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Aphelenchoides besseyi causing leaf spot on cowpea under field conditions in Brazil

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Abstract
Aphelenchoides besseyi was detected as the causal agent of foliar dark spots on cowpea plants growing in the field, and its pathogenicity was confirmed. The identity of the nematode was determined through morphological, morphometric, and molecular characterization, using species-specific primers Abess_11F/Abess_11R targeting 28S rDNA, which amplified a fragment of 570 bp. This is the first report of *A. besseyi* parasitizing cowpea under field conditions in Brazil.

Keywords Fabaceae · Foliar nematode · Identification · *Vigna unguiculata*

Aphelenchoides species are ectoparasites that infest various plant organs, including leaves and stems (Hunt et al. 2018). *Aphelenchoides besseyi* Christie is widely distributed and typically found in rice-growing regions worldwide. It can survive desiccation for several years (Hunt et al. 2018). In Northeast and North Brazil, cowpea (*Vigna unguiculata* [L.] Walp) is an important legume crop used as a source of protein for human consumption, both as a dry and green grain. During field inspections in May 2021 and June 2022, leaf samples of cowpea cultivars BRS Guariba and Corujinha exhibiting dark brown lesions, known as false angular spots (Fig. 1), were collected from two areas located within the municipality of Rio Largo, state of Alagoas, North-eastern Brazil. Approximately 20–50% of the plants were affected. Symptomatic tissue samples were transferred to Petri dishes containing distilled water for 24 h, after which an aliquot of the water suspension was examined under an inverted light microscope, revealing the presence of nematodes. A pathogenicity test was then performed by inoculating 10 healthy leaves of *V. unguiculata* (cultivars BRS Guariba and Corujinha) with infected leaf discs placed on the abaxial side of leaves (2 discs/leaf) and secured with adhesive tape. Inoculated leaves were immediately placed in plastic bags for 72 h to maintain high humidity and kept under greenhouse conditions with an average temperature of 27.05 ± 4 ºC and relative humidity of 81.9 ± 10.5%.

The nematodes were identified to the species level based on morphological and morphometric characteristics, following descriptions published by OEPP/EPPO (2017) and Subbotin et al. (2020). Morphometric measurements were taken from 20 individual female nematodes extracted from cowpea leaves using a light microscope. The following parameters were recorded: total body length (L), distance from the vulva to the anterior end (V), total body length/maximum body width (a), total body length/length of the tail (c), tail length/body width (c'), length of the post-uterine sac (PUS), vulva-anus distance (V-A), and stylet length (St). To confirm the morphological diagnosis, a molecular analysis was conducted using the species-specific primer pair Abess_11F/Abess_11R, which targets the 28S rDNA of *Aphelenchoides besseyi* (Sercero 2020). An amplicon of 570 bp, consistent with *A. besseyi*, was obtained, confirming the species identification.

Morphological observations showed slender females with L = 709.06 µm, V = 69.51%, a = 47.86 µm, c = 18.97 µm, c' = 4.56 µm, St = 11.22 µm, V-A = 146 µm, and PUS = 40 µm.

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Fig. 1 Leaf spots caused by *Aphelenchoides besseyi* in cowpea cv. BRS Guariba under field conditions in Brazil

In addition, the relative extent of the PUS was determined, because Subbotin et al. (2020) found it to be a diagnostic morphometric character for *A. besseyi*. In our population, the length of the PUS was 27.3% of the V-A distance, agreeing with the figure regarded as diagnostic for *A. besseyi*. The lateral field was composed of four notches and the nematodes had a conoid tail with star-shaped mucro. Similar symptoms to those observed under field conditions were seen three days after inoculation, i.e. foliar dark spots. In addition, specimens of the nematode were recovered from the spots, confirming their pathogenicity to the host. Control plants remained symptomless.

In Brazil, *A. besseyi* has been reported in economically important crops such as rice (*Oryza sativa* L.), causing white tip disease (Bebendo and Prabhu, 2016); in soybean [*Glycine max* (L.) Merrill], causing soybean green stem and the foliar retention syndrome (Meyer et al. 2017); in cotton (*Gossypium hirsutum* L.), causing stunting, loss of floral buds, foliage distortion and thickened nodes (Favoreto et al. 2018); and in yam (*Dioscorea cayenensis*), causing leaf spot (Noronha et al. 2020) under field conditions. Moreover, common bean (*Phaseolus vulgaris* L.) and cowpea were experimentally infected with this nematode (Favoreto et al. 2021, 2022), but to our knowledge this is the first report of *A. besseyi* parasitizing leaves of cowpea under field conditions in Brazil.

Declaration

Conflicts of interest The authors declare no conflict of interest.

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