Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neopestalotiopsis mesopotamica* Causing Root and Crown Rot on Strawberry in Ecuador

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In Ecuador, strawberry production is located in the Andean region with an area of 1,000 ha. Albion is the most popular cultivar due to its conical fruit shape, fruit size, bright red color, and sweetness. Since June 2014, farmers reported a reduction in the production cycle from 24 months to 6 to 8 months and a decreased yield of around 50% due to an unknown soil pathogen. Plant symptoms presented a reddish discoloration on new leaves, coming through the leaf apex to the petiole until turning wholly brown in old leaves, leading to plant death. Additionally, a brown-reddish spot inside the strawberry crown and root rot were reported. In 2020, in Ecuador's most extensive production area, called Yaruqui (Pichincha province), 25 diseased plants were collected for pathogen isolation. The pathogen was isolated on water agar medium from the internal tissue of crowns using 0.5-cm diseased plant fragments, previously disinfected with 2% sodium hypochlorite, and rinsed with sterile water. After 2 days, a single hyphal tip was reisolated on potato dextrose agar (PDA). A total of 18 pure isolates were grown at 25°C for 12 days, and then 3 to 7 days of blacklight treatment was applied to induce sporulation. All the isolates presented a cottony beige mycelium with undulate edges. The conidia were ellipsoidal (20.73 to 29 µm in length and 6.2 to 8.77 µm in width; $n = 60$), multiseptated (four segments) showing hyaline apical (3.8 to 5 µm) and basal (4.87 to 8 µm) cells, and three brown median cells, the second and third were darker than the fourth one, with one basal and two to four apical appendages (26.09 to 38.7 µm). According to colony and conidia morphology, the isolates were identified as *Neopestalotiopsis* sp. (Dung et al. 2016; Essa et al. 2018; Maharachchikumbura et al. 2011). Five isolates were selected randomly for DNA extraction and sequencing of the internal transcribed spacer (ITS) region (ITS4/ITS5), β-tubulin (Bt2b/T1), and translation elongation factor 1-α (TEF-1α) region (EF1-728/EF1-986) (Maharachchikumbura et al. 2014). DNA sequences obtained from each marker were identical for all isolates. Consensus sequences and

alignment were built using ClustalX in MEGA X (Kumar et al. 2018). The consensus sequences were deposited in GenBank with the following accession numbers: ITS, MZ047602; β-tubulin, MZ054301; and TEF-1α, MZ054302. A multilocus Bayesian inference phylogenetic tree was constructed using the concatenated sequences in the Beast software (version 1.8.4) (Drummond et al. 2012; Maharachchikumbura et al. 2014). The isolate in our study clustered with isolates of *Neopestalotiopsis mesopotamica* with a posterior probability of 1, confirming its identity. For Koch's postulates, healthy plants were grown in sterile soil for 4 months. Conidia of the pathogen were suspended in potato dextrose broth (PDB) (1×10^4 conidia/ml), and it was sprayed on 15 healthy plants that previously had their crowns wounded with a sterile needle (0.6 cm deep) at the four cardinal points. The control treatment (15 plants) was wounded and sprayed with PDB alone. The plants were maintained at 25°C and more than 85% relative humidity (Sigillo et al. 2020). Twelve days after inoculation, plants showed reddish discoloration on new leaves, and old leaves presented low-level wilt, rusty color, and necrotic petioles. Forty-one days later, 75% of the treated plants had severe wilt or were dead, showing root and crown rot. Control plants presented no symptoms of the disease. Reisolation of the pathogen from the diseased crown tissues was done on water agar and PDA as previously described. The isolates presented the exact morphology of pure cultures obtained from field-diseased strawberry crowns. The pathogenicity test was performed twice. To our knowledge, this is the first report of *N. mesopotamica* being the causal agent of root and crown rot on strawberries in Ecuador. *N. iranensis* and *N. mesopotamica* have been reported as causal agents of strawberries fruit rot and leaf lesions in Iran (Ayoubi and Soleimani 2016), and *N. clavisporea* was reported to be causing root and crown rot on strawberry plants in Argentina (Obregon et al. 2018). Disease diagnosis contributes to providing strategies against this new disease. Further investigations are needed to find biological/chemical techniques or cultivar resistance to control this pathogen in strawberries in Ecuador.

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