Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Neopestalotiopsis rosae* Causing Foliar and Fruit Spots on Pomegranate in Florida

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Foliar and fruit spots were observed on pomegranate trees (*Punica granatum* L.) in orchards at Balm and Zolfo Springs, FL, in 2019. Symptoms on leaves and fruits included dark brown to black irregular spots, 0.2 to 1.5 cm, with gray centers. Visible pycnidia were in the center of the lesions. Leaves became chlorotic and prematurely dropped. In a survey of 24 pomegranate cultivars, all trees were infected, and disease severity (DS) was 2 to 80%. Cultivars Bhagwa (B) and Mridula (M) were the most susceptible. Symptoms on fruits were similar to those on leaves; however, incidence on fruits was <1%. To isolate the pathogen, 5-mm² pieces of symptomatic leaves and fruit were excised from the area between diseased and healthy tissue. Pieces were surface disinfested in 70% ethanol for 30 s followed by 10% NaOCl for 2 min, rinsed in sterilized distilled water three times, dried on a paper towel, placed onto PDA, and incubated at 25°C under a 12-h photoperiod for 7 days. Cultures formed white cottony aerial mycelium with undulated edges and abundant black pycnidia. Four-septate conidia were fusiform or clavate, straight or slightly curved, measuring 29.1 (23.3 to 34.4) × 6.3 (5.1 to 6.8) µm (*n* = 100). Conidia had three median cells, which were dark brown, with a single basal hyaline appendage, 5.9 (4.1 to 7.7) µm long, and two to four (usually three) apical hyaline appendages, 20.3 (14.8 to 24.5) µm long. Morphology was consistent with *Neopestalotiopsis* (Maharachchikumbura et al. 2014). Single-spore cultures were obtained, and genomic DNA was extracted from seven isolates (one from fruit, GEV3423, and six from leaf, GEV3426 to GEV3431). ITS region and partial sequences of *TEF* and *TUB2* were amplified with the respective primer pairs ITS4/ITS5, EF1-1567/EF1-536, and Bt-2a/Bt-2b (Maharachchikumbura et al. 2014). BLAST analysis of

GEV3523 showed 100% homology for ITS, 100% for *TEF*, and 99.8% for *TUB2* to *Neopestalotiopsis rosae* isolate CBS 101057 from a rose plant in New Zealand (MT587806, MT605118, and MT597152, respectively); the six leaf isolates were 100% identical, showing 100% homology for ITS, 99.8% for *TEF*, and 98.4% for *TUB2* to the same species. Accession numbers: GEV3423 (MT587806, MT605118, MT597152); GEV3426 (MT587805, MT605119, MT597151); GEV3427 (MT587804, MT605120, MT597150); GEV3428 (MT587803, MT605121, MT597149); GEV3429 (MT587802, MT605122, MT597148); GEV3430 (MT587801, MT605123, MT597147); and GEV3431 (MT587800, MT605124, MT597146), for ITS, *TEF*, and *TUB2*. Maximum likelihood phylogenetic analysis, based on the combined alignment of ITS, *TEF*, and *TUB2*, differentiated the leaf isolates from other species, suggesting a new *Neopestalotiopsis* cryptic species. Three-month-old rooted pomegranate cuttings (cvs. B and M) were inoculated with isolates GEV3423 and GEV3427. One half of each cutting was wounded by gently rubbing sterilized sand on the leaves, and the other half remained intact. Four cuttings per cultivar were spray inoculated with a spore suspension (10⁵ conidia/ml) and four control plants with sterilized water. The experiment was performed once per cultivar. Inoculated cuttings were covered with a transparent plastic bag to maintain 100% humidity and incubated for 36 h at 26°C under a 12-h photoperiod in a growth chamber. Leaves showed small, irregular, brown spots with a gray center 3 days after inoculation. The pathogen produced pycnidia in the center of the lesions 1 week after inoculation. Symptoms developed on both wounded and unwounded leaves with 100% incidence on both cultivars inoculated separately with GEV3427 and GEV3423. DS on wounded leaves was 10 to 25% for B and 30 to 60% for M and on intact leaves 2 to 5% and 5 to 12%, respectively. GEV3423 caused average DS of 8 and 3.5% on wounded leaves of B and M, respectively, whereas GEV3427 caused 19 and 45% DS, respectively. No symptoms were observed on control plants, and no fungal growth was observed on the reisolations performed on the control plants. *Neopestalotiopsis* spp. were reisolated from leaves, fulfilling Koch's postulates. This is the first report of *N. rosae* infecting pomegranate in Florida and in the United States. It could represent a threat to pomegranate production in Florida due to its ability to cause premature defoliation.

Reference:

Maharachchikumbura, S. S. N., et al. 2014. Stud. Mycol. 79:121.

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