



BRIEF REPORT

Cladosporium species causing “*Cladosporium* rot” on “Bosc” pear fruit in Argentina



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Pyrus communis

Abstract “*Cladosporium* rot” on “Bosc” pear fruit during cold storage causes significant economic losses and has been reported in recent years in the productive valleys of Río Negro and Neuquén. The species involved were not determined. During 2016–2017, “Bosc” pears (*Pyrus communis*) in cold storage chambers exhibited external brownish black circular spots caused by *Cladosporium* spp. The objective of this work was to determine the *Cladosporium* species that caused the above mentioned symptoms. The morphological and molecular analyses of the partial sequence of the actin gene (ACT) supported the identification. *Cladosporium macrocarpum*, *Cladosporium subtilissimum* and *Cladosporium floccosum* were determined as the species involved in the disease. Although *Cladosporium* has been reported to cause pear rot, this is the first report to identify these species as causal agents of this fruit disease.

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PALABRAS CLAVE

Pudrición por
Cladosporium;
Manchas por
pudrición;
Peras «Bosc»;
Pyrus communis

Especies de *Cladosporium* causantes de podredumbre en peras «Bosc» en Argentina

Resumen En los valles productivos de Río Negro y Neuquén, se ha reportado en los últimos años la presencia de podredumbre de peras «Bosc» causada por *Cladosporium*, lo que generó significativas pérdidas económicas. Las especies involucradas no fueron determinadas. Se detectó la aparición de manchas circulares negras parduzcas en peras de dicha variedad en cámaras de almacenamiento en frío durante 2016–2017. El objetivo del presente trabajo fue determinar las especies de *Cladosporium* causantes de los síntomas mencionados. La identificación fue llevada

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a cabo por caracterización morfológica y el análisis molecular de la secuencia parcial del gen de actina (ACT). Se pudo determinar que *Cladosporium macrocarpum*, *Cladosporium subtilissimum* y *Cladosporium floccosum* fueron las especies implicadas. Si bien la podredumbre en peras causada por *Cladosporium* ha sido previamente reportada, este es el primer informe que identifica a estas especies entre los agentes causales de la enfermedad.

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Cladosporium species can cause lesions in healthy pears according to *in vitro* studies^{5,7,14}. *Cladosporium herbarum* was reported as a causal agent of *Cladosporium* rot on Bosc pear cultivars in the USA⁹. Moreover, the species *C. herbarum* and *Cladosporium* sp. were reported as postharvest phytopathogens of pears in the Netherlands¹⁵. Postharvest *Cladosporium* rots were also reported in Argentina on Beurré Bosc and Golden Russet Bosc pears in Northern Patagonia^{5,12}. A correct and accurate identification of the species is necessary because the name of the species involves a set of characteristics such as growth features, pathogenicity or production of mycotoxins, which allow to predict their behavior¹.

“Bosc” pears were affected by rot spots during cold storage (2016-2017) in the High Valley of Río Negro, a fruit producing region of Northern Patagonia in Argentina. The symptoms consisted of one or more brownish black circular spots that extended over the rind, light brown on the edges and dark brown to black in the center (Fig. 1). Therefore, the objective of this work was to determine the *Cladosporium* species that were involved in this fruit disease.

Fifteen symptomatic “Bosc” pears, stored unprocessed during approximately 2 months in bins inside conventional cold storage chambers at -0.5°C , were obtained from three commercial establishments (5 pieces from each). Fruits were superficially disinfected with a solution of sodium hypochlorite (1:10) for 5 minutes and rinsed by immersion twice in sterile distilled water. Infected internal tissue fragments were aseptically extracted from the spots and placed on potato dextrose agar supplemented with 0.1% chloramphenicol (PDA + C). Plates were incubated for 7 days at 25°C .

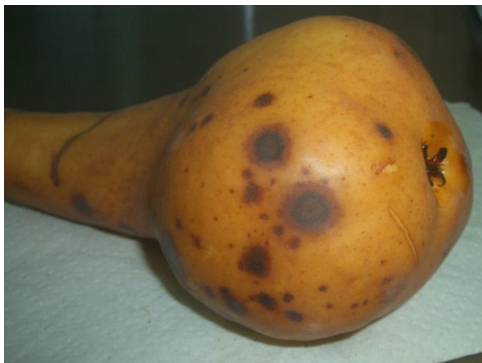


Figure 1 External symptoms of *Cladosporium* rot on Bosc pears.

The pathogens were identified at the genus level according to Pitt and Hocking⁸ as *Cladosporium* species. Characterization based on macroscopic features of the colonies clustered the isolates into nine different morphological groups (designated as G1 to G9). The microscopic characteristics of the isolates were determined in SNA (synthetic nutrient-poor agar) medium after 14 days of incubation at 25°C under close UV light³. DNA extraction was performed using the DNeasy Plant Mini Kit following the manufacturer’s instructions (Qiagen, Intl) and genomic DNA was quantified with the Qubit 2.0 fluorometer (Life Technologies, Intl.). The partial sequence of the actin gene (ACT) was amplified using the primers ACT-512F: ATGTGCAAGCCGGTTTCGC and ACT-783R: TACGAGTCCTTCTGGCCCAT to obtain resolution at the species level^{3,14}. Sequencing of the fragments was done by Macrogen Inc. (Seoul, Korea). Pathogenicity tests were performed using the toothpick technique² and verified according to Koch’s postulates.

After the molecular analysis, the nine different morphological groups were reduced to 3 species as causal agents of the disease. BLAST analysis of 200 bp fragments from the isolates with *Cladosporium* strain reference sequences obtained from the GenBank showed 100% identity to *Cladosporium subtilissimum* (isolate 53ACT GenBank Accession No. MG680545.1), *Cladosporium macrocarpum* (isolate 12ACT GenBank Accession No. MG680533.1) and *Cladosporium floccosum* (culture CPC 17802 GenBank Accession No. MF473823.1). As a result of the pathogenicity tests, the *C. subtilissimum* isolates produced a 1.5 cm lesion on the surface of the fruits with internal necrosis 1.1 cm deep (dry tissue that emerges like a plug). *C. macrocarpum* isolates produced an average lesion of 1.5 cm on the surface of the fruit with wet internal necrosis averaging 1.7 cm. *C. floccosum* isolates caused an external average lesion of 1 cm surrounded by a brown halo with dark brown internal necrosis extending 1.5 cm deep (dry tissue that emerges like a plug).

Cladosporium species are predominant in indoor and outdoor environments^{4,11,13}. *C. macrocarpum* and *C. subtilissimum* have been reported in a previous study conducted in rural environments of the High Valley of Río Negro productive region in Northern Patagonia in which eleven species were determined. Pathogenicity tests revealed that *C. macrocarpum* and *C. subtilissimum*, among other *Cladosporium* species, caused disease on pears¹⁴. The presence of these and other potentially phytopathogenic species in the air warns about the potential risk of infections by these

causal agents during cold storage and/or growing seasons in the field. Emerging diseases can be expected in the context of climate change, such as that which has been occurring in Northern Patagonia (Argentina)^{6,10}. These findings contribute to implementing appropriate preventive measures to reduce losses in pear production due to *Cladosporium* rot.

GenBank Accession numbers are MK410437-MK410445 (under examination and processing by the GenBank annotation staff).

Conflict of interest

None

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