



# First report of wilt and necrosis caused by *Diplodia seriata* on cowpea in Tennessee, USA

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Cowpea (*Vigna unguiculata*) is a productive vegetable, grain, forage or cover crop legume that is particularly adapted to subtropical and tropical climates where stresses such as drought, high temperature and marginal soil conditions are common. In 2014, several genotypes of surface-sterilised cowpea seeds were grown in a greenhouse at the University of Tennessee, Knoxville and after eight weeks, plants showed signs of wilting and necrosis of stems and leaves in concurrence with dark fungal growth (Fig. 1). Concurrently, seeds from the same lots (grown in Jackson, TN) were surface sterilised for one minute with 100 ml/l bleach (active ingredient 6% NaOCl), rinsed with sterile water, and plated onto water agar (WA). Cowpea seed produced white-to-grey mycelia that darkened upon maturation and were visually similar to the fungus observed in the greenhouse trial (Fig. 2). The fungus was isolated on WA, transferred to potato dextrose agar, and incubated until sporulation (Fig. 3).

Morphological characteristics were compared to descriptions of *Diplodia seriata* (Phillips *et al.*, 2007). Conidia were  $26.5 \times 11.9 \mu\text{m}$  ( $n = 100$ ), hyaline, tinted to dark brown, and oval to ellipsoidal. Most conidia were aseptate, a few had one septum, which has been reported for this species (Phillips *et al.*, 2013). Conidia were borne singly on conidiophores inside dark brown, globose, ostiolate pycnidia. Perithecia had asci that contained eight hyaline, aseptate, fusoid ascospores (Fig. 4). Molecular identification was performed with genomic DNA (Extract-N-Amp™ Kit, Sigma-Aldrich, USA) of fungal mycelia, PCR amplification of internal transcribed spacer (ITS) regions with primers ITS1 and ITS4 (White *et al.*, 1990), followed by amplicon sequencing. Sequences obtained shared 100% identity with several accessions of *D. seriata* (e.g. GenBank Accession Nos. MH675476.1, KY385656.1 and HQ660463.1). The amplicon sequence was deposited in GenBank (MK257126).

For initial pathogenicity assays, actively growing mycelial plugs from WA were transferred onto cowpea leaves on moist filter paper in lidded Petri dishes. Symptoms on leaves of cowpea cv. 'Early Acre' included yellowing, and pinpoint dark spots that expanded to lesions with a light brown centre and dark brown, irregular-shaped margins (Fig. 5). To fulfil Koch's postulates, the fungus was produced on oat inoculum and a conidial suspension was prepared from 300 g inoculum in 200 ml sterile water. Leaves and stems of three-week-old plants of cowpea cv. 'Early Acre' ( $n = 10$ ) were sprayed with the conidial suspension. Inoculated and control plants ( $n = 10$ , sprayed with sterile water) were individually covered with

clear plastic bags for four days, after which they were uncovered and incubated in a growth chamber for three weeks at 25°C. Leaf discoloration and wilting were evident in all inoculated plants in the first trial after one week, and in eight of ten plants in the second trial. Fungal samples from infected leaves were re-isolated and morphological and molecular identification were carried out as described above. The re-isolated fungus had 100% identity to *D. seriata*.

*Diplodia seriata* infects a wide range of woody plant species with varying severity (Díaz *et al.*, 2019; Elena *et al.*, 2015; Phillips *et al.*, 2013) and in the case of cowpea, the pathogen can affect both leaf and stem tissue causing wilt, discoloration, and significant leaf loss. To our knowledge, this is the first report of infection of cowpea by *D. seriata*.

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Figure 1

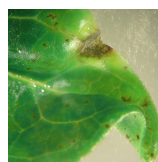


Figure 2

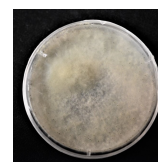


Figure 3

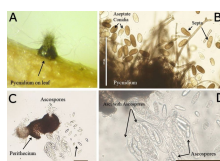


Figure 4

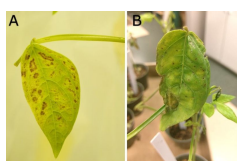


Figure 5

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