Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot on *Panax notoginseng* Caused by *Caryophylloseptoria pseudolychnidis* in Yunnan, China

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Funding: This work was supported by China Agriculture Research System (CARS-21), Major Science and Technology Project of Yunnan and Kunming (202102AE090042 and 2021JH002), and National and Provincial Excellent Scientist Supporting Programme. Plant Dis. 106:2525, 2022; published online as <https://doi.org/10.1094/PDIS-12-21-2817-PDN>. Accepted for publication 11 February 2022.

Panax notoginseng, a unique traditional medicinal plant in China, improves myocardial ischemia, protects the liver, and prevents cardiovascular diseases (Jiang et al. 2021). In July 2021, gray-brown round spots were found on the leaves of *P. notoginseng* in plantations in Lincang City (23°43'10"N, 100°7'32"E). By September, symptoms were observed on more *P. notoginseng* plants, with incidence reaching 31%. Initial symptoms on leaves were small, brown spots that expanded, with black granular bulges on the lesions, often surrounded by a yellow halo. As the disease progressed, multiple lesions merged, leaves became yellow, and abscissions occurred. To isolate the causal pathogen, 12 symptomatic leaves were randomly obtained from 12 *P. notoginseng* plants. Pieces of infected leaf tissues (about 5 mm²) were disinfected with 75% ethanol for 30 s, soaked in 2% NaOCl for 3 min, rinsed three times with sterile water, and blotted dry. Sample tissues were plated on potato dextrose agar (PDA) plates incubated at 25°C for 5 days with 12 h light/dark photoperiod. Hyphal tips from the growing edge of colonies were transferred to fresh PDA to obtain pure cultures. Eight isolates were obtained with similar colony morphology, gray (top view) or black (back view) coloration, a villous surface, and slow-growing on PDA. Conidia were hyaline, slender, obtuse to subobtusate at both ends, and 10.3 to 52.62 (av. 25.2) × 1.4 to 4.0 (av. 2.4) µm (*n* = 200). Characteristics of the colonies and conidia were consistent with

Caryophylloseptoria pseudolychnidis as described by Quaadvlieg et al. (2013) and Verkley et al. (2013). Genomic DNA of three representative isolates (LINC-4 to LINC-6) was extracted, and the rDNA-ITS region, *ACT*, and *LSU* gene regions were amplified and sequenced using the primer pairs ITS4/ITS5, 512F/783R, and LSU1Fd/LR5, respectively. Sequences were deposited in GenBank (ITS: OK614104 to OK614106, *LSU*: OK614109 to OK614111, *ACT*: OK628350 to OK628352). A BLAST search showed that all sequences had 98 to 100% homology with corresponding sequences of *C. pseudolychnidis*. ITS sequences of LINC-4 to LINC-6 showed 99.21% identity (500/504 bp) to *C. pseudolychnidis* strain CBS 128630 (NR156266). *LSU* sequences of LINC-4 to LINC-6 showed 99.76% identity (823/825 bp) to *C. pseudolychnidis* strain CBS 128630 (MH876481). For *ACT* sequences, LINC-4 and LINC-5 showed 98.53% identity (201/204 bp) to *C. pseudolychnidis* strain 128614 (KF253599); LINC-6 showed 99.02% identity (202/204 bp) to *C. pseudolychnidis* strain 128614 (KF253599). Neighbor-joining and maximum-likelihood methods were used for multilocus phylogenetic analysis of the obtained sequences using MEGA-X (Kumar et al. 2018). The three isolates were clustered in the same clade with two *C. pseudolychnidis* from the database. LINC-4 to LINC-6 were tested for pathogenicity to confirm Koch's postulates. Annual potted *P. notoginseng* were inoculated with spore suspension (10⁵ spores/ml). Each isolate was inoculated onto two leaves each of five plants. Controls were similarly mock-inoculated with sterile water. To maintain high humidity (>90% RH), all plants were placed in transparent plastic boxes in a greenhouse at 25°C with a 12 h light/dark photoperiod. Fifteen days postinoculation, inoculated leaves showed similar symptoms to those observed in the field, and control plants remained healthy. The pathogen was reisolated from symptomatic leaf spots, and the colony characteristics were the same as those of the original isolates. Morphological characteristics, molecular data, and Koch's postulates tests confirmed *C. pseudolychnidis* as the cause of *P. notoginseng* leaf spot disease. To our knowledge, this is the first report of *C. pseudolychnidis* causing leaf spot on *P. notoginseng* in Yunnan, China. The spread of the disease might pose a serious threat to *P. notoginseng* production. The occurrence and spread of this pathogen should be further studied to formulate control measures.

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The author(s) declare no conflict of interest.

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Keywords: *Caryophylloseptoria pseudolychnidis*, leaf spot disease, *Panax notoginseng*

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