Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on *Avena nuda* in Zhangbei, China

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Naked oats (*Avena nuda* L.) is an independent species of *Avena*, which can be used as both food and forage for rich nutritional value. In August 2019, leaf spot was observed at a naked oats planting base in Zhangbei County, Zhangjiakou City, Hebei Province. The incidence of disease was 40 to 50%. The symptoms of the lesions were chlorosis and gradually developing light brown spots with light yellow halos. The spots were irregular, enlarged, and even coalesced to form large areas of necrosis on leaves. To identify the pathogen, 20 symptomatic leaves were collected, and one disease spot was isolated from each samples. Small square leaf pieces (3 to 5 mm) were excised from the junction of diseased and healthy tissues with a sterile scalpel and were sterilized with 75% alcohol for 30 s followed by 0.1% mercuric chloride solution for 1 min, rinsed three times with sterile water, and then transferred onto potato dextrose agar (PDA) at 25°C for 7 days. Four fungal isolates were obtained and purified by the single-spore isolation method. All fungi had the same morphology, and no other fungi were isolated. Colonies of the isolates had round margins and thick fluffy aerial mycelia with brown coloration after 7 days on PDA. Conidiophores were brown, straight or flexuous, septate, single or in clusters. Conidia were obclavate or oval, dark brown, and size ranging from

4.61 to 15.68 × 6.61 to 35.49 μm ($n = 100$), with longitudinal and transverse septa varying from one to three and from one to seven, respectively. The transverse median septum of the central section was especially thick. On the basis of morphological characteristics, the isolates were identified as *Alternaria* spp. (Simmons 2007). To further assess the identity of the species, the genomic DNA of pathogenic isolate YM3 was extracted by cetyltrimethylammonium bromide protocol. The ribosomal DNA internal transcribed spacer (ITS) region, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), the RNA polymerase II second largest subunit (RPB2), and the plasma membrane ATPase genes were amplified and sequenced with primers ITS1/4, gpd1/2, RPB2-6F/7cR, and ATPDF1/ATPDR1, respectively (Nishikawa and Nakashima 2015; Woudenberg et al. 2015). Sequences of ITS, GAPDH, RPB2, and ATPase (MN646900, MT233043, MT233042, and MN640794) of the isolate were 99.82, 99.68, 100, and 99.51% similar to the fungus *Alternaria alternata* (MK461082.1, MK451978, KP124770.1, and MK804115). A neighbor-joining phylogenetic tree was constructed by combining all sequenced loci in MEGA7. The isolate YM3 clustered in the *A. alternata* clade with 100% bootstrap support. Therefore, the pathogen was identified as *A. alternata* based on the morphological characteristics and molecular identification. A pathogenicity test of the *A. alternata* isolates was performed by placing mycelial disks (5 mm) with conidia on the surface of the first unfolding leaves of naked oats. Each leaf was inoculated with three disks. The pathogenicity test was repeated four times, and 10 leaves were inoculated in each repetition; sterile PDA was used as the control. All treated plants were placed in a moist chamber (25°C, 16-h light and 8-h dark period). Leaf spot symptoms developed on the inoculated plants about 10 days post inoculation, whereas all control plants remained healthy. Similar isolates were reisolated from the inoculated and infected leaves and identified as *A. alternata* by DNA sequencing, fulfilling Koch's postulates. It has been reported that *A. alternata* can cause leaf spots on *A. sativa* (Chen et al. 2020). However, to our knowledge, this is the first report of *A. alternata* causing leaf spots on *A. nuda* in China. It can be concluded that *A. alternata* can cause leaf spot disease of oats (*A. sativa* and *A. nuda*). The spots disease is worthy of our attention for its harm to the production of oats.

References:

- Chen, H., et al. 2020. Plant Dis. 104:1544.
- Nishikawa, J., and Nakashima, C. 2015. Mycoscience 56:141.
- Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Center, Utrecht, The Netherlands.
- Woudenberg, J. H. C., et al. 2015. Stud. Mycol. 82:1.

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