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NEW DISEASE REPORT

First report of Neopestalotiopsis australis causing fruit rot on Byrsonima crassifolia in, Mexico

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KEYWORDS

fungal plant disease, nance

In May 2021, fruit rot was observed in nance (*Byrsonima crassifolia*) in Las Choapas, Veracruz, Mexico (17°40'53.32"N, 94°00'08.64"W). About 150 landscape trees were surveyed and the disease incidence was 50 to 70%. Symptoms appeared as irregular, brown lesions on the exocarp with a light-brown margin. Black acervuli were observed on the surface (Figure 1).

Pieces of diseased fruits (5 mm^2) were surface disinfected with 1.5% sodium hypochlorite for two minutes, rinsed three times with sterilised distilled water for one minute, then dried on sterilised paper towels, plated onto potato dextrose agar (PDA) and incubated at 22°C with a 12 hour photoperiod for three days. Mycelial plugs from the edge of fungal hyphae developing from the tissues were transferred aseptically to fresh PDA and incubated for seven days under the same conditions. Monoconidial cultures of homogenous isolates of *Neopestalotiopsis* were obtained using the single-spore method. Fungal colonies presented a dense aerial mycelium with black conidiomata exuding black conidia in a slimy, globose mass (Figure 2). Conidia were fusiform to ellipsoid, 4-septate, 21–22.5 × 7.2–7.6 μm (n = 50); basal cell conic with a truncate base, hyaline (10.5–3 μm); three median cells doliiform (14.3–15 μm), versicoloured; apical cell hyaline, subcylindrical to obconic (2–3.5 μm), with 3 tubular apical appendages, unbranched, filiform (20–22 μm); basal appendage single, tubular, unbranched (2.5–4.7 μm). The isolates were identified as *Neopestalotiopsis* sp. (Maharachchikumbura et al., 2014).

The internal transcribed spacer (ITS) region, β-tubulin (TUB), and translation elongation factor 1-alpha (TEF-1α) of two representative isolates (CPO 27.130, CPO 27.136) were amplified (Lee et al., 2004; Crous et al., 2013), sequenced and deposited in GenBank (Accession Nos. ITS: ON312539, ON312570; β-tubulin: ON316845, ON316846; TEF-1α: ON316847, ON316848). They had 98.29% nucleotide identity with ITS (KM199348.1), 99.87% identity with TUB (KM199432.1) and 96.25% identity with TEF (KM199537.1) sequences of the type isolate (CBS 114159). In addition, the isolates clustered with the ex-type strain of *Neopestalotiopsis australis* (CBS 114159) in a multilocus (ITS+TUB+TEF-1α) phylogenetic analysis (Figure 3).

To fulfill Koch's postulates, each *Neopestalotiopsis* isolate (CPO 27.130 and CPO 27.136) was inoculated on fifty detached unripe nance fruits. The detached fruits were rinsed with tap water, surface disinfected with 1% NaOCl solution for two minutes and rinsed twice with sterile distilled water. The fruits were placed in lines of ten fruits on sterilised paper in clean boxes. The fruits were wounded at a single site at the equatorial zone, the wound being made by removing a piece of fruit epidermis (1.5 mm). A mycelial plug (5 mm diameter) was removed from the margin of 5-day-old colonies growing on PDA at 22°C and was placed on the wounded site of each fruit. One nance fruit per line was inoculated with a sterile PDA plug and served as a control. Boxes were incubated at 22 ±2°C with a 12-hour photoperiod and 80% relative humidity. The inoculated fruits were monitored daily for

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FIGURE 1 Symptoms of fruit rot on naturally infected nance fruit: (a–b) lesions on 50-day-old fruits, (c) acervuli in the lesions, and (d) conidia

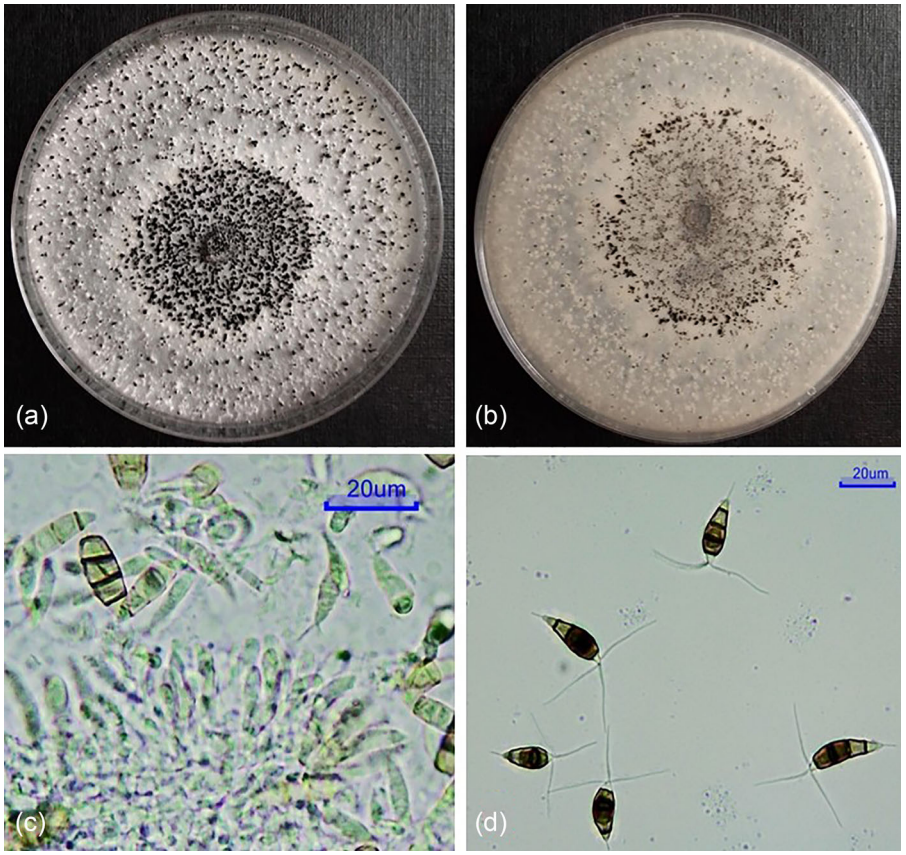


FIGURE 2 Morphological and cultural characteristics of *Neopestalotiopsis australis* isolated from naturally infected nance fruit: (a–b) 12-day-old culture on potato dextrose agar (a, upper surface and b, reverse view), (c) conidiogenous cells giving rise to conidia, and (d) conidia

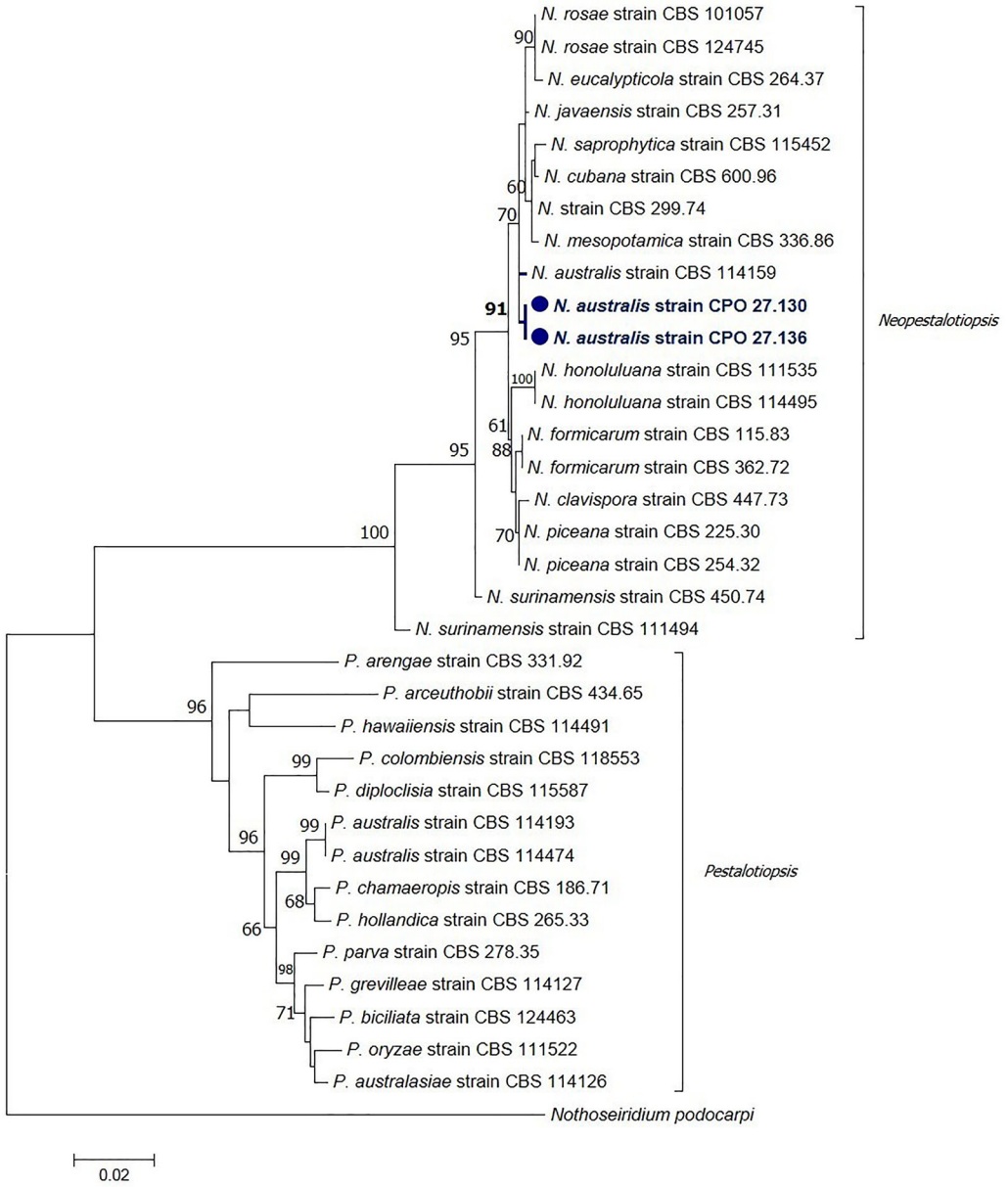


FIGURE 3 Molecular phylogenetic using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-4784.45) is shown. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 35 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1193 positions in the final dataset

development of symptoms. Eleven days after inoculation with either isolate, all the inoculated fruits developed symptoms; control fruits remained healthy (Figure 4). The frequency of fungal re-isolation was 96%, and the colony characteristics and morphometric measurements were similar to the inoculated isolates. Pathogenicity testing was performed twice with consistent results on each occasion.

To our knowledge, this is the first report of *N. australis* as the causal agent of fruit rot on nance in Mexico. To date, the disease remains confined to this location due to the implementation of management measures including pruning of dead branches, removal of affected fruits and application of copper-based fungicides.

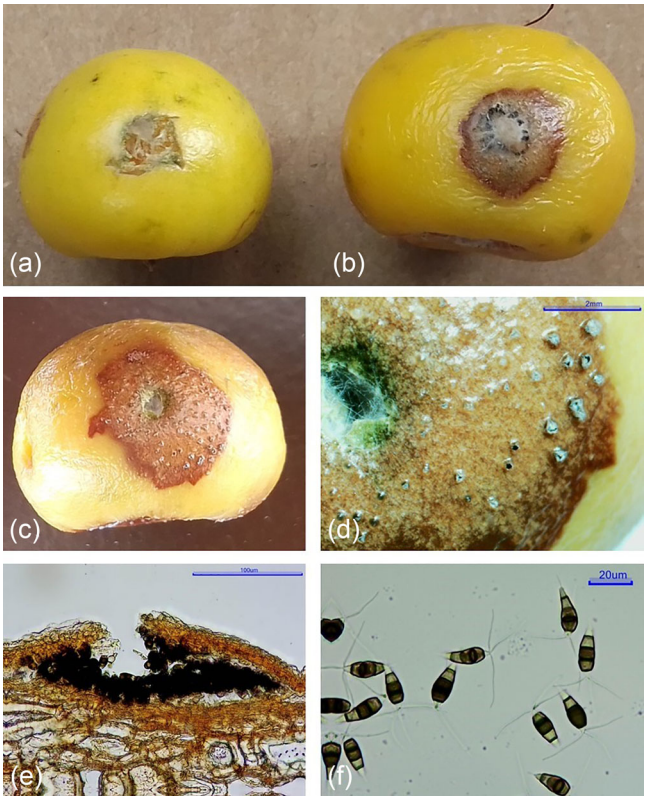


FIGURE 4 Results of pathogenicity test of *Neopestalotiopsis australis* on nance fruit: wounded control (a, c) and inoculated fruit (b, d), eight and eleven days after inoculation, respectively; (e) acervulus and (f) conidia on inoculated fruit

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