	151	NAK2001	+	+	−	−	+	−
Pcc	204d	NAK2001	+	+	d	−	+	−
Pcc	S31.1	NAK2005	+	+	−	−	+	−
Pcc	S60.3	NAK2005	+	+	−	−	+	−
Pcc	S83.1	NAK2005	+	+	−	−	+	−
Pcc	S109.1	NAK2005	+	+	−	−	+	−
Pcc	S110.1	NAK2005	+	+	−	−	+	−
Pcc	S121.1	NAK2005	+	+	−	−	+	−
Pcc	S188.3	NAK2005	+	+	−	−	+	−
Pcc	S189.1	NAK2005	+	+	−	−	+	−

Strains were tested in duplicate. All duplicate reactions were identical. d = doubtful

and ADE2 *Dickeya* spp. strains. All Pcc strains were positive with primers EXPCCF/EXPCCR, contig 1F/1R and contig 3F/3R.

Physiological and biochemical reaction patterns were in accordance with those described in the literature for Pca, Pcc and *Dickeya* spp., except that strain Pcc 204d was doubtful for the phosphatase assay and *Dickeya* strain 1651 grew in 5% NaCl. *Dickeya* strain 1741 and Pcc 91/84 were doubtful for acid production from α-methyl glucoside (Table 2). Duplicates gave identical results.

Disease development

In June first disease symptoms were found, usually recognized as stem or leaf wilting (Fig. 1a) and occasionally a dark colouring of the upper leaves. At the end of the observation period, most diseased plants showed typical blackleg symptoms (Fig. 1b), although frequently blackleg was only visible below ground level, after lifting of the wilted plant. Occasionally only weak blackleg symptoms were observed. The stems of these plants, however, showed

Fig. 1 Symptoms caused by *Pectobacterium carotovororum* subsp. *carotovorum* in a potato field crop in the Netherlands. **a** Wilting of haulms. **b** Darkening and rotting of above-ground stem tissue (blackleg). **c** Brown-discolouration of vascular tissue and pith decay

brown discolouration of vascular tissue and pith decay upon longitudinally stem cutting (Fig. 1c). No disease development was observed in the water-infiltrated tubers and untreated controls.

Pre-emergence rot was found both for tubers inoculated with Pca and Dca. For Pca 1007 the emergence was 97% in Tollebeek and 74% in Valthermond. For *Dickeya* spp. isolates 1651 and 1741, emergence was 85% and 81%, respectively, in Tollebeek and 91% and 93% in Valthermond. For sixteen Pcc isolates, Pca 1987, the water control and the two untreated controls, the emergence was >99%.

For Pca the maximum percentage of blackleg-diseased plants related to the number of emerged

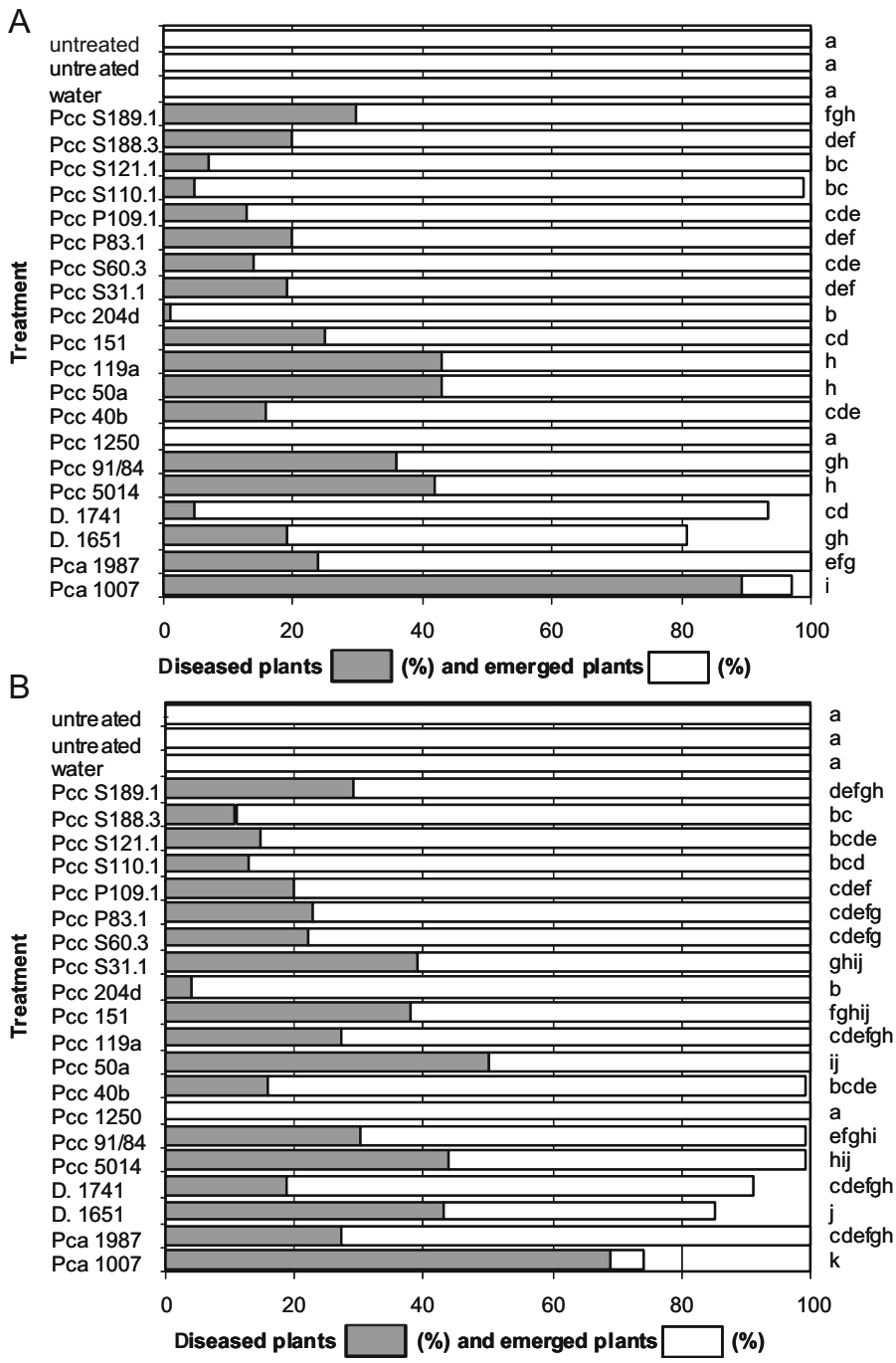

Fig. 2 Percentage of emerged plants and diseased plants calculated as percentage of emerged plants on two locations in the Netherlands, Tollebeek (**a**) and Valthermond (**b**). Pair-wise differences of the percentage of diseased plants were calculated after a logit transformation using the model described by McCullagh and Nelder (1989). Treatments with similar letters are not significantly different ($P<0.05$). *Pcc Pectobacterium carotovorum* subsp. *carotovorum*, *D. Dickeya* spp., *Pca P. atrosepticum*

plants was 89% (Pca 1007) at location Tollebeek and for *Dickeya* spp. 43% (*Dickeya* spp. 1651) at Valthermond (Fig. 2). All thirteen Dutch Pcc isolates and two out of three foreign Pcc isolates showed typical blackleg symptoms. Only Pcc 1250 from Germany did not induce any symptoms. Percentages of blackleg induced by fifteen Pcc isolates were generally high, with a maximum of 50% for Pcc 50a at location Valthermond. The average percentage of blackleg for the Pcc isolates at location Tollebeek was 24% and at Valthermond 27%.

Analysis of blackleg-diseased plants

During inspection, plants were sampled to identify the causal organism. Only early primary infections were sampled to avoid detection of secondary pathogens. The presence of Pca and *Dickeya* spp. in symptomatic plants, inoculated with Pca and *Dickeya* spp., respectively, was confirmed by enrichment ELISA and an enrichment PCR assay (Table 3). The presence of Pcc in all symptomatic plants was confirmed by enrichment PCR, using the EXPCC, the contig 1F/1R and the contig 3F/3R primer sets, except for plants inoculated with Pcc strains 151, S109.1 and S110.1. All Pcc-inoculated symptomatic plants were negative in the enrichment ELISA and PCR assays specific for Pca and *Dickeya* spp.

Discussion

In this study, for the first time it is demonstrated that in temperate climates in western Europe, Pcc strains can cause typical blackleg symptoms in potato field crops. Relatively high percentages of up to 50% blackleg-diseased plants were found in the Pcc-inoculated plants compared to 90% and 42% after Pca and *Dickeya* spp. inoculation, respectively.

The Pcc strains used were all isolated from blackleg-diseased plants, and their identity confirmed with biochemical and PCR assays. The strains were

Table 3 Identification of pathogens in symptomatic tissue of blackleg-diseased plants

Treatment		Average % diseased plants		Enrichment ELISA		Enrichment PCR		Enrichment PCR		
Bacterium	Strain	Valthermond	Tollebeek	Pca	Dcd	Pca	Dcd	Pcc, EXPCC	Pcc, Contig1	Pcc, Contig3
Pca	1007	69	89	+	−	+	−	−	−	−
Pca	1987	27	24	+	−	+	−	−	−	−
Dickeya spp.	1651	43	19	−	+	−	+	−	−	−
Dickeya spp.	1741	19	5	−	+	−	+	−	−	−
Pcc	5014	44	42	−	−	−	−	+	+	+
Pcc	91/84	30	36	−	−	−	−	+	+	+
Pcc	1250	0	0	nd	nd	nd	nd	nd	nd	nd
Pcc	40b	16	16	−	−	−	−	+	+	+
Pcc	50a	50	43	−	−	−	−	+	+	+
Pcc	119a	27	43	−	−	−	−	+	+	+
Pcc	151	38	25	−	−	−	−	−	−	−
Pcc	204d	4	1	−	−	−	−	+	+	+
Pcc	S31.1	39	19	−	−	−	−	+	+	+
Pcc	S60.3	22	14	−	−	−	−	+	+	+
Pcc	S83.1	23	20	−	−	−	−	+	+	+
Pcc	S109.1	20	13	−	−	−	−	−	−	−
Pcc	S110.1	13	15	−	−	−	−	−	−	−
Pcc	S121.1	15	7	−	−	−	−	+	+	+
Pcc	S188.3	11	20	−	−	−	−	+	+	+
Pcc	S189.1	29	30	−	−	−	−	+	+	+

For *P. atrosepticum* (Pca) and *Dickeya* spp. enrichment ELISA and enrichment PCR techniques were used; for *P. carotovorum* subsp. *carotovorum* (Pcc) enrichment PCR techniques were used, based on three different primer sets.

nd Not done

further selected for a positive reaction in PCR assays which were able to distinguish a subgroup of Pcc strains, most of which caused wilting symptoms in a field experiment in 2003 (Van der Wolf et al. 2003). Even within this selection of Pcc strains, a high and significant variation was found in the ability to cause blackleg. In general, Pcc is genetically and serologically highly diverse and therefore a variation in virulence can be expected (Togashi et al. 2001; Yap et al. 2004). In greenhouse tests in Wisconsin (USA), a high variation in virulence of Pcc strains isolated from potatoes was also found after inoculation of tubers or petioles (Yap et al. 2004).

There are also strong indications for a significant role of Pcc as a primary blackleg-causing pathogen from different surveys in Europe. In 2005, in 15% of the naturally blackleg-diseased plants in the Netherlands only Pcc could be detected using an enrichment PCR procedure (NAK, unpublished results). Similarly, in extensive surveys conducted in France between 2003 and 2005, from, on average, 20% of blackleg-diseased plants, only Pcc could be isolated (V. Helias, Rennes, INRA, personal communication).

The growing season for potatoes in the Netherlands of 2006 was characterized by slow crop development due to little rainfall and high temperatures in June and July. July had the highest temperature ever measured in the Netherlands, with a mean of 5°C above average (KNMI 2007). Possibly slow crop development in combination with the high temperatures resulted in high soil temperatures which favour the multiplication of Pcc in tubers and the onset of symptom development, as previously shown by Molina and Harrison (1980).

The location and type of soil seems to have only a minor effect on disease development, as no difference was found between disease development at the Tollebeek (clay soil) and Valthermond (peaty soil) sites.

The high disease incidences showed the efficiency of vacuum-infiltration for inoculation of potato tubers with *Pectobacterium* and *Dickeya* spp., as was previously reported (Fraaije et al. 1996, Helias et al. 2000, Toth et al. 2003).

In contrast to *Dickeya* and Pca, no pre-emergence rot was found for Pcc, possibly indicating that for Pcc the pathogenicity process starts relatively late.

Analysis of blackleg-diseased plants confirmed the presence of the different *Pectobacterium* and *Dickeya* spp. for most treatments. Only in diseased plants inoculated with Pcc isolates 151, S109.1 and S110.1, the presence of Pcc could not be confirmed with enrichment PCR, using the EXPCC, contig 1 or contig 3F primers. Possibly, after disease development, Pcc populations decline due to host defence or the activity of bacteria overgrowing Pcc in diseased stem tissue.

Our results indicate that the role of Pcc as a causal organism of potato blackleg should not be underestimated in regions with a temperate climate. It is recommended to use seed potatoes free of virulent Pcc strains able to cause blackleg. This requires the development of reliable methods able to distinguish virulent from non-virulent strains. Pcc is an opportunistic pathogen, which is widespread and can be soilborne (Peltzer and Sivasithampran 1985; Togashi et al. 2001). Methods that also detect non-virulent Pcc strains would result in a high percentage of positive samples and a degradation or rejection of many seed lots bearing no risk for blackleg development.

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