Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Black Spot on *Ophiopogon japonicus* Caused by *Alternaria alternata* in Zhejiang Province, China

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Ophiopogon japonicus (L. f.) Ker-Gawl, a traditional Chinese medicinal plant, is widely cultured in China. The root of *O. japonicus* is used as the main ingredient in many prescriptions. It is rich in chemical components for steroidal saponins, homoisoflavonoids, and polysaccharides, which have various pharmacological activities, such as cardiovascular protection, anti-inflammation, and antidiabetes (Chen et al. 2016). In May and July for 2018 and 2019, the symptoms of black spot on *O. japonicus* were observed with an incidence of 40% in Cixi County, Zhejiang Province, China. The pathogen mainly infected leaves causing severe black spots, which resulted in a 28% yield loss per acre. At the early stage of the disease, the tip of the leaf began to turn yellow, then the discoloration gradually spread to the base of the leaf, and finally the whole leaf turned reddish brown with visible black spots. Symptomatic leaves were cut into small pieces (1.0 × 1.0 cm) and disinfected successively by submersion in 75% ethanol for 30 s and 1% NaClO for 30 s under aseptic conditions. After rinsing with sterile water three times and air drying, segments were placed on potato dextrose agar (PDA) and incubated at 28°C in dark for a week. Then, pathogen on PDA was transferred onto potato carrot agar (PCA) and incubated at 23°C under the condition of alternation of day (12 h) and night (12 h) for a week. Colonies on PDA were dark gray in the center surrounded by white to gray on the upper side, and black with white margins on the back of the plate. Colonies on PCA were grayish with sparse hyphae. The conidia were obclavate or ellipsoid, pale

brown, with three to eight transverse septa and one to four longitudinal septa. Conidiophores were septate, arising singly, and measured 17.0 to 81.0 × 8.0 to 23.5 μm. Most conidia had a conical or columnar beak, approximately 0 to 23.5 × 2.5 to 9.0 μm in size. According to morphological and cultural characteristics, these isolates were preliminarily identified as *Alternaria alternata*. *A. alternata* is one of the most typical plant pathogens, more than 95% of which facultatively parasitize on plants, causing disease in numerous crops. To further confirm identification of pathogens, the internal transcribed spacer region (ITS), translation elongation factor 1-α gene (*EF-1α*), RNA polymerase II second largest subunit (*RPB2*), major allergen Alt a 1 gene (*Alt a 1*), histone 3 gene (*His*), and plasma membrane ATPase (*ATP*) were amplified with primer pairs ITS1/ITS4, EF1-728F/EF1-986R, RPB2-7cr/RPB2-5f2, Alt-for/Alt-rev, His 3-F/His 3-R, and ATP-F/ATP-R (Hong et al. 2005; Lawrence et al. 2013). BLASTN analysis of NCBI using ITS (MW989987), *Alt a 1* (MW995953), *EF-1α* (MW995955), *ATP* (MW995957), *His* (MW995954), and *RPB2* (MW995956) showed 100, 100, 97, 99, 99, and 97% identity to *A. alternata* MN249500.1, MN304714.1, MK637432.1, MK804115.1, MK460236.1, and MK605888.1, respectively. To verify pathogenicity, healthy plants (1 year old) of *O. japonicus* in 10 pots were spray-inoculated with conidial suspension (1 × 10⁶ conidia/ml). Ten plants that were treated with sterile water were used as the control. All plants were maintained in a climatic chamber (26 ± 1°C, 70 to 80% relative humidity, and a photoperiod of 16 h light/8 h dark). Fourteen days later, all inoculated plants showed typical symptoms of black spot identical to those observed in the fields. Control plants remained symptomless and healthy. The pathogenicity analysis was repeated three times. Pathogens reisolated from symptomatic plants were identified as *A. alternata* by morphology observation and sequence analysis. To our knowledge, this is the first report of black spot caused by *A. alternata* on *O. japonicus* in Zhejiang, China.

References:

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