

Identification of *Phytophthora* spp. isolated from plants and soil samples on strawberry plantations in Poland

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Abstract Crown and leather rot of strawberry caused by *Phytophthora* spp. are major soil diseases of cultivated strawberry (*Fragaria × ananassa*) in Poland. In this study, in total 45 isolates of *Phytophthora* spp. from plants of cultivars Elsanta, Honeoye, Florence, Camarosa, Roxana, Onebor, Alba, Elegance, Albion, Senga Sengana and Malwina originating from crowns (20 isolates) and fruits (25 isolates) as well as 25 soil samples from the same fruiting strawberry plantations located in central, north-eastern, and south-eastern regions of Poland were identified. Among them, 44 isolates from plant organs and eight from soil samples were identified as *Phytophthora cactorum* and from one plant and one soil sample as *P. citricola*; of those remaining from the soil, one was identified as *P. citrophthora* and one as *P. cryptogea*. Identifications were based on morphological characteristics and DNA analyses. Species-specific polymerase chain reaction confirmed the presence of *Phytophthora* spp. in all positive examined plant and soil samples and confirmed that *P. cactorum* is predominantly responsible for crown and leather rot diseases of strawberry in monitored regions of Poland. Pathogenicity tests showed that all isolates of *Phytophthora* spp. originating from soil derived from strawberry plantations were pathogenic both to strawberry and to raspberry.

Keywords Crown rot · *Fragaria × ananassa* · Identification · Leather rot · Molecular detection · *Phytophthora* spp.

Introduction

Phytophthora is a genus of plant pathogenic filamentous oomycetes (phylum: Oomycota), which, morphologically, are very similar to the filamentous fungi from the kingdom Fungi [2]. *Phytophthora cactorum* was first identified on cacti in 1870 by Lebert and Cohn [12], and it currently infects an extremely large number of plant hosts. It can limit production of many economically important crops, such as apple, pear, rhododendron, azalea, and strawberry [13]. *Phytophthora cactorum* can cause root, collar, and crown rots as well as foliar and fruit damage; thus, it is among the most serious soil-borne pathogens affecting crops worldwide [20]. In Poland, *P. cactorum* causes serious strawberry yield losses due to plant stunting and collapse, especially on susceptible cultivars, such as Elsanta, Honeoye, Elegance, Onebor. Stunting of whole plants or wilting of young leaves are the first symptoms and may appear at any time during the season. The pathogen infects the underground parts by motile zoospores and then colonises strawberry crown. As a result, a typical uniform brown discoloration is visible after the crown cross section, which may appear at the base or in the middle of the crown [4]. Severe infection of the crown leads to dysfunction of the vascular system and to dieback of roots. Leaf margins begin to turn brown and entire leaves wilt, often quickly, and, finally, the whole plant dies. Symptoms of strawberry crown rot can be similar to those elicited by *Colletotrichum acutatum* and *Verticillium dahliae*, which are commonly encountered on strawberry plantations in Poland. Therefore, identification of the causal agents of soil-borne diseases on strawberry plantations is an important issue in preventive and pest control management.

The purpose of this study was to investigate (1) which species of *Phytophthora* are causing necrosis on strawberry

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fruits and plants; (2) which species are occurring in soil samples and are involved in the decline of strawberry plants in central, north-eastern, and south-eastern regions of Poland; and (3) the pathogenicity of *Phytophthora* spp. present in soil for strawberry and raspberry plants.

Materials and methods

Sampling and isolation procedure

In 2013–2014, plants with visible disease symptoms of crown rot and/or leather rot were sampled from 25 strawberry fruiting plantations in the central, north-eastern, and south-eastern regions of Poland (Table 1).

Small fragments of infected plant organs (fruit, root crown) were surface-disinfected by dipping in 0.5 % (w/v) sodium hypochlorite for 2–5 min and rinsed with sterile water before they were placed onto potato dextrose agar (PDA, Sigma-Aldrich Sp.z.o.o, Poznań, Poland) containing streptomycin, in 90-mm Petri dishes. For each plant organ,

four Petri dishes were used, each with 10 tissue pieces. Subsequently, plates were incubated at 25 °C until the mycelial growth was observed. After 3–5 days of growth on PDA at 22 °C, isolates were identified by morphological and cultural characteristics [7, 12]. Finally, 45 isolates of *Phytophthora* spp. from fruits and/or crowns of strawberry cultivars Elsanta, Honeoye, Florence, Camarosa, Roxana, Onebor, Alba, Elegance, Albion, Senga Sengana and Malwina were obtained. The severity of leather rot on strawberry fruits was evaluated on three selected plantations by counting all healthy and infected fruits on 26 plants in each of four randomly chosen plots during harvest. The results were analysed statistically by the variance analysis method on per cent data transformed according to the Bliss function. The differences between means were estimated by the Newman–Keuls test at the 5 % level of significance.

Isolations from soil samples were made from the same 25 fruiting strawberry plantations (Table 1). Bulked soil samples (about 1.0 kg) were collected from 10 randomly chosen positions of each plantation, at a depth of

Table 1 Strawberry plantations in central, north-eastern, and south-eastern Poland included in this study

Location of strawberry plantation	Cultivar	Area (ha)	Soil texture	Soil pH	Plant material for study
Bobrowiec [C]	Honeoye	0.7	Sandy and loamy clay	6.0	Crown, fruit
Chynów [C]	Onebor	0.5	Sandy clay above clay	6.2	Fruit
Konin [C]	Honeoye	0.3	Podzol and fawn	6.2	Fruit
Godzianów [C]	Honeoye	0.5	Light soil sand	6.2	Crown, fruit
Jankowice [C]	Malwina	0.5	Sandy clay	5.0	Crown, fruit
Skierniewice 1 [C]	Elegance	3.0	Sandy clay	5.5	Crown, fruit
Skierniewice 2 [C]	Elsanta	2.0	Sandy clay	6.4	Fruit
Wilcze Średnie [C]	Florence	2.0	Sandy clay	6.0	Crown, fruit
Teodozjów [C]	Roxana	0.3	Clayey soil	5.5	Fruit
Radziejowice [C]	Elegance	0.7	Sandy clay	6.2	Crown, fruit
Karsznice [C]	Honeoye	2.0	Clayey soil	6.5	Crown, fruit
Zdziarka [C]	Alba	0.5	Sandy clay	5.0	Crown, fruit
Wilga [C]	Malwina	0.7	Sandy clay	6.0	Crown, fruit
Wilków [E]	Onebor	0.5	Sandy clay	5.5	Crown, fruit
Szydłówka [E]	Albion	3.2	Light soil sand	6.0	Crown, fruit
Tuszewo [N–E]	Camarosa	1.0	Sandy clay	5.0	Crown, fruit
Zarzecze [E]	Elegance	0.5	Clayey soil	6.0	Crown, fruit
Huszlew [E]	Elsanta	0.3	Sandy clay	5.8	Crown, fruit
Serniki [E]	Elsanta	0.4	Light soil	6.0	Crown, fruit
Zosinek [E]	Senga Sengana	0.2	Sand	6.0	Crown, fruit
Dzierzkowice-Wola [E]	Honeoye	1.0	Sandy clay	6.2	Crown, fruit
Łubianki [E]	Camarosa	1.5	Clayey soil	6.0	Crown, fruit
Tuszewo [N–E]	Camarosa	1.0	Sandy clay	5.0	Crown, fruit
Orneta [N–E]	Onebor	1.0	Sandy clay	5.5	Fruit
Mrągowo [N–E]	Honeoye	1.0	Loam	5.5	Crown, fruit
Bogumiłowice [S–E]	Honeoye	0.7	Sandy clay	6.0	Crown, fruit

C, E, N, S—region in Poland: E eastern, C central, N northern, and S southern

10–15 cm, and stored at room temperature. For *Phytophthora* spp. isolation, the baiting test according to Themann et al. [23] was conducted. Trays were filled with tap water to about 1 cm above soil level, and leaves of *Rhododendron* cultivar Nova Zembla (8–10 leaves per tray) were floated on the surface. After 5–7 days of incubation at 21–23 °C, *Rhododendron* leaves were harvested, washed under tap and deionised water, and dabbed dry. Then, segments of 5 mm diameter were cut from individual necrotic spots and placed onto PDA in 90-mm Petri dishes, 25 pieces per dish, each in five replicates. Within the next 2 days, hyphae growing from the plated segments were assessed under an optical microscope for the presence of sporangia. Small fragments from the edge of growing microorganisms were transferred onto new PDA plates in order to obtain pure cultures.

Classification of isolates into the *Phytophthora* genus

After 5–7 days of growth on PDA at 22 °C, isolates were identified by morphological and cultural characteristics [12, 18]. The isolates were cultured on PDA in darkness at 25 °C for 14 days. Characteristic morphology of *Phytophthora* spp. mycelial growth and the presence of sporangia and/or oogonia were observed. Additionally, sporangia were produced by flooding round mycelial discs of 6 × 6 mm in diameter taken from growing margins of 3- to 5-day-old colonies on PDA, 1 mm over their surface, with non-sterile soil extract [18] in 90-mm Petri dishes and incubating them in the dark at 18–22 °C. Soil extract was replaced with a fresh one every 24 hours. After three days mycelial fragments were taken from the growing edge of submerged PDA plugs and transferred onto microscopic slides. Zoosporangia and oogonia were

observed at 400× magnification under a light microscope Delta Optical Evolution 300 LED (Delta Optical, Mińsk Mazowiecki, Poland).

DNA extraction from *Phytophthora* spp. cultures

From 2- to 5-day-old *Phytophthora* spp. cultures of 45 isolates, about 50–100 mg of fresh mycelia were cut out, and total DNA was extracted following the Aljanabi and Martinez [1] protocol for DNA extraction. DNA was suspended in 50 µl of double-deionised water and kept at –20 °C for further analyses.

PCR with species-specific primers and sequence analysis

The obtained DNA was tested in PCR with the six primer sets (Table 2) specific for the following *Phytophthora* species: *P. citricola*, *P. citrophthora*, *P. cinnamomi*, *P. cryptogea*, *P. cactorum*, and *P. fragariae*. Conditions for PCR were as follows: an initial denaturation step of 3 min at 94 °C, followed by 30 cycles of 30 s of denaturation at 94 °C; 30 s of annealing at 60 °C for *P. fragariae*-specific primers or at 66 °C for the other primers; and 60 s of polymerisation at 72 °C. The final elongation step was 5 min at 72 °C. Each PCR mixture consisted of the following: 10–30 ng of total DNA template, 500 nM of reverse and 500 nM of forward primers of one species-specific set, 0.2 mM of each dNTP, 0.45 unit of DreamTaq Green DNA Polymerase (Thermo Scientific, Vilnius, Lithuania), 1× optimised DreamTaq Green Buffer, and double-distilled water in a total volume of 15 µl. All reactions were performed in a Biometra T3000 Thermocycler (Biometra, Göttingen, Germany). Then, PCR

Table 2 List of primers used in this study and their nucleotide sequences

Primer name	Primer sequence (5' → 3')	Target organism	References
<i>Phytophthora</i> spp.-specific primers			
Primer #1	GTCGACGTCCTGCTTGGCACTCTG	<i>P. citrophthora</i>	Ersek et al. [11]
Primer #2	CGGTGCTCCGCGACTGTTGTCCAC	<i>P. citrophthora</i>	Ersek et al. [11]
CITR1	TCTTGCTTTTTTGGCGAGCC	<i>P. citricola</i>	Schubert et al. [21]
CITR2	CGCACCGAGGTGCACACAAA	<i>P. citricola</i>	Schubert et al. [21]
ADF1	TACTGTGGGGACGAAAGTCCT	<i>P. cactorum</i>	Boersma et al. [5]
ADR1	CCGATTCAAAAGCCAAGCAACT	<i>P. cactorum</i>	Boersma et al. [5]
DG9	AACTGAGCTAGTAGCCTCTC	<i>P. cinnamomi</i>	Boersma et al. [5]
DC5	CGCCGACTGGCCACACAG	<i>P. cinnamomi</i>	Boersma et al. [5]
CRYF2	CGGTTTTCGGCTGGCTGGG	<i>P. cryptogea</i>	Boersma et al. [5]
CRYR2	CAGCTTGCGCCAGAACAGAC	<i>P. cryptogea</i>	Boersma et al. [5]
DC1	ACTTAGTTGGGGGCTGTCT	<i>P. fragariae</i>	Bonnants et al. [6]
DC5	CGCCGACTGGCCACACAG	<i>P. fragariae</i>	Bonnants et al. [6]
Primers for amplification of fungal and oomycetal rDNA region			
ITS1	TCCGTAGGTGAACCTGCGG	Fungi and oomycetes	White et al. [26]
ITS4	TCCTCCGCTTATTGATATGC	Fungi and oomycetes	White et al. [26]

products were separated during electrophoresis on 1.5 % (w/v) gels, stained with ethidium bromide, and visualised using a transilluminator UV (Sigma T1202; Sigma-Aldrich, St. Louis, USA). The obtainment of the proper-size PCR products was confirmed by comparison with the DNA of the reference strains, showing positive signals with the species-specific primers. For three selected isolates, GL1, BT1, and WG1 (identified as *Phytophthora* spp. in the PCR assay), and for those DNA samples derived from *Phytophthora*-like colonies growing in the baiting test for which no PCR product was obtained in species-specific PCRs, the DNA was amplified with ITS1 and ITS4 primers, specific for ITS (internal transcribed spacer) regions of filamentous fungi and oomycetes [26], and subjected to sequence analysis. The obtained DNA sequences were compared with the sequences available in the GenBank database using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov/BLAST/) and deposited in GenBank (National Center for Biotechnology Information), with accession numbers assigned to them.

Pathogenicity of *Phytophthora* spp. from strawberry plantations to raspberry and strawberry plants

Pathogenicity tests were conducted with isolates derived from soil samples from strawberry plantations: two isolates of *P. cactorum* (GL1, Serniki, and BT1, Tuszewo) and one of *P. citricola* (WG1, Serniki). In June 2014, leaf petioles of the strawberry cultivar Elsanta and stems of the raspberry cultivar Polka were taken and washed under tap water, dabbed dry, and transferred to trays onto moist, sterile blotting paper covered with a plastic net. Three-millimetre-diameter discs cut from the growing margins of 5-day-old PDA cultures of the three examined *Phytophthora* spp. isolates were placed on strawberry leaf petioles and stems of raspberry and covered with parafilm. Control plant material received plain PDA discs. Trays were covered with the foil and incubated at 24 °C. For the experiment, each of 10 leaf petioles and canes per isolate–host combination conducted in four replicates was randomised. Length of necrosis was measured on the fifth (raspberry) and eighth days (strawberry) after inoculation. The experiment was repeated twice. In order to fulfil Koch's postulates, the causal agents of observed symptoms were reisolated on PDA plates, identified using molecular tests, and used for reinfection of plants.

Statistical analyses

Results from the different pathogenicity trials were analysed using analysis of variance (ANOVA) followed by the Tukey HSD test for multiple comparisons (STATISTICA 10; StatSoft Polska, Kraków, Poland).

Results and discussion

Classification of *Phytophthora* spp. isolates on the basis of morphological traits

On the basis of morphological trait estimation during *Phytophthora* spp. growth in pure cultures (colony characters, conidial and setal morphology, and production of sporangia and/or oogonia), 44 isolates derived from examined plant tissues were classified as *P. cactorum* and one as a *P. citricola*. Among cultures growing in baiting tests, typical *Phytophthora*-like morphology was observed for 10 soil samples. For fast detection of *Phytophthora* species in strawberry plants, culture-based isolation methods are widely used. On PDA media, *P. cactorum* usually forms a white, loosely matted colony. Sporangia are distinctively papillate and are usually borne terminally. Each sporangium may contain more than 50 zoospores [10]. According to Waterhouse [25], *P. citricola* can be readily differentiated from *P. cactorum* var. *applanata* on the basis of some morphological features. In culture, *P. citricola* tends to grow in a petaloid manner, while *P. cactorum* grows uniformly fluffy. Moreover, the apex of a sporangium of *P. citricola* is flat and wide with shallow thickening, while in *P. cactorum* prominent papillae with deep thickenings can be observed. Described differentiating features were also observed for these species in our study (Fig. 1a, b, e, f). Sporangia and oogonia produced by *P. cactorum* isolates on PDA media were observed after 10 days of cultivation, whereas *P. citricola* growing on PDA produced only oogonia; sporangia of the latter species were observed on the third day of cultivation in non-sterile soil extract. The oogonia of *P. citricola* were similar in size to those of *P. cactorum* (Fig. 1i, h). Our observations of growing cultures obtained in baiting tests were similar to those previously described for *Phytophthora* spp.; therefore, it was also possible to preliminarily classify the oomycetes as *P. citricola* and *P. cactorum* and, additionally, to *P. citrophthora* and *P. cryptogea*. *Phytophthora citrophthora* showed stellate, rosettal growth on PDA medium (Fig. 1c) and was able to produce sporangia in non-sterile soil extract (Fig. 1g), but oogonia were not observed on PDA medium, nor in soil extract. Colonies of *P. cryptogea* were fairly fluffy and cottony, with lobate margins and a slight floral pattern (Fig. 1d), but they did not produce sporangia nor oogonia on PDA or in soil extract. The lack of the production of oogonia by *P. cryptogea* and *P. citrophthora* is consistent with the previous observations [12, 19] that these species are heterothallic and require crossing with an opposite mating type to form oogonia and oospores.

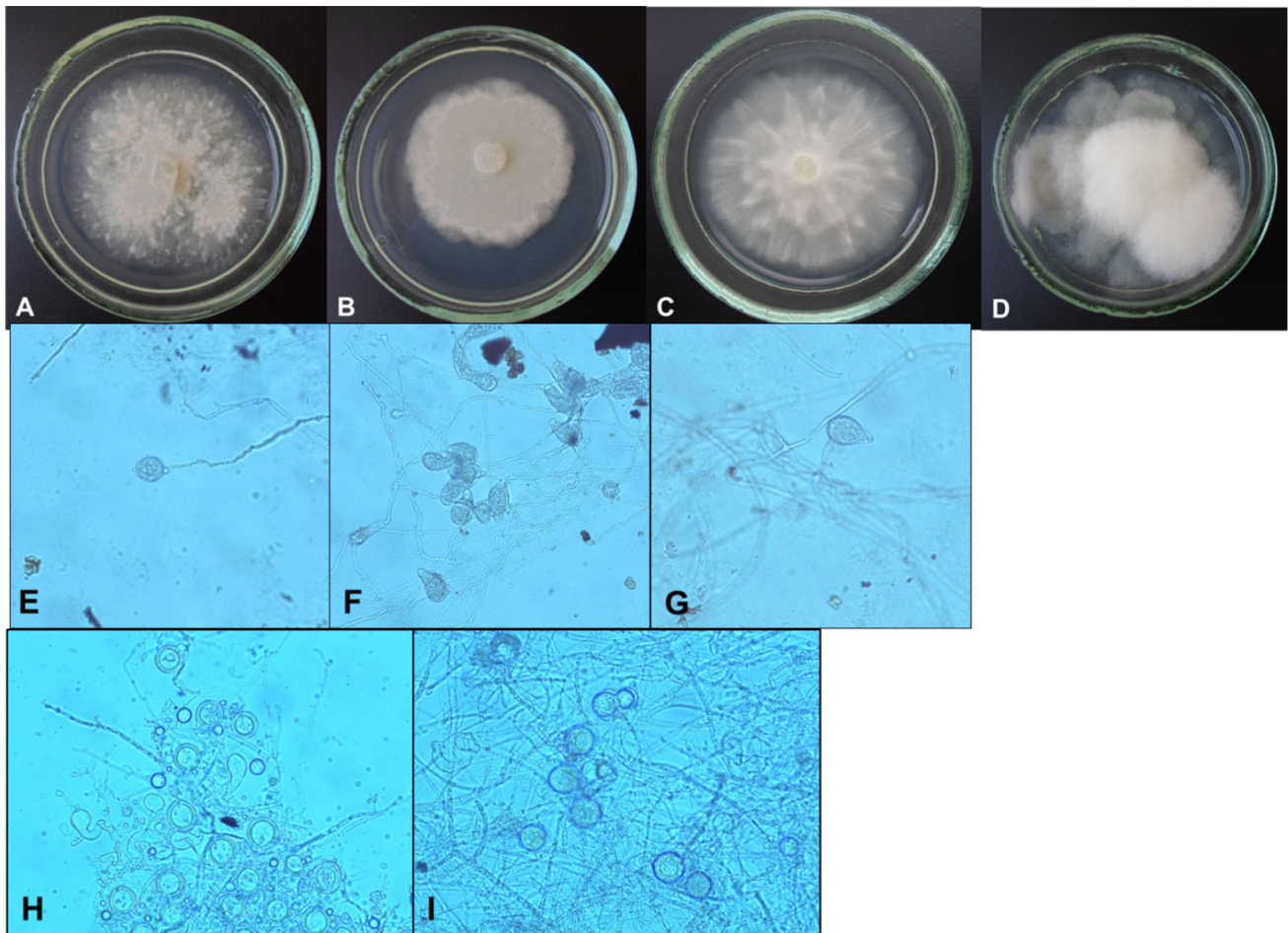


Fig. 1 Macroscopic and microscopic features of *Phytophthora* spp. growing in cultures on potato dextrose agar (PDA). The colonies photographed on the sixth day of incubation: **a** *P. citricola* (isolate WG1), **b** *P. cactorum* (isolate BT1), **c** *P. citrophthora* (strain RH04/CTPH), and **d** *P. cryptogea* (isolate from Zarzecze). Morphology of

zoospores of **e** *P. cactorum* (isolate BT1), **f** *P. citricola* (isolate WG1), and **g** *P. citrophthora* (strain RH04/CTPH). Morphology of oogonia of **h** *P. cactorum* (isolate BT1) and **i** *P. citricola* (isolate WG1). Magnification under light microscope $\times 400$

Phytophthora spp. identification in PCR assays

In PCRs with six *Phytophthora* species-specific primer sets, the presence of the proper-size products of reactions was observed on agarose gel. For 45 tested DNA samples obtained from *Phytophthora* spp. isolates from diseased plant tissues, positive signals for *P. cactorum* were observed (44 isolates) but only one for *P. citricola* (Table 3).

From 25 soil samples, only in 20 % (nine soil samples) were *Phytophthora* spp. strains trapped and recognised in the baiting test. In the PCR assay, isolates from eight plantations were identified as *P. cactorum*, from one as *P. cryptogea* (Zarzecze), from one as *P. citrophthora* (Tuszewo), and from one as *P. citricola* (Serniki) (Table 3). This may suggest that *Phytophthora* spp. propagules are transferred between plantations mostly by strawberry stocks. It has been also reported that *Phytophthora* spp. can survive in infected host tissue [14, 22].

In the case of those *Phytophthora* spp. isolates for which sequencing of the ITS rDNA region and its comparison with reference sequences in GenBank were performed, the species classification was consistent with the results of the PCR assay. BLAST analysis of obtained DNA sequences of GL1 (GenBank Acc. No. KT361202) and BT1 (GenBank Acc. No. KT361201) showed 100 % similarity with the corresponding region of reference of the *P. cactorum* strain (GenBank Acc. No. GU111587.1), while the sequence of the WG1 isolate (GenBank Acc. No. KT361200) was 100 % similar to the sequence of *P. citricola* E strain (GenBank Acc. No. AF266788.1).

For other microorganisms whose colonies were obtained in the baiting test, the sequence analysis of the ITS rDNA region demonstrated the presence of fungi or oomycetes from the genera *Pythium*, *Fusarium*, *Rhizopus*, and *Mortierella*. Species identification based on morphological and physiological characters is a primary approach, although it is mostly laborious and time-consuming. Due to high intra-

Table 3 Detection of *Phytophthora* spp. in plant and soil samples in the PCR assay

Location of plantation	<i>P. cactorum</i>	<i>P. citricola</i>	<i>P. citrophthora</i>	<i>P. cryptogea</i>
Bobrowiec	Crown, fruit			
Bogumiłowice	Crown	Crown		
Chynów	Fruit			
Dzierzkowice-Wola	Crown			
Godzianów	Crown, fruit			
Huszlew	Crown, fruit			
Jankowice	Crown			
Karsznice	Soil, fruit			
Konin	Fruit			
Lubianki	Crown			
Mrągowo	Crown			
Orneta	Fruit			
Radziejowice	Crown			
Serniki	Soil [GL1]	Soil [WG1]		
Skierniewice 1	Soil, crown			
Skierniewice 2	Fruit			
Szydłówka	Soil, crown			
Teodozjów	Fruit			
Tuszewo	Soil [BT1]		Soil	
Wilcze Średnie	Soil, fruit			
Wilga	Crown			
Wilków	Soil, crown			
Zarzecze	Crown			Soil
Zdziarka	Crown			
Zosinek	Soil, crown			

species variability and small inter-species differences between *Phytophthora* species, additional confirmation is often required. Techniques based on the PCR method offer rapid, simple, and reliable identification of fungal and oomycetal species. Not only can species be identified in pure cultures, but it is also possible to detect pathogens directly in plant material and soil, which highly facilitates the possibility for specific monitoring of these pathogens. On the basis of some species-specific regions in the genome of *Phytophthora*, Ersek et al. [11] developed species-specific primers for *P. parasitica* and *P. citrophthora* detection. The variability in the DNA regions of ITS regions of rRNA of *Phytophthora* spp. has been also examined by Schubert et al. [21] and Boersma et al. [5]; thus, it became possible to discriminate species within the *Phytophthora* genus using species-specific primers designed by those authors. Because of recent changes in the *P. citricola* complex—the new species *P. plurivora* sp. nov. was designated [18]—there was a need to additionally confirm the species affiliation of the WG1 *P. citricola* isolate by comparing its ITS1–5.8S–ITS2 rDNA region with the sequence of reference strains from this complex, available in GenBank database.

Association of *Phytophthora* spp. presence with yield loss

Excessive rainfall during the 2013 growing season was associated with heavy losses in strawberry crop due to fruit rot. Leather rot caused by *P. cactorum* accounted for up to 11 % yield loss depending on cultivar (Fig. 2) within three examined plantations. Propagules of *P. cactorum* are readily dispersed from infected strawberry fruit by

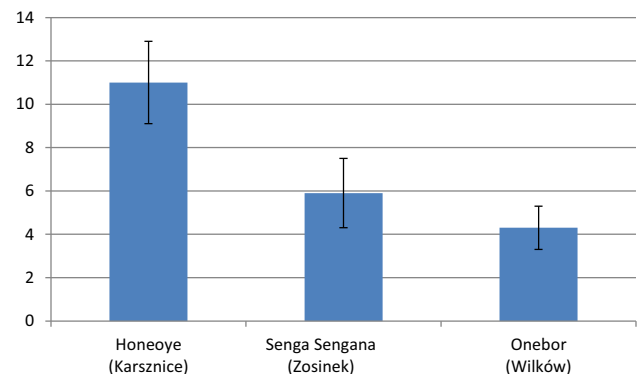
**Fig. 2** Severity of leather rot of strawberry in the 2013 season

Table 4 Length of necrosis produced on petioles of strawberry and raspberry canes after infection by *Phytophthora* spp.

Name of isolate	Source	Species of <i>Phytophthora</i>	Average length of necrosis (mm)	
			Raspberry cane	Strawberry leaf petioles
Control	–	–	0.0 a*	0.0 a
GL1	Soil	<i>P. cactorum</i>	14.6 b	42.7 b
BT1	Soil	<i>P. cactorum</i>	8.2 ab	32.0 b
WG1	Soil	<i>P. citricola</i>	19.7 b	49.5 b
			MS between groups = 119.06	MS between groups = 521.63
			df = 36	df = 36

* The same letters indicate lack of statistically significant differences between values in columns, according to the Tukey test

splashing water [16]. Gerlach et al. [14] showed that zoospores of *P. citrophthora* were readily dispersed to potted *Pieris japonica* by water. Outbreaks of fruit rot on apple caused by *P. syringae* in the UK were related to the heavy rains, and inoculum was splashed with infested leaf debris on the orchard floor [24]. *Phytophthora* spp. spread mainly through the movement of infested soil, water, and infected plants or plant material; however, there are species that are transmitted aerially [8, 15]. Especially substantial as a source of inoculum are those plants that are infected but do not show symptoms either because the disease has not yet progressed to the stage where symptoms are evident. Therefore, the major challenge in preventing the spread of *Phytophthora* is detection of the pathogen in asymptomatic tissue.

Pathogenicity of *Phytophthora* spp. from soil samples to raspberry and strawberry

The average length of necrosis on raspberry cane ranged from 8.0 to 19.7 mm, while on strawberry leaf petioles it ranged from 32.0 to 49.5 mm, depending on the isolate of *Phytophthora* spp. (Table 4). The longest necrosis was observed for isolate WG1 (*P. citricola*), and the smallest, for isolate BT (*P. cactorum*); however, this difference was not significant. All isolates of *Phytophthora* were successfully reisolated from symptomatic tissues and identified using molecular tests, fulfilling Koch's postulates.

This study shows that two *P. cactorum* and one *P. citricola* strains isolated from soil samples collected in the strawberry plantations where disease symptoms were observed were pathogenic both to strawberry plants and to raspberry plants, causing necrotic lesions on wounded leaf petioles and raspberry canes. The differentiation in *P. cactorum* isolates according to host preference was found in studies of Cooke et al. [9] and Hantula et al. [17]. Bielenin [3] reported that isolates of *P. cactorum* both from strawberry crowns and from fruit caused distinctly smaller necrosis symptoms on apple shoots than isolates from apple trees.

In conclusion, we have shown on the basis of morphology and DNA sequence analysis that the *P. cactorum* species is the most common causal agent of strawberry infections in Poland. Under favourable weather conditions for development and spread of the pathogen (high humidity and temperatures above 20 °C), it causes significant losses in yield of strawberries as well as decay of whole plants. The results of pathogenicity trials revealed that the filamentous parasitic organisms *Phytophthora* spp. (class *Oomycetes*) occurring in the soil can be pathogenic for both strawberry and raspberry plants, showing no host specificity.

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Compliance with ethical standards

Conflict of interest The authors confirm that they have no conflict of interest.

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