Canadian Journal of Plant Pathology

ISSN: 0706-0661 (Print) 1715-2992 (Online) Journal homepage: http://www.tandfonline.com/loi/tcjp20

Fruit rot of sweet cherries and raspberries caused by Penicillium crustosum and Mucor piriformis in South Patagonia, Argentina

Sofía N. López, Marcela P. Sangorrín & María Belén Pildain

To cite this article: Sofía N. López, Marcela P. Sangorrín & María Belén Pildain (2016) Fruit rot of sweet cherries and raspberries caused by Penicillium crustosum and Mucor piriformis in South Patagonia, Argentina, Canadian Journal of Plant Pathology, 38:4, 511-516, DOI: 10.1080/07060661.2016.1243582

To link to this article: http://dx.doi.org/10.1080/07060661.2016.1243582

Accepted author version posted online: 05 Oct 2016.
Published online: 14 Nov 2016.

Can. J. Plant Pathol., 2016
Vol. 38, No. 4, 511–516, http://dx.doi.org/10.1080/07060661.2016.1243582

Taylor & Francis
Taylor & Francis Group

Disease report/Rapport des maladies

Fruit rot of sweet cherries and raspberries caused by *Penicillium crustosum* and *Mucor piriformis* in South Patagonia, Argentina

SOFÍA N. LÓPEZ[1,2,4], MARCELA P. SANGORRÍN[1,3] AND MARÍA BELÉN PILDAIN[1,2,4]

[1]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[2]*Centro de Investigación y Extensión Forestal Andino Patagónico, Ruta 259 km 14.6, C.C. 14, 9200 Esquel, Chubut, Argentina*
[3]*Grupo de Biodiversidad y Biotecnología de Levaduras, Instituto de Investigación y Desarrollo en Ingeniería de Procesos, Biotecnología y Energías Alternativas (PROBIEN, CONICET-UNCo), Buenos Aires 1440, Neuquén, Argentina*
[4]*Universidad Nacional de la Patagonia S.J. Bosco, Ruta 259 km 14.6, 9200 Esquel, Chubut, Argentina*

(Accepted 27 September 2016)

Abstract: Cherries and raspberries are important fruit crops in Patagonia, Argentina. A high proportion (50%) of raspberry production is organic certified and sold for fresh and frozen domestic markets. Due to post-harvest rot diseases, cherries and raspberries from Patagonia are kept in cold storage for a short period of time. During the storage season of 2012–2013, fruit rot samples were obtained from conventional and organic sweet cherry and raspberry orchards in the Andean zone of Central Patagonia. *Penicillium crustosum* and *Mucor piriformis* were isolated from both types of production systems and identified through morphological and molecular analysis; their pathogenicity was confirmed based on their virulence (incidence and severity). To our knowledge, this is the first report confirming *Penicillium crustosum* and *Mucor piriformis* causing post-harvest disease on cherries and raspberries in Argentina, and it contributes to expanding the knowledge on emerging pathogens.

Keywords: adapted cold pathogens, organic production, post-harvest diseases, *Prunus avium*, *Rubus idaeus*

Résumé: Les cerises et les framboises sont des cultures importantes en Patagonie, en Argentine. Une forte proportion (50%) de la production de framboises est certifiée biologique et est vendue sur les marchés locaux de fruits frais et congelés. À cause de pourritures post-récoltes, les cerises et les framboises de la Patagonie sont entreposées sous froid pendant une courte période. Durant la saison d'entreposage 2012–2013, des échantillons de pourriture ont été prélevés dans des framboisières et dans des vergers des cerisiers sauvages, cultivés traditionnellement et biologiquement, dans la zone andine du centre de la Patagonie. *Penicillium crustosum* et *Mucor piriformis* ont été isolés dans les deux types de productions et identifiés par analyse morphologique et moléculaire; leur pathogénicité a été confirmée en se basant sur leur virulence (incidence et gravité). À notre connaissance, il s'agit du premier rapport confirmant l'incidence d'une maladie post-récolte causée par *Penicillium crustosum* et *Mucor piriformis* chez les cerises et les framboises en Argentine. Par ailleurs, il contribue à étendre les connaissances sur les agents pathogènes émergents.

Mots clés: agents pathogènes adaptés au froid, maladies post-récoltes, production biologique, *Prunus avium*, *Rubus idaeus*

Introduction

Berries and cherries are widely consumed all over the world. In Argentinean Patagonia, these are important fruit crops as fresh season fruit and frozen fruit, while almost 90% of raspberry production supplies the local markets. Argentinean exports of fresh cherries increased 12.4 times from 142 tons in 1994 to 1759 tons in 2013, and ranked tenth in the income generated by fresh fruit exports for the country (Bruzone 2009; Baudino 2013). In the Patagonian region, raspberry (*Rubus idaeus* L.) and

Correspondence to: María Belén Pildain. E-mail: mbpildain@ciefap.org.ar

sweet cherry (*Prunus avium* L.) are conventional and certified organic products. Both types of commodities are very perishable and can be infected by different pathogens, both in the field and, even more so, during post-harvest storage (Crisosto et al. 1993; Vaughn et al. 1993). Leak rot and blue mould caused by *Mucor* and *Penicillium* species, respectively, have been reported several times in stored cherries and raspberries fruit. *Mucor mucedo* L., *M. piriformis* A. Fisch. and *M. hiemalis* Wehmer have been associated with leak rot of raspberries in the UK (Dennis & Mountford 1975; Snowdon 1990). *Mucor piriformis* has also been reported to cause rotting of cherries in Chile, California and Norway (Michailides & Spotts 1990; Børve et al. 2000). Blue mould decay may be caused by various *Penicillium* species, *Penicillium expansum* Link being the most aggressive and commonly encountered in sweet cherry fruit (Ceponis 1987; Spotts et al. 1998). In Italy, three *Penicillium* species, including *P. expansum*, *P. chrysogenum* Thom and *P. crustosum* Thom have been associated with soft rot of sweet cherries (Sanzani et al. 2013).

During December 2012–February 2013, symptoms of decaying fruits from storage chambers at 0° in the Andean range zone of Southern Patagonia were analysed. Symptoms began as a soft and light to dark brown lesion. As infection advanced, the lesions became watery, with sporulating mycelia and, when the decayed cherry fruit was cut, the fruit flesh appeared to be completely rotten, light to dark brown, very soft or watery, and easily separated from the healthy tissue. Thus, the objectives of this study were to determine the causal agent and identify the pathogen based on morphological characteristics, pathogenicity tests and molecular biological techniques.

Materials and methods

Pathogen isolation

During surveys for post-harvest diseases of cherries and berries conducted on the Andean range zone of Patagonia, Argentina (Trevelin, 43°02′19S, 71°28′25W; Lago Puelo, 41°59′57S, 71°33′10W; El Bolsón, 41°56′42S, 71°31′13W), cherry ('Bing', 'Lapins' and 'Rainier' varieties) and raspberry ('Autumn Bliss' variety) fruits with rot symptoms were obtained from storage chambers at 0–1°C (Fig. 1*a*,*c*). Both types of fruit came from organic and conventional fields in the west area of Patagonia (Andean range zone). Small pieces (3 mm^2) of 50 symptomatic fruits were excised from the junction of diseased and healthy tissue, surface-sterilized in 70% ethanol for 30 s, washed in three changes of sterile distilled water, air dried, transferred to potato dextrose agar (PDA) and incubated for 7–10 days at 22°C. A total of 18 isolates were subcultured in Petri dishes with PDA in a growth chamber at 22°C.

Morphological and molecular identification

Fungi were morphologically identified according to macro and microscopic characteristics from cultures on PDA, Czapek yeast autolysate agar (CYA), malt extract agar (MEA) and yeast extract sucrose agar (YES) (Samson et al. 2004; Pitt & Hocking 2009). After the morphological examination, isolates were grown in 2 mL of malt-peptone, and genomic DNA was extracted using the commercial kit Ultra Clean TM Microbial DNA Isolation Kit (MOBIO Laboratories Inc., Solana Beach, CA, USA). A part of the β-tubulin gene, and the Internal Transcribed Spacer (ITS) and the intervening 5.8S RNA gene were amplified with the primers Bt2a and Bt2b (Glass & Donaldson 1995) and ITS5-ITS4 (White et al. 1990), respectively. PCR amplification was carried out in a 25 µL reaction mixture containing dNTPs (0.25 mM of each), 2.5 mM $MgCl_2$, 1 × PCR buffer supplied with the polymerase enzyme; 0.1 mM each of primer; 100–500 ng DNA; 6% bovine serum albumin (BSA, Promega, Madison, WI) and 1.25 U GoTaq polymerase (Promega). PCR reactions were performed in a thermal cycler (MyCycler™, BioRad). Conditions for PCR amplification and sequencing were as described by Kim et al. (2007) and Kwasna et al. (2006). The amplified fragments were purified and sequenced with an ABI 3700 automated sequencer (Perkin-Elmer, Foster City, CA) at the DNA Synthesis and Sequencing Facility, Macrogen (Seoul, Korea). Sequences were submitted to GenBank (KU961521–KU961529). Phylogenetic relationships were inferred with maximum likelihood (ML). The best-fit models of evolution were K80 (ITS dataset) and TrN +G (β-tubulin dataset), determined in jModelTest (Posada 2008). Branch support was determined with non-parametric bootstrapping implemented in RAxML 7.2.8 (Stamatakis 2014), using the default parameters, executed on the CIPRES (cyberinfrastructure for phylogenetic research) Science Gateway 3.1 (Miller et al. 2010) with bootstrap statistics calculated from 1000 bootstrap replicates.

Pathogenicity tests

To determine pathogenicity, healthy cherry fruits 'Bing' were randomized and sterilized with 2% NaOCl for 30 s, wounded (3 × 3 mm wound) with a sterile stick and inoculated with 10 µL of 10^6 conidia mL^{-1} from 7-day-old sporulating cultures of each isolate. Fruits were

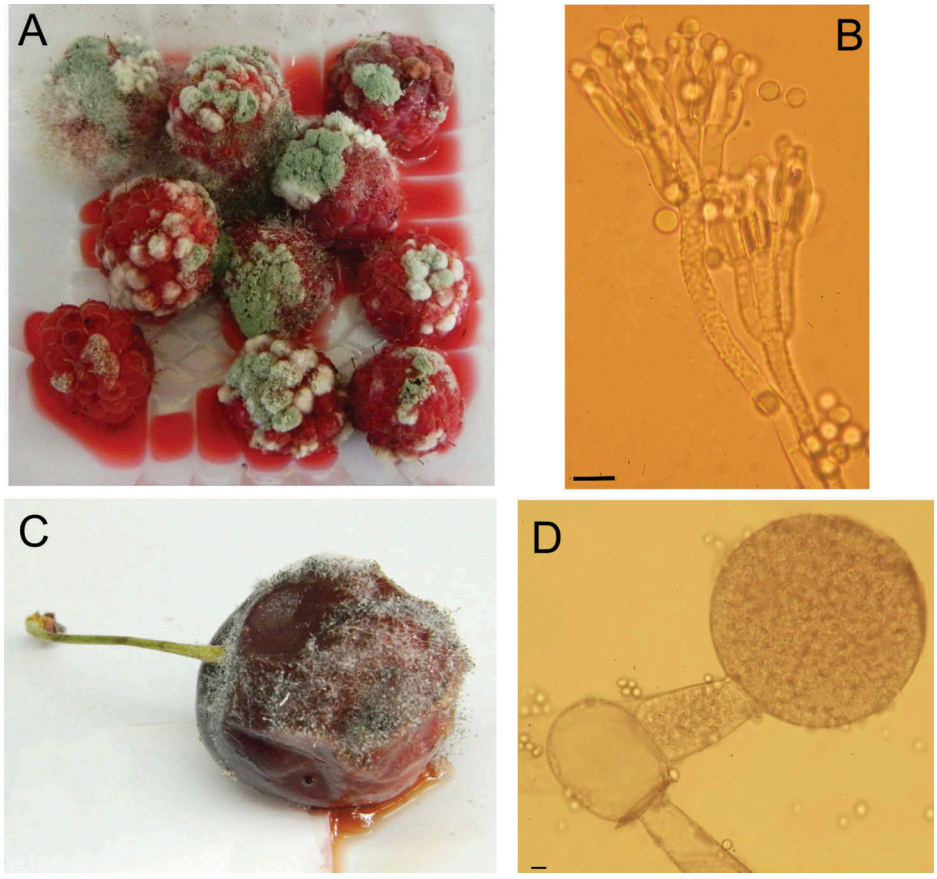

Fig. 1. (Colour online) Decay symptoms and morphological features. **(a)** *Penicillium crustosum* white mycelium and green sporulation and rot symptoms on inoculated raspberries in pathogenicity tests. **(b)** *Penicillium crustosum* conidiophores and conidia. **(c)** *Mucor piriformis* mycelia and symptoms in later stage on naturally infected cherry. **(d)** Sporangiophores, sporangia, collumelae and sporangiospores of *Mucor piriformis*. Scale bar = 10 µm.

placed in plastic trays inside polyethylene bags, avoiding contact between wounds and bags, and kept in cold storage conditions (30 days at 0°C followed by 3 days at 22°C), and under ambient conditions (7 days at 22°C) in refrigeration chambers. Severity (wound diameter) was determined. Raspberry fruits 'Autumn Bliss' were spray-inoculated with a conidial suspension containing 10^6 conidia mL^{-1} from 7-day-old sporulating cultures of each isolate (Verma & Sen 2008). Fruits were placed in plastic trays inside polyethylene bags as with cherries, and kept in two storage conditions: (a) at 0°C for 5 days and for 2 days at 22°C, (b) at commercial conditions during 5 days at 22°C. Disease incidence was defined as the number of diseased wounds/total number of wounds × 100. Distilled water was used as negative control. Three replicates of 10 fruits were used. The pathogen was re-isolated; its morphology and DNA sequence was compared with the original isolate. The experiment was conducted in December 2013 and repeated in February 2014 with a different lot of fruit, belonging to 'Lapins' variety, for confirmation.

Results and discussion

Fungal identification and isolation

From the sampled fruit, 23% of cherries and 16% of raspberries developed fungi different to *Botrytis cinerea* Pers.:Fr. (2%), the main worldwide post-harvest pathogen associated with both types of fruit. Two different fungi were identified in both fruits: *Penicillium crustosum* (five isolates: two from organic and conventional cherries, three from organic certified raspberries) and *Mucor piriformis* (10 isolates: two from organic cherries, five from conventional cherries and three from organic certified raspberries). Isolates of *P. crustosum* were morphologically identified according to Frisvad & Samson (2004). Isolates ccCIEFAP479 and ccCIEFAP483 presented

white mycelia, with sporulation, yielding greyish green colonies on all media. Colonies were radially sulcate and velutinous, with clear exudate, and produced a yellow to orange reverse colour on CYA and YES. Conidiophores were terverticillate, stipes septate with rough walls, and ampulliform phialides. Conidia were smooth, spherical to subglobose, in columns, measuring 2.9–4.9 (3.6) µm (n = 30) (Fig. 1*b*). BLAST analysis revealed that the sequences were 100% identical to *Penicillium crustosum* (GenBank accession number AY674353, sequence of the type material CBS115503), and the phylogenetic analyses confirmed these relationships (Fig. 2*a*).

On the other hand, *M. piriformis* was determined based on cultural characteristics according to Michailides & Spotts (1990). Colonies of the isolates ccCIEFAP484 and ccCIEFAP569 were dark grey, fast growing, and produced dark brown sporangia on small peg-like branches of the vegetative hyphae. Sporangiophores were tall and short, branched and unbranched, short sporangiophores branched sympodially. Sporangia were globose, black-brown at maturity, 85–212 µm in diameter. Collumelae were variable in shape, cylindrical-ellipsoidal or pyriform, 34.7–61.8 (43.1) × 23.2–60.8 (51.7) µm. Sporangiospores were ellipsoidal, subspherical and smooth 3.9–10.7 (6.2) × 4.9–12.6 µm (n = 30) (Fig. 1*d*). Chlamydospore-like resting structures, isogametangia and zygospores were not evident in culture. BLAST analysis of the DNA sequences identified the 10 isolates as *M. piriformis sensu lato* (99% identical to the ITS-5.8S rDNA sequence of *M. piriformis* isolate

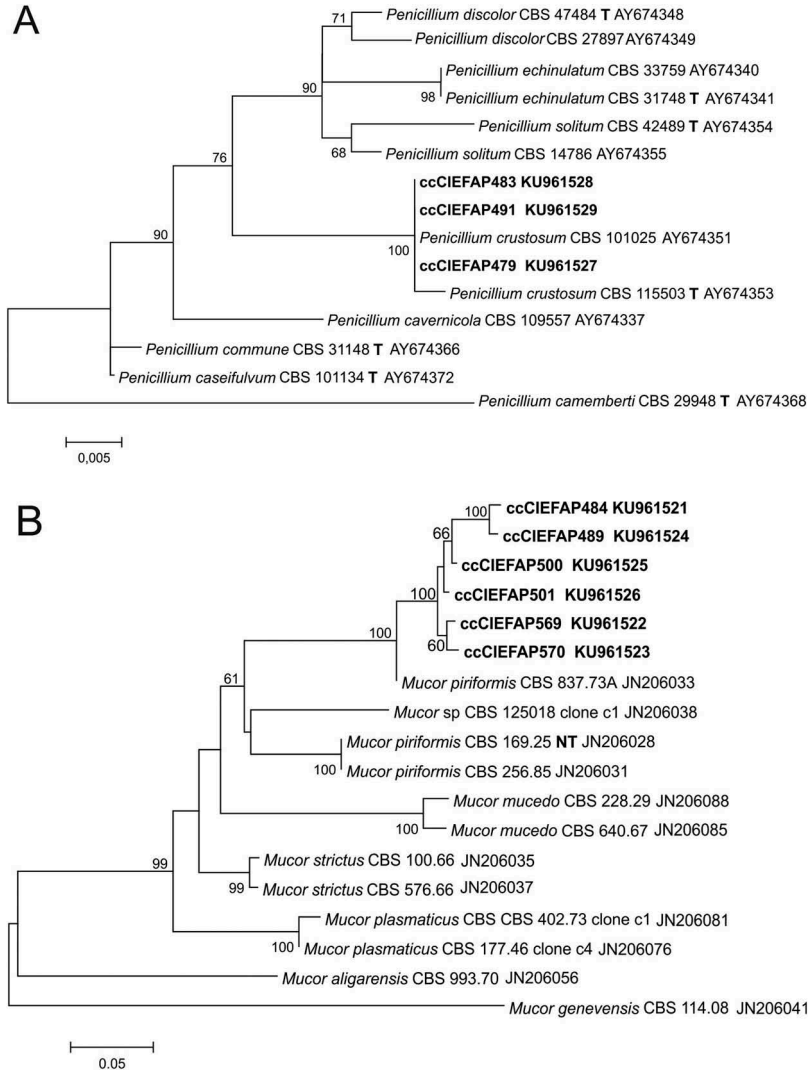

Fig. 2 Maximum likelihood trees from (**a**) *Penicillium crustosum* sequences and related species generated from β-tubulin gene data. (**b**) *Mucor piriformis* ITS sequences. Bootstrap values resulting from 1000 replicates are shown at the branch points.

CBS837.73A, GenBank accession number JN206033, from red currant). In the phylogenetic tree, the representative isolates were placed within a clade comprising the sequence of the neotype isolate of *M. piriformis* (GenBank accession number JN206028) (Fig. 2*b*).

Pathogenicity tests

Decay observed on inoculated fruits was similar to symptoms originally observed on 'Bing' cherries and 'Autumn Bliss' raspberries from storage and the pathogens were re-isolated from inoculated fruits. *Mucor piriformis* and *P. crustosum* isolates were able to cause symptoms on cherry under cold-storage and ambient conditions. The average severity rate (± SE) for *M. piriformis*on cherry fruits was 20 ± 0 mm at both temperatures in both experiments. For *P. crustosum*, disease severity values were 6.13 ± 1.34 and 5.16 ± 0.96 mm at 0°C, and 13.6 ± 1.11 and 8.11 ± 0.98 mm at 22°C across both types of fruit, temperatures and experiments; the disease incidence was 100% for both pathogens. Symptoms of decay were observed in all inoculated fruits, but not in the controls. *Mucor piriformis* rot was light brown, watery, soft and covered with fuzzy mycelia. Coloured sporangiophores bearing terminal sporangia protruded through the skin (Fig. 1*c*). Decayed areas produced by *P. crustosum* were light to medium brown, soft and watery, and with blue green sporulation on the surface of the lesion (Fig. 1*a*). Both fungi were re-isolated from infected tissues and showed the same morphological and molecular characteristics as the original isolates.

Both *Mucor* and *Penicillium* contain species considered as high risk for developing resistance to fungicides (FRAC 2010). Currently, there is no fungicide registered in Argentina to control post-harvest pathogens on cherries and raspberries. *Penicillium crustosum* causes blue mould on pome fruits and is also regularly found on cheese, nuts and in soil (Frisvad & Samson 2004; Pitt & Hocking 2009). The fungus produces a wide range of mycotoxins such as penitrem A, roquefortine C, terrestric acid and cyclopenol, which impact human health (Frisvad & Samson 2004). *Mucor piriformis* causes Mucor rot of pome and stone fruits during storage and has been reported in Australia, Canada, Germany, Northern Ireland, South Africa and the USA (Michailides & Spotts 1990; Sholberg & Michailides 1997). Cherries and raspberries are cold stored at 0°C to extend their shelf life in the markets. Our results show that both *M. piriformis* and *P. crustosum* isolates are pathogenic under ambient and cold-storage conditions. As a handling measure, the use of plastic bags and boxes help contain and prevent secondary spread. As far as we know, this is the first report of rot caused by *P. crustosum* in cherries and raspberries in Argentina and worldwide. *Mucor piriformis* appears to be associated with Mucor rot of cherries in Chile and California, USA (Michailides & Spotts 1990). However, this is the first report for Argentina, and also for raspberries.

Isolates of *M. piriformis* and *P. crustosum* from organic and conventional cherries and raspberries proved to be pathogenic. Both production systems yielded the presence of pathogens, suggesting there is no effect of the type of production on the fungal diversity. Studies performed on vines under organic and conventional treatments have shown that fungal population was influenced by handling practices; on the contrary, for citrus in Portugal, different production systems had no influence on fungal communities (Nunes et al. 2010; Schmid et al. 2011).

Acknowledgements

This work was supported by the ANPCyT Grant PICT 2012-0733 and UNPSJB Grant FCN039/13. The authors are grateful to Humus, Valle del Medio, Gaiman cooperative and Hishashi fruit producer.

Funding

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica [PICT 2012-0733]; Universidad Nacional de la Patagonia San Juan Bosco [FCN039/13];

References

Baudino AM. 2013. International competitiveness of companies in the production sector and processing strawberries Coronda region during 2010-2012. Positioning through the formation of a cluster [dissertation]. Rosario (Argentina): Universidad Abierta Interamericana. Spanish.

Børve J, Sekse L, Stensvand A. 2000. Cuticular fractures promote postharvest fruit rot in sweet cherries. Plant Dis. 84:1180–1184.

Bruzone A. 2009. Raspberries, blackberries and currants. Aliment Argent. 44:25–29. Spanish.

Ceponis MJ. 1987. Disorders in sweet cherry and strawberry shipments to the New York market, 1972–1984. Plant Dis. 71:472–476.

Crisosto CH, Garner D, Doyle J, Day KR. 1993. Relationship between fruit respiration, bruising susceptibility and temperature in sweet cherries. Hort Sci. 28:132–135.

Dennis C, Mountford J. 1975. The fungal flora of soft fruits in relation to storage and spoilage. Ann Appl Biol. 79:141–147.

[FRAC] Fungicide Resistance Action Committee. 2010. Pathogen risk list [Internet]. [revised 2015 Jun 2; cited 2015 Jun 17]. Available from: www.frac.info

Frisvad J, Samson R. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud Mycol. 49:1–52.

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. 61:1323–1330.

Kim WK, Sang HK, Woo SK, Park MS, Paul NC, Yu SH. 2007. Six species of *Penicillium* associated with blue mold of grape. Mycobiology. 35:180–185.

Kwasna H, Ward E, Bateman GL. 2006. Phylogenetic relationships among Zygomycetes from soil based on ITS1/2 rDNA sequences. Mycol Res. 110:501–510.

Michailides TJ, Spotts RA. 1990. Postharvest diseases of pome and stone fruits caused by *Mucor piriformis* in the Pacific Northwest and California. Plant Dis. 74:537–543.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE) New Orleans, USA. p. 1–8.

Nunes C, Duarte A, Manso T, Weiland C, García JM, Cayuela JA, Salazar M. 2010. Relationship between postharvest diseases resistance and mineral composition of citrus fruit. In International symposium on mineral nutrition of fruit crops. VIth ed. Faro, Portugal. p. 417–422.

Pitt JI, Hocking AD. 2009. Fungi and food spoilage. 3rd ed. New York: Springer.

Posada D. 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol. 25:1253–1256.

Samson RA, Hoekstra ES, Frisvad JC, editors. 2004. Introduction to Food- and Airborne Fungi. 7th ed. Utrecht: Centraalbureau voor Schimmelcultures.

Sanzani SM, Montemurro C, Di Rienzo V, Solfrizzo M, Ippolito A. 2013. Genetic structure and natural variation associated with host of origin in *Penicillium expansum* strains causing blue mould. Int J Food Microbiol. 165:111–120.

Schmid F, Moser G, Müller H, Berg G. 2011. Functional and structural microbial diversity in organic and conventional viticulture: organic farming benefits natural biocontrol agents. Appl Environ Microb. 77:2188–2191.

Sholberg PL, Michailides TJ. 1997. First report of Mucor rot in commercially sold cherries caused by *Mucor piriformis*. Plant Dis. 81:550.

Snowdon AL. 1990. A colour atlas of post-harvest diseases and disorders of fruits and vegetables. In: Volume 1: General introduction and fruits. CRC Press, Boca Raton, USA.

Spotts RA, Cervantes LA, Facteau TJ, Chand-Goyal T. 1998. Control of brown rot and blue mold of sweet cherry with preharvest iprodione, postharvest *Cryptococcus infirmo-miniatus*, and modified atmosphere packaging. Plant Dis. 82:1158–1160.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30:1312–1313.

Vaughn SF, Spencer GF, Shasha BS. 1993. Volatile compounds from raspberry and strawberry fruit inhibit postharvest decay fungi. J Food Sci. 58:793–796.

Verma P, Sen N. 2008. The impact of plant growth regulators on growth and biochemical constituents of coriander (*Coriandrum sativum* L.). J Herbs Spices Med Plants. 14:144–153.

White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal DNA for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to the methods and applications. New York: Academic Press; p. 315–322.