Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neofusicoccum parvum* Causing Leaf Spot on Coral Trees (*Viburnum odoratissimum*) in China

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Coral trees (*Viburnum odoratissimum*), as a class of evergreen shrubs, are mainly planted in landscapes in numerous cities in China. During September 2020, we investigated four major parks in Hefei (Bao Park, Hefei Botanical Garden, Luzhou Park, and Peninsula Park) and the campus of Anhui Agricultural University (approximately 0.5 ha) (31°49'21.30"N, 117°13'18.25"E). The results showed that the incidence rate of leaf spot disease reached 60% among approximately 100,000 coral trees planted in these areas. Coral trees begin to show leaf spots in August. In early stages of coral tree infection, the symptoms appeared as small brown spots ranging in length from 2 to 3 mm on the leaves. After the disease patches expanded and darkened, the leaves eventually withered and fell, seriously affecting the aesthetic value. To identify the fungal pathogen, the five-point sampling method was used to take typical similar leaf samples from five regions, and six samples were taken from each site, so a total of 150 samples were obtained. Fragments of sample leaves were surface sterilized with 1% NaClO, plated on potato dextrose agar, and incubated at 25°C in the dark. A total of 275 strains were obtained from 150 samples. According to the morphological characteristics, 275 strains were purified and divided into four types. Four representative strains (MI1, K1, F1, D1) were selected from four types for further pathogenicity testing and identification. The pathogenicity test was conducted in triplicate by inoculating wounded leaves of 1-year-old potted *V. odoratissimum* with 20 µl of

a conidial suspension (10⁶ conidia/ml). Controls were inoculated with sterile water. The specimens were placed in a growth chamber while maintaining 90% relative humidity and 28°C. After five days, the characteristic lesions were observed only on inoculated MI1 spore suspension leaves. The same fungus was reisolated from the lesions, thus fulfilling Koch's postulates. The pathogenic fungi accounted for 60% of all strains. Fungal colonies were circular and had abundant white aerial mycelium, and colonies changed from white to pure black after maturity. Conidia were fusiform (16 to 17 × 5 to 6 µm), thin-walled, transparent, and without diaphragms. Molecular identification was performed by partially sequencing the internal transcribed spacer (ITS) gene, the translation elongation factor 1-alpha (*EF1-alpha*) gene, and the β-tubulin (*TUB2*) gene using the primers ITS1/ITS4 (White et al. 1990), EF1-728F (Phillips et al. 2013)/EF1-986R (Carbone and Kohn 1999), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. The obtained ITS sequence (MW767713) showed 99% identity with *N. parvum* CMW28429 (KU997429.1), the *EF1-alpha* sequence (MZ398261) showed 99% identity with *N. parvum* isolate A4 (FJ528597.1), and the *TUB2* sequence (MZ398260) showed 99% identity with *N. parvum* isolate BO52 (KU554657.1). By combining the sequences of individual fragments of each fungus in the order ITS, *EF1-alpha*, and *TUB2*, MEGA 6.0 was used to analyze the sequence of kinship using the maximum likelihood method, and the repeat value of bootstraps was 1,000. A polygenic phylogenetic tree analysis based on multilocus alignment (ITS, *EF1-alpha*, and *TUB2*) was constructed with some strains of Botryosphaeriaceae species. The results of the phylogenetic tree showed that MI1 and *N. parvum* clustered into a branch. To our knowledge, this is the first report of *N. parvum* causing leaf spot on *V. odoratissimum* in China.

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