Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Pseudoestalotiopsis* and *Neopestalotiopsis* Species Causing Leaf Spot of *Camellia chrysantha* in China

J. Zhao,¹ D. P. Zhang,¹ T. Liu,^{1,†} W. C. Liu,¹ L. Q. Pan,² N. Y. Liao,² and D. W. Liu¹

¹ Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

² Fangcheng Golden Camellia National Nature Reserve Management Office, Fangchenggang, Guangxi Zhuang Autonomous Region 538021, China

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Camellia chrysantha (Hu) Tuyama (golden camellia) is an important ornamental and medicinal plant grown in southern China, which is often referred to as “the queen of camellia” (Zhang et al. 2017). In October 2019, leaf spot symptoms were observed with 20 to 30% disease incidence in the famous golden camellia growing area of Fangchenggang city of Guangxi Zhuang Autonomous Region in China. The diseased leaves showed brown spots, which gradually expanded to large, circular to irregular necrotic lesions surrounded by dark brown halos during the period from July to October each year. For pathogen isolation, small tissue pieces (5 × 5 mm) from disease leaf lesions were sterilized by 0.3% sodium hypochlorite for 2 min and 70% ethanol for 40 s, rinsed three times with sterile distilled water, and then incubated on potato dextrose agar plates. A total of 13 *Pestalotiopsis*-like fungal isolates were obtained and purified through single-spore culture. The fungal isolates produced white to creamy colonies with concentric rings and wavy edges after 7 days of incubation. These isolates were preliminarily identified as belonging to *Pseudoestalotiopsis* or *Neopestalotiopsis* species based on conidial characters (Maharachchikumbura et al. 2014). All of these isolates produced five-celled conidia, which were fusiform or ellipsoid, straight or slightly curved, with three colored median cells and two colorless terminal cells. The three median cells of eight

Pseudoestalotiopsis isolates were concolorous, dark brown to olivaceous, whereas those of five *Neopestalotiopsis* isolates were versicolor, with two upper cells dark brown and one lower cell light brown. Molecular identification was conducted by DNA sequence analyses of three different phylogenetic markers, including the internal transcribed spacer, partial translation elongation factor 1- α , and partial β -tubulin gene regions (Maharachchikumbura et al. 2012). Phylogenetic analyses of the combined sequences data of the three genes indicated that the eight *Pseudoestalotiopsis* isolates were located in one clade with the known *P. ampullacea* strains such as HN41-1, YB36-2, GX17-1, and LC6618 retrieved from GenBank, whereas the five *Neopestalotiopsis* isolates were attributed to one clade with the known *N. clavispora* strains including CBS 447.73, FJ2-G-2, and YN27-2-2 (Chen et al. 2018; Shu et al. 2020). The sequences in this study were all submitted to GenBank (accession nos. MN886776 to MN886814). Pathogenicity tests were conducted on healthy leaves detached from 2-year-old golden camellia plants (cv. Fangpu). Conidial suspensions (10⁶ conidia/ml) of all the 13 isolates were prepared and inoculated on the middle of artificially wounded leaves. Control leaves were inoculated with sterile distilled water only. The experiment was repeated three times with five leaves per treatment. After 7 days of incubation at 25°C and 90% relative humidity, all of the inoculated leaves developed leaf spot symptoms similar to those observed initially, whereas control leaves remained symptomless. The fungi were reisolated from lesions of the disease leaves, and their colony and microscopic morphology features were identical to the original inoculated isolates, which confirmed the pathogenicity of *P. ampullacea* and *N. clavispora*. These two species have been reported to cause mango leaf spot (Shu et al. 2020) and *Camellia sinensis* gray blight (Chen et al. 2018). To our knowledge, this is the first report of both *P. ampullacea* and *N. clavispora* causing leaf spot of *C. chrysantha* in China.

References:

- Chen, Y. J., et al. 2018. Plant Dis. 102:98.
- Maharachchikumbura, S. S. N., et al. 2012. Fungal Divers. 56:95.
- Maharachchikumbura, S. S. N., et al. 2014. Stud. Mycol. 79:121.
- Shu, J., et al. 2020. Plant Dis. 104:1207.
- Zhang, K., et al. 2017. Ecol. Inform. 42:32.

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[†]Indicates the corresponding author.
E-mail: ltng11@163.com (T.L.)