Diseases Caused by Fungi and Fungus-Like Organisms

First Report on *Neopestalotiopsis aotearoa* of Rubber Tree in China

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Natural rubber is an important industrial raw material and an economically important perennial in China. In recent years, A new leaf fall disease, caused by *Neopestalotiopsis aotearoa* Maharachch., K.D. Hyde & Crous, has occurred in Indonesia, Malaysia, Thailand, Sri Lanka, and other major rubber planting countries. In May and July 2020, this disease was first found on 2-year-old rubber seedlings in two plantations located in Ledong and Baisha counties in Hainan Province, China. In the two plantations of ~32 ha, 15% of the rubber seedlings had the disease and the defoliation was >20%. The infected leaves turned yellow and watery, and dark brown and nearly round lesions 1 to 2 mm in diameter were formed on the leaves. When the humidity was high, the center of the lesion was gray-white, and the lesions had many small black dots, black margins, and yellow halos. When the disease was severe, leaves fell off. To identify the pathogen, leaf tissues were collected from lesion margins after leaf samples were surface sterilized in 75% ethanol, rinsed with sterile water three times, and air dried. The leaf tissues were plated on PDA and incubated at 28°C for 7 days. Fungal cultures with similar morphology were isolated from 90% of tested samples, and two isolates (HNPeHNLD2001 and HNPeHNLD2002) were used in pathogenicity and molecular tests. Rubber leaves (clone PR107) were inoculated with conidial suspension (10⁶ conidia/ml), and ones inoculated with PDA were the control. Each treatment had three leaves, and each leaf was inoculated with three spots and incubated at 28°C under high-moisture conditions. Five days later, leaves inoculated with conidial suspension showed black leaf spots resembling the disease in the field, whereas the control leaves remained symptomless. The fungal cultures isolated from the inoculated

tissues had identical morphology compared with the initial isolates. Colonies on PDA were 55 to 60 mm in diameter after 7 days at 28°C, with undulate edges, pale brown, thick mycelia on the surface with black, gregarious conidiomata; the reverse side was similar in color. Black conidia were produced after 8 days of culture on PDA. Conidia were fusoid, ellipsoid, straight to slightly curved, four-septate, and 18.35 to 27.12 (mean 22.34) × 4.11 to 7.03 (mean 5.41) µm. The basal cells were conic with a truncate base, hyaline, rugose, thin-walled, and 4.35 to 6.33 µm long (mean 4.72 µm). Three median cells were doliform, 12.53 to 18.97 µm long (mean 15.26 µm), hyaline, cylindrical to subcylindrical, thin- and smooth-walled, with two to three tubular apical appendages, arising from the apical crest, unbranched, filiform, 14.7 to 25.3 µm long (mean 19.94 µm). The basal appendages were singular, tubular, unbranched, centric, 3.13 to 7.13 µm long (mean 5.48 µm). Morphological characteristics of the isolates were similar to the descriptions of *N. aotearoa* (Maharachchikumbura et al. 2014). The rDNA ITS region, TEF gene, and TUB2 gene were amplified using the primer pairs ITS1/ITS4, EF1-728F/EF1-986R, and T1/Bt-2b (Pornsuriya et al. 2020), respectively. The sequences of these genes were deposited in GenBank (ITS, MT764947, MT764948; TUB2, MT796262, MT796263; TEF, MT800516, MT800517). According to the latest classification of *Neopestalotiopsis* spp. (Maharachchikumbura et al. 2014) and multilocus phylogeny, isolates HNPeHNLD2001 and HNPeHNLD2002 were clustered in the same branch with *N. aotearoa*. Thus, the pathogen was identified as *N. aotearoa*, which is different from *N. cubana* and *N. formicarum* reported in Thailand (Pornsuriya et al. 2020; Thaochan et al. 2020). *Neopestalotiopsis* leaf spot disease of rubber tree (*Hevea brasiliensis*) was one of the most serious and destructive leaf diseases in major rubber planting countries in Asia (Tajuddin et al. 2019). The present study of leaf fall disease on rubber tree caused by *N. aotearoa* is the first report in China. The finding provides the basic pathogen information for further monitoring the disease and its control.

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