Diseases Caused by Fungi and Fungus-Like Organisms

Neopestalotiopsis rosae Causing Black Spot on Leaf and Fruit of Pecan (*Carya illinoensis*) in China

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Pecan (*Carya illinoensis* K. Koch) is very productive and brings good economic returns for mountain residents. In recent years, symptoms were observed on both the leaves and fruit of pecan in orchards in Fuyang, Anhui Province, and Jiande, Zhejiang Province. Long fusiform to irregular shaped, 1 to 3 mm rust-colored spots on leaves first appeared between veins. The area around spots gradually changed from green to yellow, making a yellow halo. Spots often merged into a big lesion. On the fruit, symptoms first appeared as irregular black spots. The spots gradually spread to most of the fruit and the core of the fruit turned black at the late stage of pathogenesis. The normal leaf infection rate was <10%, but the infection rate of fruit could reach 5 to 15%, which leads to drastic quality reduction and significant yield losses. To isolate the pathogens, fresh infected tissues were cut from samples, disinfected, and cultured on 2% water agar at 28°C until the colony could be spotted. Fungal isolates were obtained by mycelia tip transfer. Similar cultural and morphological characters showed on 39 of 45 isolates. The mycelia grew rapidly on PDA plates, and colony diameter could reach 8.0 cm after culture at 25°C in the dark for 5 days. Mycelia on the surface of the colony were cottony, had obvious growth rings, and turned yellow from the middle on aged cultures. Pycnidia were brown to black and began to form on PDA after 15 days of culture in the dark. Conidia were fusoid, straight to slightly curved, 4-septate, and 23.5 × 26.3 × 7.6 to 9.0 µm (average 24.9 × 8.3 µm [*n* = 60]). Basal cells were hyaline, conic with a truncate base, thin-walled,

and averaged 4.4 to 5.1 µm long (*n* = 60). The three median cells were doliiform and brown with darker septa. The apical cell was hyaline, cylindrical, with two to three tubular apical appendages arising from the apical crest, unbranched, filiform, and averaged 21.7 to 29.2 µm (*n* = 60) long. The cultural and morphological characteristics of the strains matched previous descriptions of *Neopestalotiopsis* sp. (Maharachchikumbura et al. 2014). Three partial genes, ITS, β-tubulin (TUB), and translation elongation factor 1-α (TEF1-α), were amplified and sequenced with primers (Lee et al. 2006; Maharachchikumbura et al. 2014), and the sequences were submitted to GenBank (accession nos. ITS: MW504791; TEF1-α: MW633956; TUB2: MW633958). BLAST results against the GenBank database showed more than 99% identity with sequences from *Neopestalotiopsis* sp. A multilocus phylogenetic analysis of the three genes showed the isolate was closest to *Neopestalotiopsis rosae*. The strains were finally identified as *N. rosae* by morphological and molecular analysis. Pathogenicity testing was performed by spreading 20 µl of conidial suspension (1 × 10⁶ conidia ml⁻¹) on the surface of wounded or unwounded leaves and fruits of pecan variety MaHan. Wounds were made by scratching with sterile needles. A 20 µl drop of conidial suspension was spread on the wounded or unwounded area. The same size water drops were spread on the surface of wounded and unwounded tissues as controls. Then, plant parts were wrapped with polyethylene film to keep moisture. Ten trees were selected for inoculation, and 10 leaves and fruits were randomly selected separately for wounded and unwounded inoculation and corresponding controls in each tree. Similar symptoms appeared 3 dpi on leaves and 9 dpi on fruits, appearing on >90% of wounded and <50% of unwounded inoculated leaves or fruits. Controls remained symptomless. *N. rosae* was consistently reisolated from inoculated parts with symptoms, but not from the control, confirming *N. rosae* as the pathogen causing black spot on the leaf and fruit of pecan. To the best of our knowledge, this is the first report of *N. rosae* causing black spot disease of pecan. In recent years, both the infection area and severity of this disease have grown rapidly, so the disease may become a big problem in local orchards.

References:

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

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