Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neopestalotiopsis clavispora* Causing Calyx and Receptacle Blight on Strawberry in China

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Strawberry (*Fragaria × ananassa*) is widely cultivated in Liaoning Province, the most important strawberry production area in China. In November 2020, extensive brown spotting on calyxes and receptacles and dark brown necrotic lesions on leaves of ‘Yanli’ strawberry plants were observed in the greenhouse, College of Horticulture, in Shenyang Agriculture University of Liaoning Province, China. The incidence ranged from 15 to 20% in the greenhouse. As the disease progressed, the spots extended to cover entire receptacles and entire leaves. Fresh samples (5 mm²) were cut from the margin of healthy and infected tissues. The isolation and purification methods were as described previously (Obregón et al. 2018). Fungal isolations were subcircular with irregular edges. The colonies showed white aerial mycelium at the early stage and formed concentric circles after 5 days of culture at 28°C on PDA. Black viscous acervuli appeared scattered on the surface of the colony after ~7 to 10 days of culture. Acervuli were 162.4 µm in average diameter, *n* = 30. Conidia were spindle shaped, five cells, four septa, 7.0 µm (average width), and 24.0 µm (average length), *n* = 30. Apical and basal cells were lighter in color, mostly hyaline, and the middle three cells were darker in color, mostly brown. There were two to four colorless, transparent unbranched accessory

filaments at the top, 30.8 µm in average length, and the basal cell had a small appendage, 9.0 µm in average length, *n* = 30. For fungal identification to species level, genomic DNA was extracted by the 2% CTAB method. The internal transcribed spacer (ITS) region, *β-tubulin* gene (*TUB2*), and translation elongation factor-1 α (*TEF-1 α*) were amplified (Qiu et al. 2020). Purified PCR products were sequenced in a single direction. BLASTn searches of GenBank, using the partial ITS (MW861730), *β-tubulin* (MW856302), and *TEF-1 α* (MW861729) sequences of representative isolate NP102 as the queries, revealed 97, 100, and 100% sequence identity, respectively, to *Neopestalotiopsis clavispora* strain WJ31 accessions MG729690, MG740736, and MG740759. A phylogenetic tree was constructed by MEGA X (Kumar et al. 2018). A pathogenicity assay was carried out by spraying a spore suspension (1×10^6 spores/ml) of an 8-day-old fungus culture on six healthy ‘Yanli’ strawberry plants. An additional six control plants were only sprayed with sterile distilled water. The inoculated plants were maintained in a light incubator at $25 \pm 1^\circ\text{C}$, 90% relative humidity, and a photoperiod of 12 h. Calyx and receptacle blight and leaf spot symptoms resembled those observed in the greenhouse on all inoculated tissues after 10 days, whereas calyxes, receptacles, and leaves on control plants remained asymptomatic. The experiment was repeated twice. *N. clavispora* was successfully reisolated from the infected tissues, fulfilling Koch’s postulates. Researchers in Spain (Chamorro et al. 2016) first reported *N. clavispora* on strawberry. Two years later, *N. clavispora* was found in Argentina (Obregón et al. 2018). The reports characterized it mainly as a root rot pathogen. In January 2021, *Neopestalotiopsis* spp. were reported as a pathogen of strawberry leaves and fruits in Florida (Juliana et al. 2021). Our studies indicated that isolate NP102 was more aggressive. In the warm and humid greenhouse, the disease struck suddenly at the early stages of strawberry fruiting. Unlike previous reports, symptoms were a calyx and receptacle blight as well as a leaf spot. This pathogen can cause serious economic losses to strawberry growers. To the best of our knowledge, this is the first report of calyx and receptacle blight by *N. clavispora* on strawberry in China.

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