Title First report of *Pestalotiopsis clavispora* and *pestalotiopsis* spp. causing postharvest stem end rot of avocado in Chile

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Abstract

Avocado (*Persea americana*) production in Chile has increased to more than 33,500 ha. Chilean avocadoes are sent to markets 15 to 45 days away by overseas transport to the United States, Europe, and Asia. Although apparently healthy avocadoes were harvested in 2009, a 10 to 14% incidence of stem end rot appeared after 15 days of cold storage. Symptoms appeared as small, irregular, brown lesions on the peel at the stem end. Lesions enlarged rapidly, became sunken and soft, eventually comprising the entire fruit as ripening progressed. A white mycelium often developed around the stem cavity. A dark brown necrosis of the pulp was observed that comprised a big part of the pulp as the fruits matured. Isolations were performed from 'Hass' avocadoes that developed stem end rot after fruits were kept in humid chambers for 15 days at 5°C plus 6 days at 20°C (n = 50) to simulate a transport period from Chile to U.S. markets or from diseased fruits (n = 50) kept for 15 days at 20°C. Fruits were surface disinfected for 60 s in 75% ethanol, and small pieces of tissue were excised from the margins of the pulp lesions and then plated onto potato dextrose agar (PDA) plus 1 ml/liter of Igepal CO-630 (Sigma-Aldrich, Atlanta, GA) (MPDA). Fungal colonies that developed on PDA were white and cottony, turning slightly yellow after 15 days. Black acervuli appeared after 15 days at 20°C. Conidia (n = 40) were fusiform, (22.2) 27.0 to 30.4 × (6.3) 7.0 to 9.8 µm with a length/width ratio of 3.4 ± 0.4. All isolates had five-celled conidia. Apical and basal cells were colorless, while the three median cells were dark brown. Conidia had one basal appendage (9.3 ± 3.3 µm) and two to four long apical appendages (34.5 ± 6.9 µm). On the basis of colony and conidia morphology, most of these isolates were initially identified as *Pestalotiopsis clavispora* (G.F. Atk) Steyaert, but other nonidentified species of *Pestalotiopsis* were also found (3). Identification was confirmed by amplifying and sequencing the internal transcribed spacer (ITS) region of rDNA using ITS1/ITS4 primers of *P. clavispora* isolate PALUC-12 (Accession No. HQ659767). A BLAST search of the NCBI database showed that isolate PALUC-12 had 100% homology with *P. clavispora* (No. EU342214.1). Pathogenicity tests were conducted on surface-disinfected (75% ethanol, 30 s) fruits by

placing agar pieces (3 mm in diameter) from 7-day-old cultures and a 20-µl drop of 10^6 conidia/ml on wounded and unwounded stem cavities and equatorial area of five avocado fruits of 'Hass', per isolate tested, at the commercial maturity stage. Inoculated fruits were placed in moist chambers at 25°C for 10 days. Necrotic lesions resembling symptoms that occurred in storage fruits were observed on wounded fruits. No symptoms were observed on unwounded fruits inoculated in the equatorial zone. However, unwounded fruits inoculated in the stem cavity developed a slight necrosis probably because of undetectable wounds made at harvest. Koch's postulates were confirmed after the reisolation of *P. clavispora* and *Pestalotiopsis* spp. from diseased fruits. *P. versicolor* has been reported in South Africa (1), but to our knowledge, this is the first report of *P. clavispora* causing stem end rot of avocado. *P. clavispora* has been reported on blueberry in Chile (2).