Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neofusicoccum parvum* Causing Brown Spots on Gallnuts of *Rhus potaninii* in China

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Chinese gallnuts have been used as a source of tannic acids, which are widely used in medicinal and chemical products (Zhang et al. 1999). In summer 2020, a severe outbreak of brown spots was observed on Du-ensiform gall of *Rhus potaninii*, at the Dongyue village, Emei county, Sichuan province, China. Spots irregular brown to blackish brown mainly occurred on the gall surfaces and spread quickly on about 40% of Du-ensiform galls. Nine symptomatic galls were randomly collected in the field. Tissues from the infected galls (~3 × 5 mm) were surface disinfected by 75% ethyl alcohol and 10% sodium hypochlorite and then plated on potato dextrose agar (PDA) at 25°C in the dark for 6 days. Similar fungal colonies were isolated from 56% of the infected galls. Five isolates from different galls showed similar colony morphology. After subculturing of hyphal tips on PDA, the initially whitish colonies showed abundant gray to black aerial mycelium after 5 to 7 days, with no sporulation. A representative isolate (RC82) was randomly selected to induce sporulation on sterilized pine needles placed on water agar at 25°C in the dark. Black pycnidia (up to 450-µm diameter) developed after incubation for 28 days. Conidiogenous cells were holoblastic, hyaline and short subcylindrical. Conidia were hyaline, fusiform or elliptic, thin-walled and aseptate, and measured $15.2 \pm 0.9 \times 5.3 \pm 0.3$ µm ($n = 126$). The morphological characteristics were consistent with *Neofusicoccum parvum* (Crous et al. 2006; Pennycook et al. 1985). To classify this fungal pathogen genetically, sequence analyses were conducted using the ITS rDNA region and the gene coding for EF-1α with DNA samples from four isolates of infected samples. Genomic DNA was amplified with primers ITS1/ITS4 and EF1-728F

(Carbone et al. 1999)/EF-2 (O'Donnell et al. 1998). The sequences obtained were deposited in GenBank (accession nos.: ITS, MZ269214 to MZ269217; and EF-1α, MZ269219 to MZ269222, respectively). BLASTn searches revealed 100% similarity of each gene to related sequences of multiple reference isolates of *N. parvum*. Based on neighbor-joining phylogenetic tree analyses of the combined ITS and EF-1α datasets, the four isolates clustered with *N. parvum* strains CMW9081 (ex-type), CMW27135, CAA856, and so on, with clear separation from other closely related *Neofusicoccum* spp. To perform pathogenicity tests, three isolates were randomly selected. The tests were conducted with mycelial plugs of a 7-day-old colony placed onto the surface of wounded and/or unwounded galls and host leaves. A total of five replicates were included/treatment. Sterile PDA agar plugs were inoculated as controls. The galls and leaves were incubated at 25°C in plastic containers lined with wet filter paper for high humidity. Initial symptoms appeared within 3 days on all wounded galls and leaves, whereas the unwounded galls and leaves and the controls remained asymptomatic. Some of the unwounded galls and leaves showed symptoms within 4 to 10 days after incubation. The fungus was reisolated from the lesions of inoculated tissues. The reisolated fungal colonies showed identical morphology and 100% sequence similarity of ITS and EF-1α with the initial isolate. No fungus was isolated from the controls. *N. parvum* is a common and cosmopolitan species on a wide variety of hosts (Phillips et al. 2013). To our knowledge, this is the first report of *N. parvum* causing gallnut brown spots on *R. potaninii* worldwide. It indicates that crop management practices need to be evaluated to control and/or prevent the disease.

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