

1 First report of black spot on *Ophiopogon japonicus* caused by *Alternaria*
2 *alternata* in Zhejiang Province, China

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9 *Ophiopogon japonicus* (Linn. f.) Ker-Gawl, a traditional Chinese medicinal plant, is
10 widely cultured in China. The root of *O. japonicus*, is used as the main ingredient in
11 many prescriptions. It is rich in chemical components for steroidal saponins,
12 homoisoflavonoids and polysaccharides, which have various pharmacological
13 activities, such as cardiovascular protection, anti-inflammation and anti-diabetes
14 (Chen. et al. 2016). In May and July for 2018 and 2019, the symptoms of black spot
15 on *O. japonicus* were observed with an incidence of 40% in Cixi County, Zhejiang
16 Province, China. The pathogen mainly infected leaves causing severe black spots,
17 which resulted in a 28% yield loss per acre. At the early stage of the disease, the tip of
18 the leaf began to turn yellow, then the discoloration gradually spread to the base of the
19 leaf and finally the whole leaf turned reddish brown with visible black spot.
20 Symptomatic leaves were cut into small pieces (1.0 cm × 1.0 cm) and disinfected
21 successively by submersion in 75% ethanol for 30s and 1% NaClO for 30s under
22 aseptic conditions. After rinsing with sterile water three times and air drying,
23 segments were placed on potato dextrose agar (PDA), and incubated at 28 °C in dark
24 for a week. Then, pathogen on the PDA were 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 3~8 transverse septa and 1~4 longitudinal septa. Conidiophores were septate, arising singly, and measured $(17.0\sim81.0) \times (8.0\sim23.5)$ μm . Most conidia had a conical or columnar beak, approximately $(0\sim23.5) \times (2.5\sim9.0)$ 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 pathogen, 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-1 α*), RNA polymerase II second largest subunit (*RPB2*), major allergen Alt a 1 gene (*Alt a 1*), Histon 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, ATP-F/ATP-R (Lawrence D.P. et al. 2013; Hong, S.G., et al. 2005). BLASTN analysis of NCBI using ITS (Accession NO. MW989987), *Alt a 1* (Accession NO. MW995953), *EF-1 α* (Accession NO. MW995955), *ATP* (Accession NO. MW995957), *His* (Accession NO. MW995954) and *RPB2* (Accession NO. MW995956) showed 100%, 100%, 97%, 99%, 99% and 97% identity to *A. alternata* MN249500.1, MN304714.1, MK637432.1, MK804115.1, MK460236.1, MK605888.1, respectively. To verify

47 pathogenicity, healthy plants (1-year-old) of *O. japonicus* in ten pots were
48 spray-inoculated with conidial suspension (1×10^6 conidia/ml). Ten plants, which
49 were treated with sterile water, were used as the control. All plants were maintained in
50 a climatic chamber (26 ± 1 °C, 70–80% relative humidity and a photoperiod of 16:8
51 [L: D] h). Fourteen days later, all inoculated plants showed typical symptoms of black
52 spot identical to those observed in the fields. Control plants remained symptomless
53 and healthy. The pathogenicity analysis was repeated three times. Pathogens
54 re-isolated from symptomatic plants were identified as *A. alternata* by morphology
55 observation and sequence analysis. To our knowledge, this is the first report of black
56 spot caused by *A. alternata* on *O. japonicus* in Zhejiang, China.

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