Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Fruit Black Spot of Pecan (*Carya illinoensis*) Caused by *Botryosphaeria dothidea* in China

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Pecan (*Carya illinoensis* [Wangenh.] K. Koch) planting is a profitable business supporting thousands of mountain-dwelling farmers. From 2017 to 2020, black spot symptoms on pecan fruits were found in Jiande (29°25'17.7"N, 119°18'10.7"E) and Jinhua (28°32' to 29°41'N, 119°14' to 120°47'E), Zhejiang Province. Irregular-shaped black lesions initially developed on infected fruits. Infection progressed until fruits were dark, rotten, and mummified. Fruit infection rate was about 10%, causing about \$800 in losses per hectare. Pieces of infected pericarps were surface sterilized for 1 min in 2% NaOCl, rinsed three times with sterile water, plated on potato dextrose agar (PDA), and incubated at 25 ± 2°C. Fungi were isolated from the cultures by transferring mycelial tips. In total 25 isolates (92.5% of samples) showed the same cultural and morphological characters. Hyphae of the isolates were hyaline at first and later became gray and grayish black and developed into villous colony with dense aerial mycelium. Isolates were cultured on PDA for 7 days at 25°C in dark conditions, and then for another 7 to 10 days in continuous light until conidia were formed. Conidia were fusiform, straight, hyaline, unicellular, obtuse at the top and truncated at the base, and measured 16.23 to 25.46 µm width × 5.17 to 8.87 µm length (18.13 × 5.54 µm average, *n* = 60, five isolations). DNA was isolated from mycelia and three loci, including the internal transcribed spacer (ITS1, 5.8s, and ITS2), a 200-bp intron of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and partial beta-tubulin (TUB2), were amplified using the primer pairs ITS4/ITS5 (White et al. 1990), GDF1/GDR1 (Guerber et al. 2003), and T1/Bt2b (Glass and

Donaldson 1995; O'Donnell and Cigelnik 1997), respectively. Assembled sequences MT791339 (ITS), MT771644 (GAPDH), and MT792797 (TUB) were deposited in GenBank and showed 99.3 to 100% similarity with published reference sequences of *Botryosphaeria dothidea*. Phylogenetic analysis showed that isolate PCG0901 and *B. dothidea* clustered in the same clades. Pathogenicity tests were performed, inoculating fruits from healthy plants with both mycelial plugs (14 days old) and conidial suspension (1 × 10⁶ conidia/ml). Three trees were randomly selected for each treatment, and from each tree 10 fruits with similar maturity level without visible infections were selected for the inoculations. The fruits were surface sterilized by wiping with 75% alcohol and then rinsed with sterilized water. After being dried in the air, the sterilized fruits were wounded with needles. Subsequently, the mycelial plugs or conidial suspension (20 µl each) were put or dropped on each wound. The negative control fruits were wounded and treated with agar plugs. The treated fruits were covered with plastic bags and incubated in moist chambers at 25°C with a humidity of 90%. Typical symptoms appeared on 27 of 30 fruits inoculated with mycelial agar plugs and on 25 of 30 fruits inoculated with conidia suspension. Symptoms were the same as the ones observed in the forest. Fruits used as negative controls remained symptomless. The same fungus was reisolated from the inoculated fruits, whereas no fungus was reisolated from the control plants, confirming pathogenicity of the isolate PCG0901 and thus completing Koch's postulates. The isolates from both the original and artificially inoculated fruits were identified as *B. dothidea* (Crous and Palm 1999) based on the results of morphological and molecular analyses. To the best of our knowledge, this is the first report of black spots on fruits of *C. illinoensis* caused by *B. dothidea* in China. The disease has the potential to become a big problem for China's rural pecan growers, considering the crop damage observed and the importance of this pathogen in other crops. Identification of *B. dothidea* as a pathogen of *C. illinoensis* is important for implementing control management strategies for fruit black spot disease.

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