Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Brown Rot of Fruit on *Vitis amurensis* Caused by *Monilinia polystroma* in China

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Vitis amurensis Rupr. (Amur grape) is a wild grape genetic resource widely distributed in Heilongjiang, Jilin, Liaoning, and Inner Mongolia, among other places in China (Song et al. 2009) and the Russian Far East and Korean Peninsula. In September 2018, brown rot symptoms were observed at ripening stage on the fruits of a 5-year-old Amur grapevine germplasm resources nursery of the cultivar 'Beibinghong' and a few Russian resources in Zuojia Town, Jilin City, Jilin Province, China. The diseased fruit surface became brown with soft rot and produced buff to brownish-gray sporodochia with conidia. Around 180 plants of Beibinghong were examined, which had 8% incidence. Forty-five samples were collected from symptomatic fruits of 15 randomly sampled Beibinghong grape clusters, cut into 5-mm² pieces of diseased tissue, surface sterilized with 1% NaOCl for 2 min, rinsed three times with sterile water, dried on sterilized filter paper, and plated on potato dextrose agar (PDA). Thirteen monospore isolates were obtained using the single-spore isolation method with incubation at 25°C and a 12-h light/12-h dark cycle. The average colony diameter was 46 to 49 mm after 4 days of culture on PDA. Colonies were white to grayish with even margins. Irregular black stromatal crusts were observed on the reverse side of dishes 10 days after inoculation. Conidial spores were produced when cultured on cherry agar at 25°C under near-ultraviolet light. Spores were single-celled and hyaline, limoniform or ellipsoid, and were produced in branched moniloid chains, 12 to 22 × 8 to 13 µm (mean: 15.4 ± 1.03 × 9.01 ± 0.72 µm, *n* = 50). When conidia were cultured on water agar at 25°C for 18 h, the germ tubes were straight, 700 to 1,000 µm long, and

often with two germ tubes per conidium. Morphological characteristics were consistent with those of *Monilinia polystroma* (Van Leeuwen et al. 2002). To confirm the species identification, two DNA regions of the selected isolate VAMPWYZSH8 were amplified by polymerase chain reaction (PCR) and sequenced: the internal transcribed spacer region (ITS) was generated using primers ITS1/ITS4 (Munda 2015), and β-tubulin (TUB2) was amplified using primers Bt2a/Bt2b (Zhu et al. 2016). A BLAST analysis of the nucleotide sequence of the PCR products revealed 100% identity with two *M. polystroma* sequences in the NCBI GenBank (KJ814976 for ITS, KR778970 for TUB2). Our sequences were deposited in GenBank with accession numbers MT038413 for ITS and MT038414 for TUB2. On the basis of these results, the isolate was identified as *M. polystroma*. To confirm pathogenicity, 78 fresh and healthy bunches of Beibinghong grapes at ripening were collected, surface disinfected by immersion in 1% NaOCl for 1 min, rinsed three times with sterile water, and then allowed to air dry. Under dry aseptic conditions, the fruits were inoculated using the pinprick method. Each wound was inoculated with 10 µl of conidial suspension (10⁶ spores/ml) and incubated at 25°C with about 90% relative humidity and natural light. Inoculation with water was used as a control, and the experiment was repeated three times. After a 10-day incubation, typical symptoms of brown rot developed on inoculated fruits, whereas control fruits were symptomless. The fungus was consistently reisolated only from diseased fruits and showed the same morphological characteristics as the original isolates, thus fulfilling Koch's postulates. This is the first report of *M. polystroma* on *V. amurensis* in China. The resulting disease decreases fruit quality and yield, necessitating the development of effective control measures.

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