Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neopestalotiopsis rosae* Causing Leaf Blight and Crown Rot on Strawberry in Taiwan

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For the past 30 years, the most predominant strawberry cultivar in Taiwan has been ‘Taoyuan No. 1’, which produces fruit with rich flavor and aroma but is highly susceptible to anthracnose (Chung et al. 2019). Because epidemics of anthracnose became more destructive, farmers switched to anthracnose-tolerant cultivar ‘Xiang-Shui’ (~50 and ~80% of the cultivation area in 2018 and 2019, respectively). Since 2018, severe leaf blight and crown rot symptoms have been observed all year in Xiang-Shui in Miaoli, Nantou, Hsinchu, Taipei, Taoyuan, and Chiayi Counties. The disease became more prevalent and severe during 2019 to 2020 and caused up to 30% plant loss after transplanting. Symptoms appeared as brown necrotic lesions with black acervuli on leaves, slightly sunken dark-brown necrosis on stolons, and sunken reddish-brown necrosis on fruit. The diseased crown tissue showed marbled reddish-brown necrosis with a dark-brown margin, and plants with severe crown rot usually showed reddish-brown discoloration on leaves (the leaves initially turned reddish-brown between the veins and could become entirely scorched at later stages). To isolate the causal agent, small fragments of diseased leaves, crowns, stolons, and fruits were surface disinfested with 0.5% sodium hypochlorite for 30 s, rinsed with sterile water, and then placed on 1.5% water agar. Single hyphal tips extended from tissues were transferred to potato dextrose agar and cultured for 7 days at 25°C under a 12-h/12-h photoperiod. In

total, 20 isolates were obtained from diseased leaves, crowns, stolons, and fruits. Colonies were white with cottony aerial mycelium, irregular margins, and black acervuli distributed in concentric rings. Conidia were fusiform to ellipsoid (five cells), with one basal appendage and three or four (usually three) apical appendages. From colony and conidial morphology, the causal agent was identified as *Neopestalotiopsis* sp. (Maharachchikumbura et al. 2014). The internal transcribed spacer (ITS) region, β -tubulin (*TUB*), and translation elongation factor 1- α (*TEF-1 α*) of three isolates (ML1664 from diseased crown tissue collected in Hsinchu County; ML2147 and ML2411 from diseased leaves collected in Miaoli County) were sequenced (GenBank nos. MT469940 to MT469948). All three isolates clustered with the ex-type strain of *Neopestalotiopsis rosae* in the multilocus (ITS + *TUB* + *TEF-1 α*) phylogenetic tree. To fulfill Koch’s postulates, spore suspensions of ML1664 and ML2147 at 1×10^6 conidia/ml were used to spray inoculate Xiang-Shui seedlings at the three- to four-leaf stage until run-off (two trials, five seedlings per trial). Inoculated plants were put in a plastic bag (>90% relative humidity) at 25°C under a 12-h/12-h photoperiod. After 10 to 14 days, 80% of inoculated plants showed leaf or crown symptoms similar to those in the field. Control plants sprayed with sterile water showed no symptoms (four to five seedlings per trial). The fungi were reisolated from necrotic lesions with 100% frequency ($n \geq 3$ isolates per trial), and morphological characters and ITS sequences were identical to the original ones. This is the first report of *N. rosae* causing leaf blight and crown rot in strawberry in Taiwan. *N. rosae* and *N. clavispora* have been reported as new threats to strawberry in several other countries (Gilardi et al. 2019; Rebollar-Alviter et al. 2020). Clarification of the pathogen provides a basis for developing strategies to control the emerging disease. Further studies are needed to evaluate the resistance/susceptibility of major strawberry cultivars and the fungicide sensitivity of the pathogen.

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