

1 First Report of *Alternaria alternata* Inciting Leaf Spot of Dangshen
2 (*Codonopsis pilosula*) in China

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7 As a commonly traditional Chinese medicine, the perennial herb Dangshen
8 (*Codonopsis pilosula*) has superior curative effects including regulating immunity,
9 strengthening the spleen, and tonifying lungs (Bai et al. 2020). To imitate natural
10 ecological conditions, plants were grown on hillside fields with stems prostrate on the
11 ground, tangle-up with each other. In August 2020, leaf spots were observed on *C.*
12 *pilosula* in Wutai county, Shanxi province, China, and indicated a high disease
13 incidence (70%-80%) in investigated fields (6.67 ha). Small brown necrotic spots,
14 occasionally enclosed by chlorotic halos, were observed on leaves, stems, and sepals.
15 For identification of the pathogen, 15 small pieces (5×5 mm) of symptomatic tissues
16 from 5 randomly-collected diseased plants were surface sterilized, placed on potato
17 dextrose agar plates, and incubated for 4 d in darkness at 25 °C to obtain the colonies.
18 Cultures were purified by single spore isolation from these colonies. A total of 15
19 isolates named as Dcp-3, and Dcp-5~Dcp-18 were recovered. They produced ovoid or
20 obclavate spores with 15.9-57.5×9.1-20.1 μm in size, 1-6 transverse septa, and 0-4
21 longitudinal septa. The conidial chains with 4 to 6 spores had numerous secondary and
22 occasionally tertiary chains on potato carrot agar plates. Because all isolates had
23 identical morphological traits, five genes from the representative isolate Dcp-3, actin
24 (ACT), *Alternaria* major allergen (*Alt a1*), plasma membrane ATPase (ATP), histone
25 3 (H3), and rDNA ITS, were amplified with primer pairs ACTDF1/ACTDR1, Alt-
26 for/Alt-rev, ATPDF1/ATPDR1, H3-1a/H3-1b, and ITS1/ITS4, respectively (Hong et
27 al. 2005; Lawrence et al. 2013; Ma et al. 2020). BLASTn searches indicated species of
28 Dcp-3 could not be accurately confirmed by rDNA ITS, ATP, ACT, and *Alt a1*

(GenBank accession nos. OM334894, OM362504, OM326344, OM362500). Phylogenetic analysis showed it was most closely related to *Alternaria alternata*, *A. arborescens*, and *A. tenuissima* based on concatenated sequences of above four genes. The H3 sequence (OM362508) shared 100% homology with that of *A. alternata* (MN481948). The phylogenetic tree using H3 also confirmed Dcp-3 as *A. alternata*. Healthy, two-year-old *C. pilosula* were transplanted to a greenhouse. A surface-sterilized leaf was sprayed with 50 μ L spore suspension (10^6 conidia/mL) of Dcp-3. A leaf sprayed with isometric sterile water was used as controls. Each treatment used six plants (five leaves per plant). Plants were covered with sterilized plastic bags and incubated at 22 °C. The test was repeated twice. A week later, control leaves were healthy, but brown necrotic spots similar to field symptoms emerged on treated leaves. The *A. alternata* isolates were re-isolated from the border of lesions, and confirmed by morphological and molecular characteristics mentioned above, fulfilling Koch's postulates. Leaf spot of *C. pilosula* caused by *Septoria codonopsidis* has been reported in China (Wang et al. 2011). However, to our knowledge, this is the first report of *A. alternata* inciting leaf spot of *C. pilosula* in China. Our report would promote growers to enhance the field management and consider associated strategies on controlling *Alternaria* leaf spot of *C. pilosula*.

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The author(s) declare no conflict of interest.

References:

- Bai, R. B., et al. 2020. **Food Funct.** 11:3306. <https://doi.org/10.1039/c9fo02969a>
- Hong, S. G., et al. 2005. **Fungal Genet Biol.** 42:119. <https://doi.org/10.1016/j.fgb.2004.10.009>
- Lawrence, D. P., et al. 2013. **Mycologia** 105:530. <https://doi.org/10.3852/12-249>

- 56 Ma, G. P., *et al.* 2021. **Plant Dis.** 105:60. <https://doi.org/10.1094/PDIS-01-20-0130-RE>
- 57 Wang, Y., *et al.* 2011. **J. Shanxi Agr. Sci.** 39:866. [https://doi.org/10.3969/j.issn.1002-](https://doi.org/10.3969/j.issn.1002-2481.2011.08.28)
- 58 2481.2011.08.28. (in Chinese)

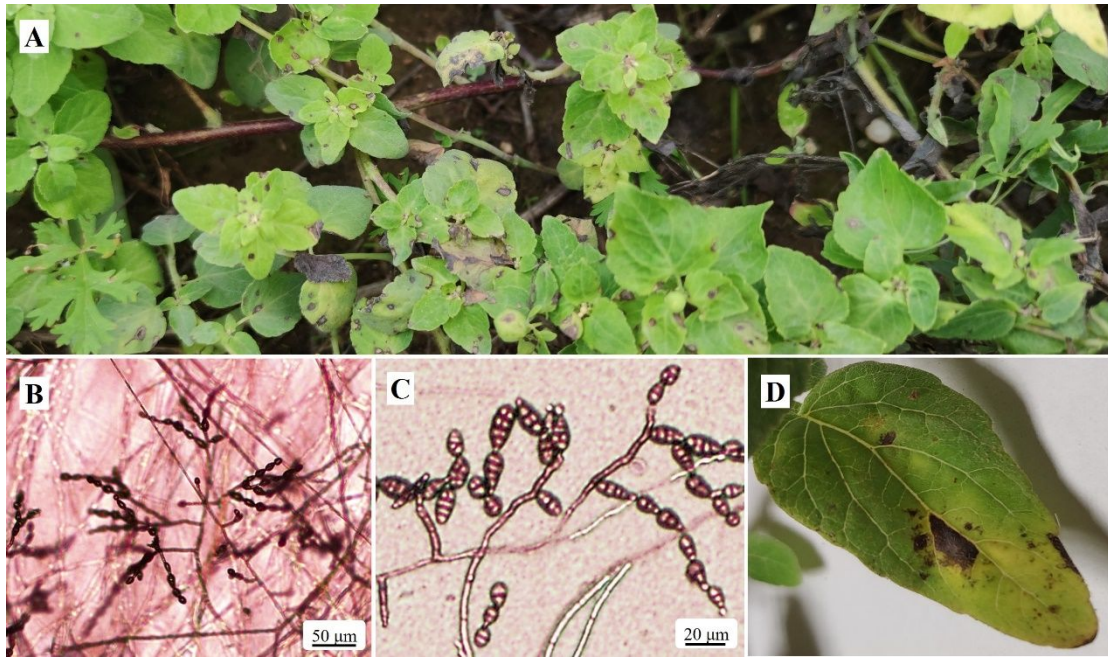


Fig. S1 (A) leaf spot symptoms on *C. pilosula* in the field. (B-C) sporulation patterns and conidia of *Alternaria alternata* isolate on potato carrot agar (PCA) plate, respectively. (D) leaf spots caused by *A. alternata* on *C. pilosula* in pathogenicity test.

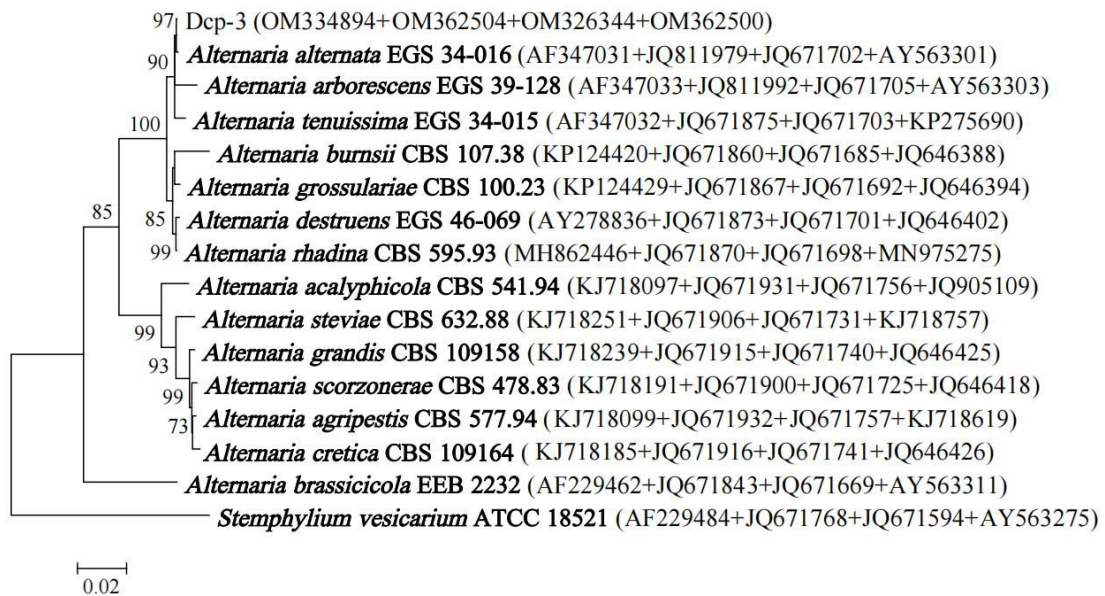
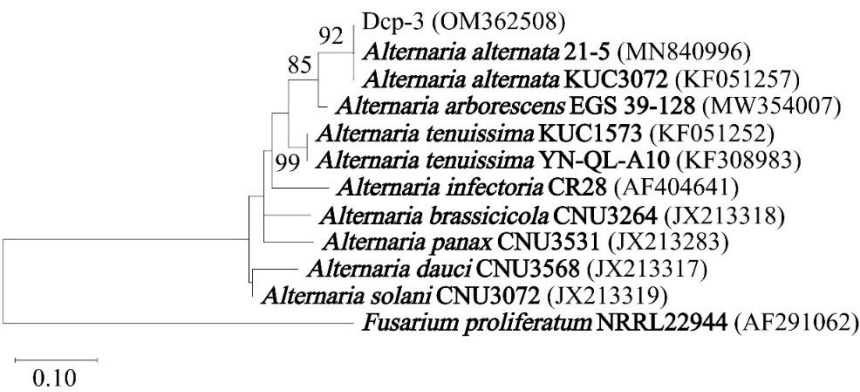


Fig. S2 Phylogenetic tree based on the concatenated rDNA ITS, plasma membrane ATPase, actin, and *Alt a 1* of Dcp-3 constructed using maximum likelihood analysis with Tamura-Nei model.



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11 Fig. S3 Phylogenetic tree based on the histone 3 gene of Dcp-3 constructed using
12 maximum likelihood analysis with Tamura-Nei model.