Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Aureobasidium pullulans* var. *pullulans* Causing Spot Blight of Pear (*Pyrus pyrifolia*) in Zhejiang Province of China

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Asian pear (*Pyrus pyrifolia* Nakai ‘cuiguan’), is widely grown in Zhejiang province of China. In April 2019, symptoms consisting of small, round, black leafspots and blighted flower petals were observed on over 30% of Cuiguan pear trees in an orchard (ca. 0.8 ha) near Cixi city, Zhejiang province, China. Initially, leaf spots were observed on leaf petioles, which, with time, enlarged and coalesced into necrotic streaks (1 to 2 cm) along the length of the petioles. Irregular, reddish brown spots developed on flower petals and hastened their senescence. Additional symptoms included round, black spots on leaves (2 to 3 mm in diameter) and necrosis of shoot tips. Symptomatic tissues from petals, petioles, and leaves were plated onto potato dextrose agar (PDA). After five days of incubation at 26°C, slimy fungal colonies (48 × 48 mm) with pinkish to orange-colored mycelia and with regular annulations were isolated from all tissues. After 10 days, cultures were shiny and dark brown in the center. The color of conidia ranged from hyaline to dark brown. Hyaline conidia were blastic, unicellular, ellipsoidal, smooth, with lengths that ranged from 11.03 to 27.14 (avg. 18.38) µm, and widths that ranged from 3.45 to 8.86 (avg. 6.04) µm (*n* = 50). Dark brown conidia were one to two celled, 10.89 to 26.03 (avg. 17.41) µm in length and 4.26 to 12.15 (avg. 6.94) µm in width (*n* = 50), and with a slight septal constriction. Conidiogenous cells were clavate, hyaline, aseptate, and with a smooth top with 3 to 11 spores. Indistinct scars remained when the conidia dislodged from the conidiogenous cells. Single spore isolation was used to obtain pure cultures. Mycelia and conidia were

scraped from cultures and DNA was extracted using Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech). Amplified PCR products from the internal transcribed spacer (ITS) region ITS1F/ITS4 (White et al. 1990), the partial 28S rDNA (LSU) NL1/NL4 (Boekhout et al. 1995), the β-tubulin (*TUB*) gene Bt2a/Bt2b (Glass and Donaldson 1995), and the partial elongase gene (*ELO*) ELO2-F/ELO2-R (Zalar et al. 2008) were sequenced (Tsingke Biotechnology Co., Hangzhou, Zhejiang). A BLAST search (GenBank accession nos. MT107050, OK485685, OK631951, and OK631950) showed 99% homology with *Aureobasidium pullulans* reference isolate CBS584.75 and EXF-150., which was consistent with the morphological data (Gostinčar et al. 2014). Three-year-old seedlings from Cuiguan pears were sprayed with conidial suspension (10⁶ conidia/ml) on both sides of leaves without wounding. In a greenhouse (26°C, natural light), six inoculated plants and three noninoculated plants (sprayed with sterile distilled water) were enclosed in plastic bags to maintain humidity for 72 h. At 5 days after inoculation, shoot tips blackened and began to wilt. At 15 days after inoculation, symptoms similar to those on the original sample developed on inoculated petioles and leaves, whereas the control plants remained healthy. No symptoms were observed on leaves that were mature at the time of inoculation. *Aureobasidium pullulans* var. *pullulans* was reisolated from all inoculated plants. Overall, this disease shortened the life of pear flowers and reduced fruit. To our knowledge, *A. pullulans* var. *pullulans* has not previously been reported as a pathogen of *P. pyrifolia*.

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The author(s) declare no conflict of interest.

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