Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Alternaria alternata* Causing Leaf Spot on Cockscomb in Northeast China

Ruiting Wang,¹ Changchun Dai,¹ Chuzhen Xu,¹ Baiyan Xue,¹ Yonggang Li^{1,†} and Lei Sun²

¹ Agricultural College, Northeast Agricultural University, Harbin 150030, P.R. China

² Institute of Soil Fertilizer and Environment Resources, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, P.R. China

R. T. Wang and C. C. Dai contributed equally to this work and are considered co-first authors.

Funding: This study was funded by the National Natural Science Foundation of China (31971760) and Heilongjiang Collaborative Innovation and Extension System of Modern Agricultural Industry Technology of Forage and Feed. Plant Dis. 104:3255, 2020; published online as <https://doi.org/10.1094/PDIS-05-20-0954-PDN>. Accepted for publication 7 July 2020.

Cockscomb (*Celosia cristata* L.) is grown in the garden as an ornamental plant and also used in traditional medicine for the treatment of headache, sores, skin eruption, painful menstruation, and carpal tunnel syndrome (Islam et al. 2016). In July 2019, a leaf spot of cockscomb was observed in three nurseries with approximately 40% of the 150 plants diseased in Harbin (44°40'N, 125°42'E), China. Lesions began as tiny brown spots surrounded by a yellow halo and expanded gradually to circular or approximately circular necrosis with tawny to brown and gray center (0.2 to 0.9 cm in diameter). Ten diseased tissues from 10 individual plants (two plants per nursery) were cut into 0.25-cm² pieces and surface sterilized in 75% ethanol for 45 s, rinsed three times in sterile distilled water, cultured on potato dextrose agar (PDA), and incubated at 26°C in the dark for 3 days. Six fungal isolates were isolated, were all morphologically similar, and were subcultured by transferring hyphal tips. Fungal colonies were olive-green with white margin, black pigment, and abundant aerial hyphae on PDA. Conidia were black to brown, obclavate to obpyriform, in chains, with one to seven transverse and one to three longitudinal septa, measuring 5.6 to 16.8 µm in width and 9.5 to 37.9 µm in length ($n = 50$). The isolated fungi were identified as a species of *Alternaria* based on cultural and

morphological characteristics (Simmons 2007). For molecular identification, genomic DNA was extracted from a representative isolate JGH1, amplified, and sequenced using primers ITS1/ITS4 (White et al. 1990), EF1-728F/EF1-986R (Carbone and Kohn 1999), and Gpd1/Gpd2 (Woudenberg et al. 2013). The consensus sequences (accession nos. MT279999 for ITS, MT321480 for TEF1, and MT428544 for GAPDH) were aligned using BLASTn in GenBank, obtaining 100% identical to *Alternaria alternata* strains ACT-6 (accession no. MN548785.1, 563/563 nucleotides), JDD14-3 (MK637432.1, 268/268 nucleotides), and F217055 (MK649882.1, 423/423 nucleotides), respectively. Koch's postulates were satisfied by the inoculation of leaves growing for 45 days in 15-cm pots containing a commercial potting mix (one plant per pot) with two repetitions. All leaves of three healthy plants were inoculated by spraying 20 ml of a spore suspension (10⁵ spores/ml). Three plants were inoculated with sterile distilled water as a control. After inoculation, all plants were incubated in a greenhouse (16-h/8-h light/dark, 22 to 28°C, 70% RH) and covered with plastic bag, which was removed after 48 h. After 8 days, typical symptoms showed on the inoculated leaves, whereas no symptoms were observed on the control leaves. *A. alternata* was reisolated from the diseased inoculated leaves and identified by morphology and molecular biology. There has already been a report about *A. alternata* on *C. cristata* in Poland (Mulencko et al. 2008). To our knowledge, this is the first report of *A. alternata* causing leaf spot on cockscomb in China. Occurrence of the new disease caused by *A. alternata* should give full consideration for the use of proper disease management to protect cockscomb.

References:

- Carbone, I., and Kohn, L. M. 1999. Mycologia 91:553.
- Islam, S., et al. 2016. BMC Complement. Altern. Med. 16:400.
- Mulencko, W., et al. 2008. Page 752 in: A Preliminary Checklist of Micromycetes in Poland, Vol. 9. W. Szafer Institute of Botany, Polish Academy of Sciences, Krakow, Poland.
- Simmons, E. G. 2007. *Alternaria: An Identification Manual*. CBS Fungal Biodiversity Center, Utrecht, the Netherlands.
- White, T. J., et al. 1990. Page 315 in: *PCR Protocols: A Guide to Methods and Applications*. Academic Press, San Diego, CA.
- Woudenberg, J. H., et al. 2013. Stud. Mycol. 75:171.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: *Alternaria alternata*, leaf spot, *Celosia cristata*

[†]Indicates the corresponding author.
E-mail: neaulyg@126.com (Y.G.L.)