Phacidiopycnis pyri causing *Phacidiopycnis* rot on pear fruit in Argentina

María Cecilia Lutz[1,2] · **Carla Nahir Basso**[1,2] · **María Julia Ousset**[2] · **Leticia Dorotea Vexenat De Giorgi**[1,2] · **María Cristina Sosa**[1,2]

Abstract

Previously unknown postharvest rots in Packham's Triumph pear were detected in a batch of long-term cold-stored fruit during 2022 in North Patagonia, Argentina. Rots were characterized according to their symptoms and the causal agent was isolated and identified through cultural and molecular methods. Pathogenicity tests were performed on different pear cultivars both at 20 °C and under storage conditions (0 °C − 95% RH). Koch's postulates were fulfilled by re-isolation of the pathogen. Our results indicated that *Phacidiopycnis pyri* was the causal agent of postharvest rot in the fruit examined. This would constitute the first report of *P. pyri* as a causal agent of postharvest decay in Argentina.

Keywords *Pyrus communis* · *Phacidiopycnis* · Postharvest rot

Argentina is one of the main pear-producing and exporting countries in the world. The largest volume of pear production (*Pyrus communis*) in the Alto Valle region, the main producing area in Argentina, is exported during the winter season of the Northern Hemisphere. The pear cultivar Packham's Triumph is one of the region's main crops and has

María Cecilia Lutz and María Cristina Sosa contributed equally to this work.

✉ María Cecilia Lutz
m.cec.lutz@gmail.com

✉ María Cristina Sosa
mcristinasosa10@gmail.com

Carla Nahir Basso
carlanahirbasso@gmail.com

María Julia Ousset
juousset@gmail.com

Leticia Dorotea Vexenat De Giorgi
letivex@gmail.com

[1] Facultad de Ciencias Agrarias, Universidad Nacional del Comahue, Instituto de Biotecnologia Agropecuaria (IBAC), Ruta 151, Km 12, Cinco Saltos, Rio Negro 8303, Argentina

[2] Centro de Toxicologia Ambiental y Agrobiotecnologia del Comahue (CITAAC), CONICET - Universidad Nacional del Comahue, Buenos Aires 1400, Neuquen, C.P. 8300, Argentina

long-term cold storage capacity. Postharvest diseases due to fungal pathogens such as *Botrytis cinerea* and *Alternaria* spp., are common during storage of pear (Sholberg et al. 2003; Sosa et al. 2016). Postharvest rots by *B. cinerea* are associated to infections through both wounds and activation of latent infections affecting mainly stem and calyx ends (Lennox et al., 2004).

Phacidiopycnis rot caused by *P. pyri* has been reported at postharvest in fruit of the cultivar Beurre d'Anjou in the United States (Xiao and Boal 2004) and Canada (Sholberg et al. 2010), entailing significant losses during long-term storage. Similar to *B. cinerea, P. pyri* (whose sexual morph was known as *Potebniamyces pyri*) can cause rot through infections at the calyx, stem, or on fruit skin wounds (Liu and Xiao 2009). According to Lui and Xiao (2009), fruit infection by *P. pyri* occurs in the orchard and remains latent, with symptoms appearing after months of storage. During October 2022, significant losses were observed in a batch of bin-stored pear on long storage, with 3% incidence of unknown rot, mostly associated to the stem-end and less frequently to the calyx-end. Such rot did not present the characteristics typical of previous postharvest diseases reported for the region. Thus, the objective of the present work was to identify the causal agent and characterize the symptoms of a postharvest rot as yet undescribed in Argentina, affecting Packham's Triumph pear under long-term cold storage.

🖄 Springer

Fig. 1 Packham's Triumph pear found on bin box with decay symptoms after 7 months of cold storage (A). Fruits with symptoms of stem infections (B) and complete decay (C)
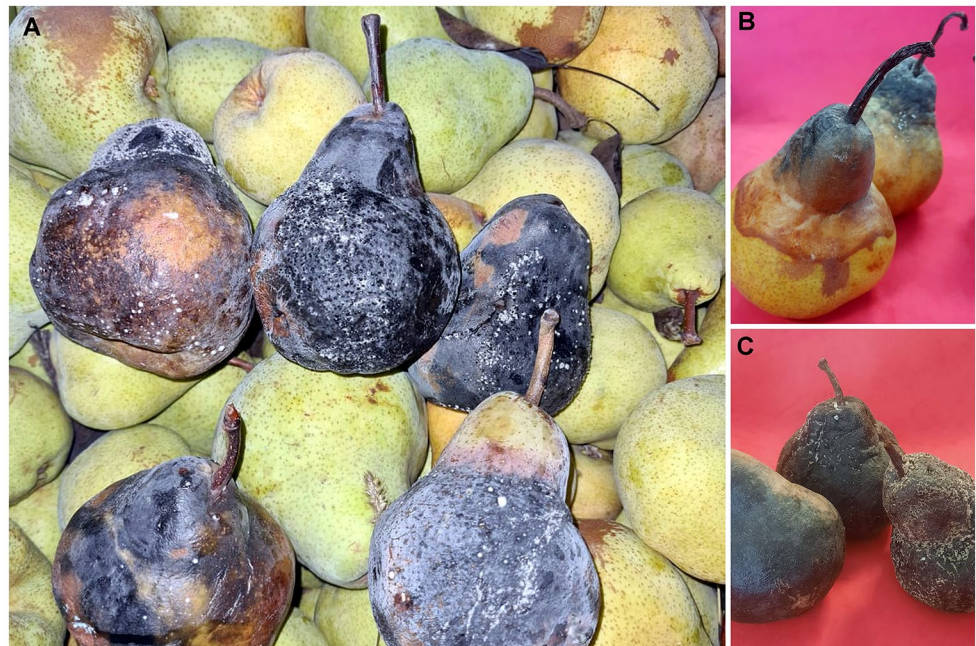
Fig. 2 Symptoms and signs of infection of pear fruit by *Phacidiopycnis pyri* of the decay (after 7 month's storage) from the stem, black in the upper part of the fruit and lighter, brown with diffuse edges towards the middle (A). Detail of internal rot (B). Totally decayed fruit exhibiting mycelial development (greyish white) and pycnidia (C and D)

Symptoms of stem-end, calyx-stem, and whole fruit rot were observed in a batch of Packham's pear fruit stored in bins (350−80 kg) after 7 months of cold storage (-1°/0° C −95% RH) (Fig. 1). The fruit had been harvested at the beginning of March 2022 and thermonebulised with fludioxonil and 1 methylcyclopropene in the processing line prior to packaging.

Rot presented a leathery and spongy texture, slightly firmer than gray mold rot, with brown to black specks, a water-soaked advance zone, and brown to black internal decayed tissue not separable from healthy tissue. Initially, the decaying tissue was brown, and turned black over time while the fruit stayed spongy. At advanced decay stages, the presence of white-grayish mycelia was detected, followed by emergence of black pycnidia with white-cream exudates (conidia) clearly visible to the naked eye (Fig. 2). Rotten fruit did not have a distinct odor. The symptoms observed in the fruit were the same as those produced by the *Phacidiopycnis pyri*, described by Xiao and Boal (2004).

Isolation of the fungus was performed from fruit tissues showing advanced rot. To this end, the surface of the fruit was disinfected with 70% ethanol, and tissue samples (3 × 3 mm) were incubated for 4 weeks at 20 °C under a light-dark cycle (12:12 h) on Petri dishes containing acidified Potato Dextrose Agar (PDA).

The microscopic and morphological characteristics of the PDA-grown colonies suggested that the fungal isolates corresponded to *Phacidiopycnis pyri*, as reported by Xiao (2006). Initially, colonies were white to colourless, with little or no aerial mycelia, and later turned gray to dark, radially outward from the colony centre. After 2

weeks of incubation, pycnidia were observed in the central area of the colonies, which formed and exuded both macroconidia and microconidia in droplets (Liu and Xiao 2009; Sholberg et al. 2010). Pycnidia were spherical and 10.5 ± 2.0 to 36.8 ± 4.5 μm (average 25.3 ± 5.0 μm, $n = 30$) in diameter. Macroconidia were hyaline, ovoid, and measured $4.8–6.5 \times 3.1–4.2$ μm ($n = 50$). After 4 weeks of incubation on PDA, the colonies did not reach the edge of the plates.

The isolates 1902, 1903, and 1906 were selected for molecular identification. Genomic DNA was extracted according to Liu et al. (2000). The ITS and TUB loci were amplified with the primer sets ITS1/ITS4 (White et al. 1990) and BT2a/BT2b (Glass and Donaldson 1995), respectively. Purified products were sequenced and analyzed with BLAST software (NCBI). DNA sequences were deposited into the GenBank database, with accession numbers OQ280985 (1902), OQ280986 (1903), and OQ280987 (1906) for ITS, and OQ291281 (1902), OQ291282 (1903), and OQ291283 (1906) for TUB. Sequences were used as a query to search the NCBI database. Partial ITS genes revealed 99.80%-99.40% similarity with CBS:322.63 and CBS:282.55 reference strains of *Potebniamyces pyri*, currently named as *Phacidiopycnis pyri*. In GenBank, no β-tubulin sequences derived from BT2a/BT2b primers had been submitted for *P. pyri* isolates prior to those derived from this study. The isolates 1902, 1903 and 1906 were deposited in the Instituto Spegazzini Culture Collection of La Plata, Argentina (LPSc) as LPSc 1741, LPSc 1742 and LPSc 1743, respectively.

The referred ITS and TUB sequences were manually edited and aligned using MAFFT v. 7 (Katoh et al. 2019) and phylogenetic analyses performed by the maximum likelihood method were run with Molecular Evolution Genetic Analysis version 11 (MEGA11) software (Tamura et al. 2021). The ITS sequences were compared with published sequences deposited in GenBank, whereas the TUB sequences were compared with the MG952723.1 strain of *Phacidiopycnis washigtonensis* from *Malus domestica*. In both ITS and TUB analyses, *Botrytis cinerea* was used as outgroup (Fig. 3). The phylogenetic analyses for the ITS gene region provided conclusive information regarding the identity of isolates.
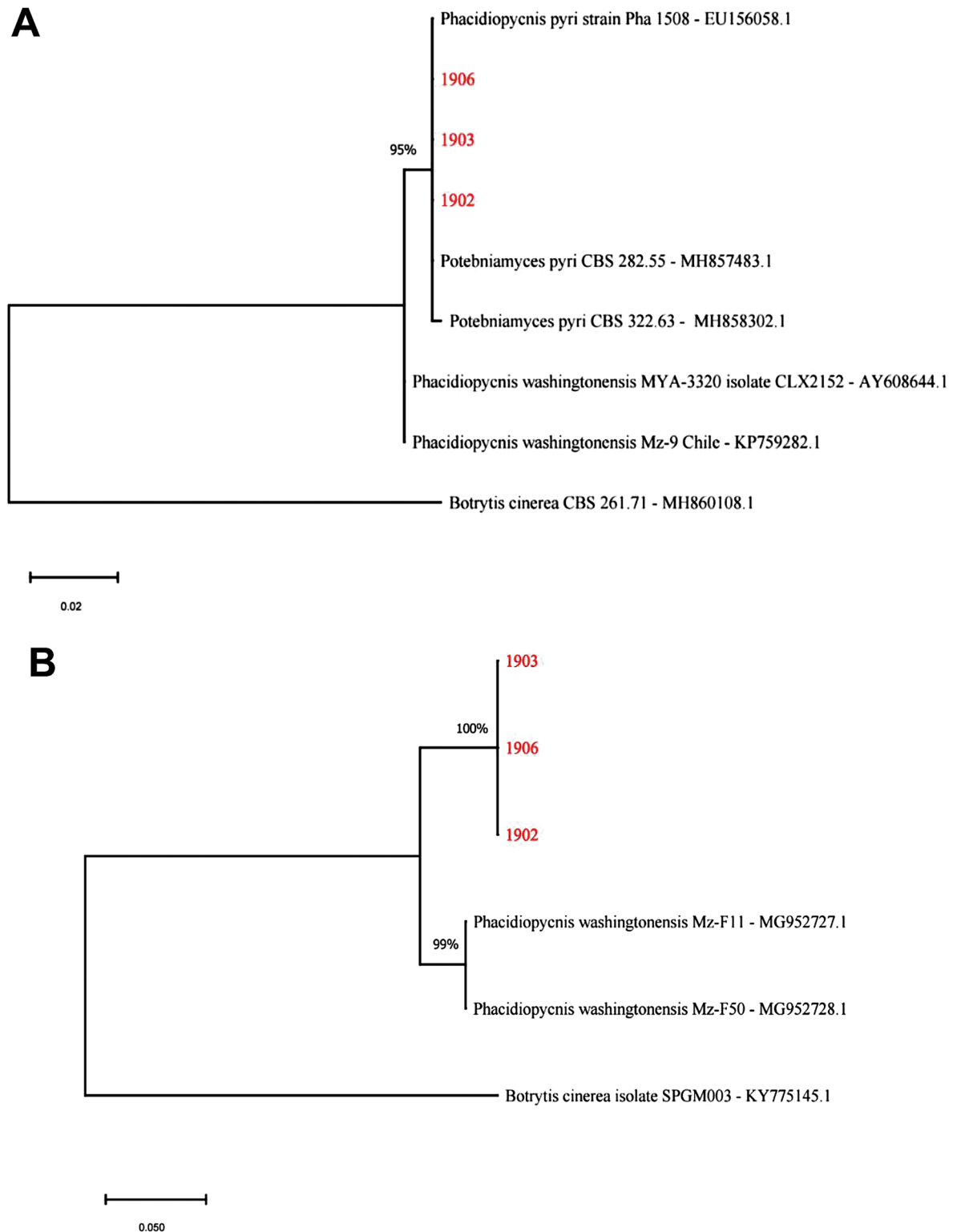
For pathogenicity assays, pear fruit were inoculated with conidia of the isolates 1902, 1903, and 1906, obtained from 21-day-old cultures in 12:12-h light-darkness. Three fruits each of the cultivars d'Anjou and Packham's Triumph, with three replicates (total 9 fruits per variety), were inoculated. The d'Anjou cultivar was incorporated in the experiment, as it was reported as highly susceptible (Sholberg et al. 2010). Fruit was inoculated with 10 μL of sterile distilled water (SDW) or suspension of 1×10^3 conidia mL^{-1} on the stem-end, calyx-end, and onto artificial wounds (3×3 mm) located at the equatorial zone. As a control, the fruit was inoculated with SDW (total 9 fruits per variety). Fruit was incubated at 20 °C for 7 days, then at 0 °C for 30 days, and finally at 20 °C for an additional 20 days. Isolates evaluated were pathogenic, although they differed in terms of symptom severity and site affected in each cultivar. Stem-end and wound rots occurred in the two varieties. On inoculated wounds, the largest rot diameters were observed in d'Anjou fruit (Table 1). In Packham's, rots were only observed in fruit stored cold and then incubated at room temperature. Stem-end rot developed with no statistical differences between the two cultivars (Table 1). In turn, in d'Anjou fruit cold stored and then, incubated at room temperature, the evaluated isolates more aggressively caused calyx-end rots. From symptomatic fruits, the fungus was re-isolated on PDA and incubated as described above. Fulfilling the Koch postulates, the colonies obtained were identical to the original ones.

This report confirmed of *Phacidiopycnis pyri* as causal agent responsible of sporadic and so far, undescribed rot that significantly affected stored fruit of Packham's cultivars in Argentina. In our region, application of postharvest fungicides does not control this rot. As the symptoms produced in the initial stages can be confused with those caused by *B. cinerea* (Xiao and Boal 2004), the incidence of *P. pyri* infections may be underestimated.

To our knowledge, this is the first report of *Phacidiopycnis pyri* causing *Phacidiopycnis* rot on pear fruit in Argentina. Indeed, we are not aware of previous reports of *P. pyri* infections in pear fruit cv. Packham's in other countries. Epidemiological studies in pear orchards where the fungus infected pear fruit from trees with cankers and branch dieback (Sholberg et al. 2010) and analysis of disease incidence in lots of different pear fruit cultivars during medium-long storage are necessary to establish the importance of the pathogen in our region, which produces and exports high-quality pears to northern markets in counter-season.

A

Phacidiopycnis pyri strain Pha 1508 - EU156058.1

1906

1903

1902

Potebniamyces pyri CBS 282.55 - MH857483.1

Potebniamyces pyri CBS 322.63 - MH858302.1

Phacidiopycnis washingtonensis MYA-3320 isolate CLX2152 - AY608644.1

Phacidiopycnis washingtonensis Mz-9 Chile - KP759282.1

Botrytis cinerea CBS 261.71 - MH860108.1

0.02

B

1903

1906

1902

Phacidiopycnis washingtonensis Mz-F11 - MG952727.1

Phacidiopycnis washingtonensis Mz-F50 - MG952728.1

Botrytis cinerea isolate SPGM003 - KY775145.1

0.050

Fig. 3 Phylogeny, inferred with the Maximum Likelihood method with a bootstrap tree formed from 1000 replicates, based on Kimura 2-parameter model for ITS (A) and TUB (B) sequences. Initial tree(s) for the heuristic search were obtained automatically by applying the neighbour-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Sequences from this study are shown in red. The tree is rooted with sequences derived from *Botrytis cinerea*. Numbers next to branches indicate bootstrap support (%) and the bar shows the number of substitutions per site

Table 1 Mean lesion size (mm) recorded in pear cultivar Beurrè d'Anjou and Packham's Triumph following inoculation with three different *Phacidiopycnis pyri* isolates under specific incubation conditions

Pear cv.	Incubation conditions	Inoculation site		
		Wounds*♯	Calyx-end ♯	Stem-end ♯
Beurrè d'Anjou	7 days 20 °C	42.00 ± 9 d	3.00 ± 1 b	4.00 ± 1.5 b
	30 days 0 °C	17.30 ± 5 c	0.00 a	2.00 ± 1.0 a
	30 days 0 °C + 20 days 20 °C	37.30 ± 6 d	5.00 ± 1 c	4.50 ± 0.5 b
Packham's Triumph	7 days 20 °C	10.00 ± 5 b	0.00 a	5.00 ± 1.0 b
	30 days 0 °C	0.00 a	0.00 a	2.00 ± 1.0 a
	30 days 0 °C + 20 days 20 °C	15.33 ± 4 bc	3.00 ± 1 b	5.50 ± 1.0 b

* Wounds located at the equatorial zone and near the stem end were evaluated.

♯ Means followed by the same letter in the column do not differ by the Tukey test ($p \leq 0.05$)

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol 61(4):1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Katoh K, Rozewicki J, Yamada KD (2019) MAFFT Online Service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinf 20:1160–1166. https://doi.org/10.1093/bib/bbx108

Lennox CL, Spotts RA (2004) Timing of preharvest infection of pear fruit by *Botrytis Cinerea* and the relationship to postharvest decay. Plant Dis 88(5):468–473. https://doi.org/10.1094/pdis.2004.88.5.468

Liu Q, Xiao CL (2009) Infection of 'd'Anjou' Pear Fruit by *Potebniamyces pyri* in the Orchard in Relation to *Phacidiopycnis* Rot during Storage. Plant Dis 93(10):1059–1064. https://doi.org/10.1094/PDIS-93-10-1059

Liu D, Coloe S, Baird R, Pedersen J (2000) Rapid mini-preparation of fungal DNA for PCR. J Clin Microb 38(1):471-471. https://doi.org/10.1128/JCM.38.1.471-471.2000

Sholberg PL, Bedford KE, Stokes S (2003) Effect of preharvest application of cyprodinil on postharvest decay of apples caused by *Botrytis Cinerea*. Plant Dis 87(9):1067–1071. https://apsjournals.apsnet.org/doi/pdf/https://doi.org/10.1094/PDIS.2003.87.9

Sholberg PL, Stokes SC, O'gorman DT (2010) First report of *Phacidiopycnis* rot of pears caused by *Potebniamyces pyri* (anamorph *Phacidiopycnis pyri*) in British Columbia. Can J Plant Pathol 32(3):334–341. https://doi.org/10.1080/07060661.2010.499258

Sosa MC, Lutz MC, Sanchez A, Condoplo Lefort N (2016) Postharvest losses by Complex of *Phytophthora Sp* and *Botrytis Cinerea* in Long Storage Pear Fruit in the Northpatagonia, Argentina. ISHS Acta Hortic 1144:35. https://doi.org/10.17660/ActaHortic.2016.1144.35

Tamura K, Stecher G, Kumar S (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol 38(7):3022–3027. https://doi.org/10.1093/molbev/msab120

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DG, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, London, pp 315–322

Xiao CL (2006) Postharvest fruit rots in d'Anjou pears caused by *Botrytis Cinerea*, *Potebniamyces pyri*, and *Sphaeropsis pyriputrescens*. Plant Health Prog 7(1):40. https://doi.org/10.1094/PHP-2006-0905-01-DG

Xiao CL, Boal RJ (2004) Prevalence and incidence of *Phacidiopycnis* rot in d'Anjou pears in Washington State. Plant Dis 88(4):413–418. https://doi.org/10.1094/PDIS.2004.88.4.413