First Report of *Neopestalotiopsis clavispora* Causing Leaf Spot on Macadamia in Brazil

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During March of 2015, irregular light brown spots on leaves of macadamia (*Macadamia integrifolia*) were observed in Vitória da Conquista, Bahia, Brazil. The disease severity was estimated at around 25%. Initial disease symptoms were characterized by small spots measuring 6 to 12 mm, which enlarged and coalesced covering an extensive leaf area. Lesions showed abundant acervuli on the adaxial surface. From diseased leaves, direct isolations were performed by picking up conidia from acervuli and placing them on potato dextrose agar (PDA). Cultures were incubated at 25°C with a 12 h photoperiod for 10 days and four single spore isolates were obtained. Isolates showed similar morphological characteristics and the representative isolate MAC-01 was further investigated. Fourteen-day-old colonies grown on PDA were white with cottony aerial mycelium and abundant black globular acervuli. Conidia were clavate to fusiform, 4-septate, straight or slightly curved and measured 17.9 - 27.5 μm long × 5.1 - 6.8 μm wide ($n = 100$). The three median cells were dark brown, while the basal and apical cells were hyaline. Conidia had a single basal appendage (3.5 to 7.4 µm long; *n* = 100) and 2 to 3 apical appendages (16.3 to 29.3 µm long; *n* = 100). Morphological features were consistent with those of *Neopestalotiopsis clavispora* reported by Maharachchikumbura et al. (2014). To confirm species identification, the internal transcribed spacer region (ITS), a partial sequence of the β-tubulin gene (TUB2) and a partial sequence of translation elongation factor 1-alpha gene (*tef1-α*) were PCR-amplified and sequenced (Maharachchikumbura et al. 2014). The resulting sequences were deposited in GenBank under accession numbers KX721071 - KX721073. BLAST searches showed 98-100% identity with the existing sequences of *N. clavispora* deposited in GenBank (Accession Nos. ITS: JX398978; TUB2: JX399013 and; *tef1-α*: JX399044). Phylogenetic Bayesian inference analysis from a combined ITS, TUB2 and *tef1-α* sequence alignment showed that the examined isolate belonged to the *N. clavispora* species. The *N. clavispora* clade was well supported with a Bayesian posterior probability value of 1. To confirm pathogenicity, detached healthy leaves of macadamia were superficially disinfected with 1% sodium hypochlorite solution for 2 min, washed with sterile water, air-dried at room temperature, placed in Petri dishes containing sterile filter paper moistened with sterile water, and then inoculated with the pathogen. For that, 5 mm mycelial plugs were excised from a 10-day-old colony grown on PDA and placed on the adaxial surface of 25 leaves. Plates were incubated at 25°C with a 12 h photoperiod for 10 days. As a control treatment, five additional detached leaves were mock inoculated with a PDA plug. The experiment was performed twice. Two days after inoculation, all inoculated leaves showed irregular light brown spots, measuring up to 8 mm, similar to those observed in the affected orchard. As the disease progressed, spots enlarged and acervuli were formed. Control leaves were asymptomatic. *N. clavispora* was re-isolated from the symptomatic tissues and identified as previously described, thus satisfying Koch's postulates. *N. clavispora* has been reported as the causal agent of leaf spot on *Carya illinoensis* (Lazarotto et al. 2012) and *Mangifera indica* (Ismail et al. 2013). To our knowledge, this is the first report of *N. clavispora* causing leaf spot on macadamia in Brazil. Further studies are necessary to assess the geographic distribution and the importance of the disease in Brazilian macadamia orchards.

References:

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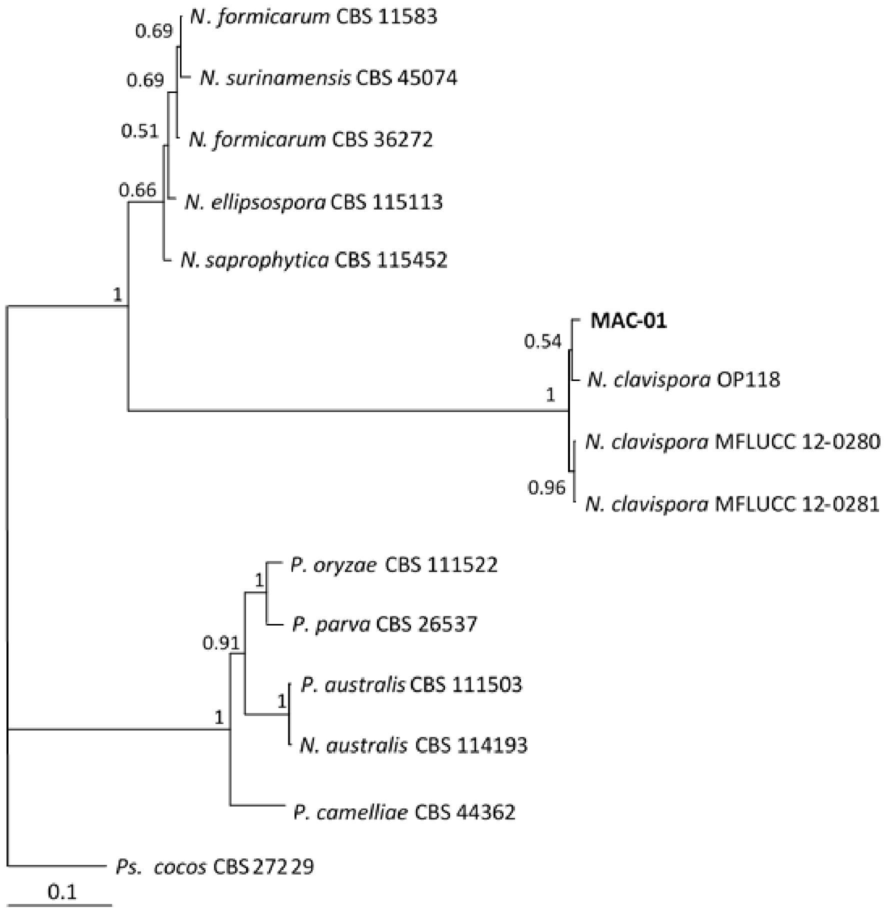

Supplementary Figure S1. Phylogenetic tree derived from a Bayesian analysis of an alignment based on ITS, TUB2 and tef1-ɑ sequences of Neopestalotiopsis, Pestalotiopsis and Pseudopestalotiopsis isolates. The numbers on the nodes are Bayesian posterior probability values. Isolate in bold was obtained in the present study.

163x154mm (96 x 96 DPI)