Plant Disease • 2022 • 106:1555-1557 • https://doi.org/10.1094/PDIS-06-21-1232-SC

Short Communication

Aphelenchoides besseyi Parasitizing Cowpea (*Vigna unguiculata*) in Brazil

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Abstract

Several species of nematodes are known to cause losses to cowpea (*Vigna unguiculata*) throughout the world. In Brazil, *Aphelenchoides besseyi* was described as causing damage on soybean, cotton, and common bean, but no report was found about the parasitism of this nematode in cowpea. This study aimed to verify the host reaction of cowpea cultivars to *A. besseyi*. The experiment was conducted under greenhouse conditions, using two *A. besseyi* populations as inocula, obtained from symptomatic soybean and cotton plants collected in naturally infested fields. Cultivars Imponente, Aracê, Guariba, Tumucumaque, Nova Era, and Tracuateua were inoculated with 500 *A. besseyi* of each population, separately, into soil, and after 30 days from the inoculation nematodes were extracted from shoot systems. Both populations were able to parasitize all the cowpea cultivars. Independently of the cultivar, cowpea plants exhibited symptoms of leaf deformation similar to those described for soybean, cotton, and common bean and, in addition, severe brooming was observed, with the interior of the stems being porous and necrotic. To our knowledge, this is the first report of parasitism by *A. besseyi* of cowpea in Brazil, under greenhouse conditions, increasing the list of hosts of this nematode.

Keywords: disease symptoms, Fabaceae, foliar nematode, grain legume, green stem and foliar retention

The Brazilian cowpea (*Vigna unguiculata*) production area was 1.32 million hectares in 2020/2021, and the main producer regions are the Northeast and North, with 85.7% of the cropped area, and the Midwest with 13% (Companhia Nacional de Abastecimento 2021). Some of these areas are sowed after soybean or in crop rotation with cotton.

Several species of nematodes are known to cause losses to cowpea throughout the world (Jain 1983; Ponte 1987), but there is no report of *Aphelenchoides besseyi* parasitizing this plant. In Brazil, this nematode was described causing damage to soybean (*Glycine max*) and cotton (*Gossypium hirsutum*), under field conditions, and on common bean (*Phaseolus vulgaris*), under greenhouse conditions, with severe deformation of leaves and pods and extensive abortion of flowers (Favoreto et al. 2018, 2021; Meyer et al. 2017). In soybean, yield losses of 60% have been attributed to *A. besseyi* in Brazil and symptoms associated are referred as "soybean green stem and foliar retention syndrome" (Meyer et al. 2017); in cotton, yield losses can reach 85% (Favoreto et al. 2018).

Management of *A. besseyi* in infested soybean or cotton fields is difficult because the main soybean cultivars are susceptible to this nematode, and no information is available regarding the susceptibility of cotton cultivars (Favoreto and Meyer 2018). Maize (*Zea mays*), pearl millet (*Pennisetum glaucum*) cultivar ADR 300, sorghum (*Sorghum bicolor*) cultivars BRS 659 and ADV 2499, tropical grasses *Urochloa ruziziensis* and *U. brizantha*, and tropical legumes *Crotalaria juncea*, *C. spectabilis*, and *C. ochroleuca* were all reported as poor hosts of one population of *A. besseyi* from soybean plants, and could be used in crop rotation management schemes (Favoreto and Meyer 2018).

Because of the potential economic importance of *A. besseyi* for Brazilian agriculture, studies have been conducted aiming to verify the host reaction of several plant species to this nematode; results showed that cowpea hosts *A. besseyi*. One experiment was conducted under greenhouse conditions, using as inoculum two *A. besseyi* populations, obtained from symptomatic soybean and cotton plants collected in naturally infested fields. Both populations were identified through morphological (Subbotin et al. 2021) characteristics and through a molecular approach using the species-specific primer for *A. besseyi* located in the 28S rRNA (Sercero 2020), resulting in a fragment of 570 bp from *A. besseyi*.

Subsequently, both populations were multiplied on cultures of *Fusarium* sp. on potato dextrose agar (Merck Millipore Corporation, Darmstadt, Germany) in Petri dishes (Favoreto et al. 2011) and maintained in a Bio-Oxygen Demand Incubator for 30 days at 25°C (±1°C), in the dark. Inoculum was prepared by washing the condensed water droplets containing the nematodes with distilled water, as proposed by Meyer et al. (2017), and specimens were quantified on a Peters slide under a light microscope.

Four cowpea seeds (cultivars Imponente, Aracê, Guariba, Tumucumaque, Nova Era, and Tracuateua) were sown in 3.5-liter plastic pots, containing a mixture of soil and sand (2:1) previously autoclaved (120°C/1 h). Seedlings were thinned to one per pot after germination. Seedlings were inoculated 15 days after sowing by pipetting a suspension containing 500 *A. besseyi* of each population in the soil in one small, 2- to 4-cm–deep hole next to the seedling root system. The plants were maintained under greenhouse conditions with 89% of relative humidity throughout the experimental period (Favoreto et al. 2021). The experiment was arranged in a completed randomized block design, with six replicates per cultivar/nematode population.

Thirty days after inoculation (DAI), shoot systems were cut just below the first node, then weighed and processed according to the

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Funding: This work was supported by the Conselho Nacional de Desenvolvimiento Cientifico e Technológico under grant no. 308814/2018-5.

The author(s) declare no conflict of interest.

Accepted for publication 23 December 2021.

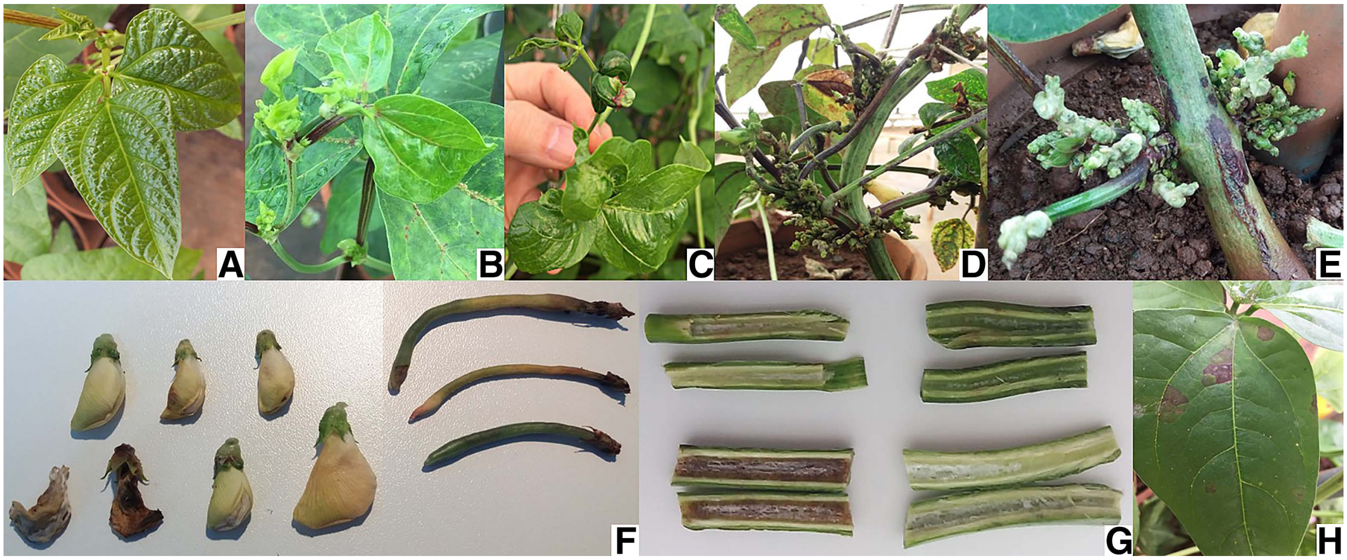

Fig. 1. Symptoms caused by the parasitism of *Aphelenchoides besseyi* in cowpea plants. **A,** Initial symptoms of leathery leaves with deformations in the leaf surface, blistering, and vein thickening. **B,** Bunchy leaves, and enlargement of nodes. **C,** Leaf deformation. **D and E,** Brooming. **F,** Aborted flowers and necrotic, brown to reddish pods. **G,** Stems with porous and necrotic interior. **H,** Angular reddish-brown foliar lesions.

method described by Coolen and D'Herde (1972). The nematodes recovered from each plant were quantified using a Peters slide under a light microscope.

Independently of the cultivar, cowpea plants initially exhibited leathery leaves with deformations in the leaf surface, such as blistering and vein thickening (Fig. 1A). Later, we observed bunchy leaves (Fig. 1B), enlargement of nodes (Fig. 1B to E), and severe brooming (Fig. 1D and E). At flowering stage, an intense abortion of flowers was observed, with few and necrotic, brown to reddish pods (Fig. 1F). In the evaluation made at 30 DAI, the interior of the stems was porous and necrotic (Fig. 1G) and angular reddish-brown foliar lesions were also observed on older leaves (Fig. 1H).

Both populations were able to parasitize all the cowpea cultivars evaluated, and the number of nematodes extracted from shoot tissues at 30 DAI varied from 57.3 (cultivar Nova Era inoculated with cotton population) to 390.0 (cultivar Aracê inoculated with soybean population; Fig. 2).

A. besseyi is known as an important pathogen for rice, causing the white tip disease (Adamo 1976). However, this nematode has been reported causing leaf deformations, enlargement of nodes, and abortion of flowers on economically important crops in Brazil such as soybean and cotton under field conditions, and on common bean under greenhouse conditions (Favoreto et al. 2018, 2021; Meyer et al. 2017). This is a concern for Brazilian growers and researchers, because little information is available regarding the biochemical and molecular aspects of the interaction between *A. besseyi* and these novel hosts, making it difficult to develop the management tools necessary to reduce the damages associated with this nematode.

In addition, the list of new hosts for *A. besseyi* in Brazil seems to be gradually increasing. To our knowledge, this is the first report, under greenhouse conditions, of the pathogenicity of *A. besseyi* in cowpea in Brazil and worldwide. Shoot symptoms here observed under greenhouse conditions are remarkably similar to those observed in soybean, cotton, and especially common bean, starting as leaf blistering and vein thickening, evolving to an intense abortion of flowers that leads to a few numbers of pods, and some angular leaf lesions, as reported in common bean in Costa Rica and Brazil (Chaves et al. 2013; Favoreto et al. 2021). In cowpea, we also observed a severe brooming that was not observed in common bean (Favoreto et al. 2021) and was less intense in soybean and cotton (Favoreto et al. 2018; Meyer et al. 2017).

Symptoms of soybean green stem and foliar retention syndrome are observed especially in the Brazilian Northern region, including the north of the State of Mato Grosso, causing yield

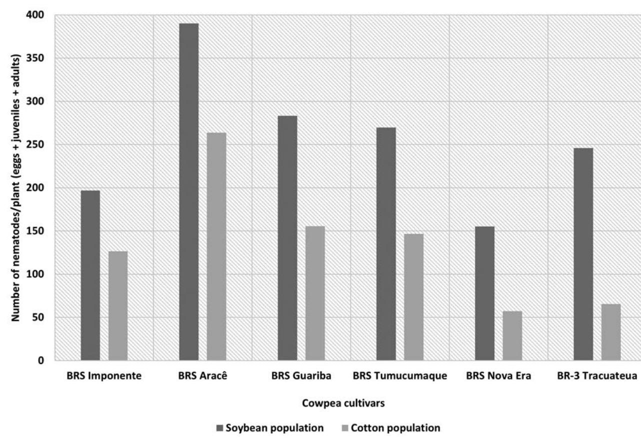

Fig. 2. Number of *Aphelenchoides besseyi* extracted from shoot parts of cowpea cultivars.

losses of up to 100% (Meyer et al. 2017). Cowpea is cropped in some of these regions (Companhia Nacional de Abastecimento 2021), especially Mato Grosso, and the presence of *A. besseyi* causing damage on soybean in these localities increases the risk of cowpea production also being affected by this nematode. This first report of its pathogenicity to cowpea in Brazil is important because it presents the plasticity of *A. besseyi* in parasitizing new hosts and highlights the importance of including this nematode in the nematode management program throughout the different crop seasons in Brazil.

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