New Disease Reports

First report of the root-knot nematode *Meloidogyne enterolobii* parasitising sweet pepper (*Capsicum annuum*) in Niger

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Sweet pepper (*Capsicum annuum*) is one of the most valuable export commodities in Niger. In 2013, during disease surveillance activities on sweet pepper crops, a severe infestation of root-knot nematodes was found in the main growing area, the district of Diffa (Latitude: 13° 18' 55.30" N: Longitude 12° 36' 40.86" E). Above-ground symptoms were stunted growth, yellowing leaves, chlorosis and even plant death (Fig. 1). These symptoms were accompanied by numerous root galls (Fig. 2).

Egg-laying female nematodes were dissected from galled roots following staining with acid fuchsin. The posterior region of mature females was cut and cleared in a solution of lactic acid to remove remaining tissues. A total of 40 perineal patterns were mounted in glycerine and observed using a DMI2000 compound microscope. Males and freshly hatched second stage juveniles (J2) were fixed in 4% hot (60-80°C) formaldehyde and processed for slide mounting before undergoing morphological and morphometric study. The perineal patterns of females were round to ovoid, dorsal arch rounded, striae fine widely spaced and mostly lacking obvious lateral field or with single line and ventral arches (Fig. 3 A-D). There was a wide variation in patterns observed, as reported by Filho *et al*. (2016). The males (Fig. 3E) (n = 20) had body lengths ranging from 1331 to 1802 µm (1612.8 ±275) and the distance of the dorsal pharyngeal gland orifice ranged from 3.4 to 4.7 µm (4.5 ±2). The second stage juveniles (Fig. 3F) (n = 20) had body lengths ranging from 395 to 410 µm (402.4 ±18.7) and the tail length ranged from 50 to 56 µm (55.6 ±5.2). These observations and morphological measurements in general conformed to those described for *Meloidogyne enterolobii* (Yang & Eisenback, 1983; Karssen *et al*., 2012).

A PCR test based on the ribosomal intergenic spacer region is recommended for molecular identification to species (EPPO, 2016). To provide support for the identification, the primer pair C2F3/1108 (Powers & Harris, 1983) was used to amplify the COII/16S rRNA gene of the mitochondrial DNA of populations from the same field sample. Genomic DNA of previously identified *M. arenaria*, *M. incognita* and *M. javanica* cultures from the University of Bonn, Germany were used in PCR for comparison. An amplicon of c. 700 bp specific for *M. enterolobii* was produced with nematodes isolated from galled roots compared to amplicons of 1.1 kb from a sample of *M. arenaria*, and 1.7 kb from samples of *M. incognita* and *M. javanica* (Fig. 4) (Long *et al*., 2014). The sequence described in this study has been deposited in GenBank (Accession no. MF927970). A BLAST search indicated that the sequence had 99% identity with isolates of *M. enterolobii* from Kenya (KX214350).

In vitro testing of the pathogenicity of *M. enterolobii* to sweet pepper was done in a growth chamber using soil autoclaved at 120°C for three hours. Plants which were inoculated with infective juveniles of *M. enterolobii* sourced from the field population developed typical galled root symptoms (Fig. 5A) as observed in the field. DNA from juveniles extracted from the infested pots was tested by PCR as described previously and all produced amplicons of 700 bp. In comparison, no disease symptoms were observed in the control plants (Fig. 5B). To our knowledge, this is the first report of *M. enterolobii* in Niger.

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Figure 1

Figure 2

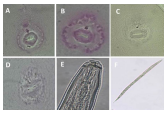

Figure 3

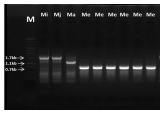

Figure 4

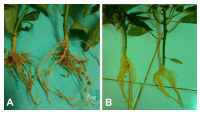

Figure 5

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