Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Spot Needle Blight on *Pinus thunbergii* Caused by *Aureobasidium pullulans* in China

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Pinus thunbergii Parl., black pine, is widely distributed in China. This pine tree can promote soil conservation and shows excellent potential for reforestation and planting in shelter belts. Lumber from this species can be used for building construction and furniture production. In August 2020, needle blight symptoms were found on several trees of black pine in Sichuan Province, China. Further surveys showed that these symptoms were common, and the disease incidence was less than 30%. The tips of old needles first turn grayish green, developing brown bands ranging from 1 to 2 mm wide. To determine the cause, 20 needle samples with typical symptoms were disinfected with 75% ethanol, and sections of the tissue were cut from the margin of diseased and asymptomatic tissue with a sterile scalpel. The pieces of tissue were surface sterilized a second time by submerging them in 75% ethanol for 45 s and soaking in a 1.5% NaClO bleach solution for 90 s, rinsed with sterile water, and then dried. Small cut tissues were placed on potato dextrose agar at 25°C for 10 days. Pure cultures were obtained by isolating single spores. The colonies initially appeared white to cream, yeast-like, turning brown in the center after 10 days. Conidia were hyaline, smooth-walled, single-celled, and ellipsoid, ranging in size, 7.5 to 16 × 3.5 to 7 µm; the chlamydospores were oval and brown (Zalar et al. 2008). DNA was extracted from the mycelium with the CTAB method. The internal transcribed spacer (ITS) region of rDNA and the partial β-tubulin gene of a representative isolate (SC05) were amplified by PCR using the ITS1/ITS4 and Bt2a/Bt2b primer pairs,

respectively (Wu et al. 2017). The sequences were submitted to GenBank (accession nos. MW228368 and MW256762) and showed high similarity with BLAST sequences of *Aureobasidium pullulans* (ITS, KR704881 [100%]; β-tubulin, MT671934 [99.49%]). Phylogenetic analysis based on the combined sequences revealed that all the representative isolates recovered from symptomatic *P. thunbergii* needle blight showed at least 97% bootstrap support with those of the *A. pullulans* isolates in the NCBI database but were significantly different from those of other species of *Aureobasidium*, including two recently reported isolates from *P. tabuliformis*. Morphological and molecular characteristics confirmed the isolates as *A. pullulans* (Jiang et al. 2019). For the pathogenicity testing, a conidial suspension was prepared with a concentration of 2.0×10^7 conidia/ml. The suspension was sprayed onto three 1-year-old seedlings' needles, and the control was sprayed with distilled water. Three replicates for each inoculated and noninoculated plant were kept in humid chambers in a glasshouse. After 10 days, typical symptoms appeared on inoculated needles, whereas control needles remained symptomless. The fungus, *A. pullulans*, was reisolated from those lesions, confirming Koch's postulates. No symptoms were observed on control plants. *A. pullulans*, a ubiquitous saprobic fungus on many fruits, was rarely reported to cause disease on pine needles. It has only been reported during invasion of ozone-injured needles in *P. strobus* (Costonis and Sinclair 1972) and needles damaged by acid rain in *P. sylvestris* (Ranta 1990). To our knowledge, this is the first report of brown spot needle blight on *P. thunbergii* caused by *A. pullulans* in China. The disease represents a threat to pine forests, and more research on the pathogenesis and management is needed.

References:

- Costonis, A. C., and Sinclair, W. A. 1972. Eur. J. For. Pathol. 2:65.
- Jiang, N., et al. 2019. Phytotaxa 402:199.
- Ranta, H. M. 1990. Environ. Pollut. 67:349.
- Wu, J.-B., et al. 2017. Plant Dis. 101:249.
- Zalar, P., et al. 2008. Stud. Mycol. 61:21.

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