BRILL

Nematology 24 (2022) 695-705

Nematology
brill.com/nemy

The differential impact of four tropical species of root-knot nematodes (*Meloidogyne* spp.) on biofortified cassava

Aminat K. OYETUNDE [1,2], Steve O. AFOLAMI [3], Peter KULAKOW [2] and Danny COYNE [4,5,*]

[1] *Department of Biological Sciences, Faculty of Science, Augustine University, P.M.B. 1010, Ilara-Epe 106101, Lagos State, Nigeria*
[2] *International Institute of Tropical Agriculture (IITA), Head Quarters and West Africa Hub, P.M.B. 5320, Oyo Road, Ibadan 200001, Oyo State, Nigeria*
[3] *Department of Crop Protection, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Ogun State, Nigeria*
[4] *IITA, Kasarani, P.O. Box 30772-00100, Nairobi, Kenya*
[5] *Nematology Research Unit, Department of Biology, Ghent University, 9000 Gent, Belgium*
ORCID iDs: Oyetunde: 0000-0002-6522-2347; Coyne: 0000-0002-2030-6328

Received: 2 November 2021; revised: 8 March 2022
Accepted for publication: 9 March 2022; published online: 10 May 2022

Summary – Cassava plays an important food security role in Africa. Although a hardy crop in general, average yields are low, while traditional cultivars tend to be low in nutrients and vitamins. Substantial efforts have therefore been made to improve the nutritional quality of cassava through the development of biofortified cultivars. Although root-knot nematodes (RKN) are among the various important constraints affecting production, details on the impact of different species of RKN on cassava productivity are scarce. In this study, six popular cultivars of biofortified cassava were evaluated for their response to *M. arenaria*, *M. enterolobii*, *M. incognita*, *M. javanica* and a combination of all four species, in pots. All tested cultivars were susceptible to the four *Meloidogyne* species, but some cultivars showed a tolerance to *M. arenaria* infection. Galling damage was observed on feeder roots of inoculated plants, with nematode reproduction factors ranging between 2.3 and 9.5. Plant height, stem girth and fresh plant mass were significantly lower for most cultivars by as much as 70% following RKN infection. The highest root galling and damage were observed in plants following inoculation with a combination of the four species. As individual species inoculations, *M. incognita* and *M. javanica* were the most damaging, with the least damage observed in plants inoculated with *M. arenaria* only. These results confirm the pathogenicity of *M. arenaria*, *M. incognita* and *M. javanica* and further illustrate the potential of *M. enterolobii* to impact cassava production, while combined species infections demonstrate the greater levels of damage that these may cause.

Keywords – food security, *Meloidogyne arenaria*, *Meloidogyne enterolobii*, *Meloidogyne incognita*, *Meloidogyne javanica*, nutritional insecurity.

Cassava is an important crop grown across sub-Saharan Africa, which is well adapted to diverse soil and environmental conditions, as well as to complex traditional farming systems. It is a major source of energy for over 600 million people on the continent (Afuape, 2009), even though it is generally low in nutritional value (HarvestPlus, 2014). Cassava is grown principally for its swollen storage roots, while cassava leaves are also consumed in some areas, particularly in parts of Africa (Dahniya, 1983). Cassava leaves are an important vegetable in Congo, Sierra Leone and Tanzania (Okigbo, 1980) and have a nutritive value similar to other dark green leaves and are an extremely valuable source of vitamins A (carotene) and C, iron, calcium and protein (Latham, 1979). The consumption of cassava leaves helps many Africans compensate for the lack of protein and some vitamins and minerals in the roots. In some Africa countries, farmers plant tree cassava – *mpiru* – for the production of leaves and stems. Producers earn additional income by selling cassava leaves and stems. However, while cassava is renowned for its ability to thrive under marginal conditions and for its resilience, in Africa yields tend to be poor and way below potential (FAO, 2018). Numerous reasons underlie this, such as losses to pests and

* Corresponding author, e-mail: D.Coyne@cgiar.org

DOI 10.1163/15685411-bja10161

diseases. Although not well known, the damage caused by root-knot nematodes (RKN; *Meloidogyne* spp.) to cassava is becoming increasingly recognised (Coyne & Affokpon, 2018; Akinsanya & Coyne, 2021). Furthermore, in order to raise the nutritional value of cassava, substantial efforts have been made towards breeding biofortified cultivars, mostly with enhanced levels of provitamin A carotenoids (Montagnac *et al.*, 2009). The specific enhancement of nutritional elements through genetic improvement is referred to as biofortification (Tanumihardjo *et al.*, 2008). These cassava cultivars were developed by conventional plant breeding methods and released for use in Nigeria and DR Congo (Busani, 2011; Levitt, 2011). Micronutrients that are especially targeted for biofortification include vitamin A, iron and zinc, which are aimed at tackling vitamin A deficiency (Saltzman *et al.*, 2013), an important public health problem in sub-Saharan Africa. It was recently highlighted how the yield and nutritional quality of biofortified cassava cultivars could be reduced by RKN (Akinsanya *et al.*, 2020a, b).

Meloidogyne spp. mostly attack the feeder roots on cassava, although storage roots and stems can be affected, causing deformities and swellings, or galls, decay and root death (Gapasin, 1980; Caveness, 1982; Coyne *et al.*, 2003). Damage to the root system disrupts translocation of water and nutrients, as well as facilitating entry of secondary root rot pathogens. Consequently, storage root number and yield can be affected, resulting in, at times, substantial losses from RKN infection (Coyne & Affokpon, 2018). In addition to storage root losses, even of up to 87%, reduced storability and post-harvest losses can be high, due to higher levels of rots and rapid deterioration under severe nematode attack (Caveness, 1982; Théberge, 1985). In Kenya, severe damage to a small number of cassava germplasm lines (*ca* 1%) was observed in a breeder's selection trial without the species involved being identified (Coyne *et al.*, 2004). Similarly, in Nigeria RKN are becoming regarded as important pest constraints to cassava (Akinsanya & Afolami, 2019; Akinsanya *et al.*, 2020a), but where the species involved have not been determined. The release of cassava cultivars into geographic situations where assessment has not been comprehensively conducted has also highlighted the damaging nature of *Meloidogyne* spp. on otherwise high yielding and promising cassava lines (Coyne *et al.*, 2004, 2005); knowledge on the species involved and their damage potential will be useful for deployment of new cultivars.

The main species of *Meloidogyne* recorded infecting cassava are *M. javanica* and *M. incognita*, which can occur as single species infections or as multiple species infections, including in combination with *M. arenaria* and *M. hapla* (Coyne & Affokpon, 2018). *Meloidogyne enterolobii* has also been reported from cassava fields in Brazil (Rosa *et al.*, 2014) but without any information on its pathogenicity or damage potential to cassava. In West Africa, *M. enterolobii* is among the most commonly occurring species of *Meloidogyne*, recovered from a range of crops (Pagan *et al.*, 2015; dos Santos *et al.*, 2019) and it has recently been confirmed infecting cassava and causing significant damage in Nigeria (Oyetunde *et al.*, 2021). In light of the damage potential of *M. enterolobii* across a range of crops, as well as its prevalence in West Africa, the opportunity to gain an understanding of its interaction with cassava was taken in the current study.

Akinsanya *et al.* (2020a, b) recently demonstrated that improved, biofortified cassava cultivars can be quite profoundly affected by *M. incognita* from studies in pots and in field plots in Nigeria. Both yield, post-harvest quality and nutritional quality were affected following nematode infection. However, how this damage may differ following infection by different species of RKN has not been well studied. The current study was therefore undertaken to determine the differential effects of *M. incognita*, *M. javanica*, *M. arenaria* and *M. enterolobii* individually and in combination on the growth and development of the same popular, biofortified cultivars that Akinsanya *et al.* (2020a, b) used.

Materials and methods

Experimental site and details

The study was conducted in the screenhouse at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (7°22′39″N 3°54′21″E; 181 m a.s.l.). Experiments were maintained for 4 months each, first in June 2018 and repeated in April 2019. The study consisted of two factors: RKN treatments (*M. enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and combination of the four species) and cultivar (six biofortified cultivars plus a susceptible standard ('IITA-TMS-IBA 30572') as control; Table 1). Cassava cultivars were selected from among the most popular biofortified cultivars cultivated in Nigeria with a popularly grown RKN susceptible standard (Akinsanya & Afolami, 2018). Treatments were arranged in a completely randomised design with four replicates each

Table 1. Root galling damage of biofortified cassava cultivars following inoculation with 1000 eggs of *Meloidogyne enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and their combination (250 eggs of each species) in 10 l pots[1].

Cultivar	*M. enterolobii*			*M. incognita*			*M. javanica*			*Meloidogyne* spp.			DoR[5]	*M. arenaria*			DoR[5]
	TD[2]	GI[3]	RF[4]	TD[2]	GI[3]	RF[4]	TD[2]	GI[3]	RF[4]	TD[2]	GI[3]	RF[4]		TD[2]	GI[3]	RF[4]	
'IITA-TMS-IBA 011368'	5585.0^{ab}	3.0^{a}	5.6^{ab}	6442.5^{ab}	4.0^{a}	6.4^{c}	6133.8^{a}	3.0^{a}	6.2^{a}	7901.3^{a}	4.0^{a}	8.0^{a}	S	3515.0^{ab}	2.0^{b}	3.6^{abc}	T
'IITA-TMS-IBA 011412'	5881.3^{a}	3.0^{a}	5.9^{ab}	9497.5^{a}	5.0^{a}	9.5^{a}	6481.3^{a}	3.0^{a}	3.9^{b}	5611.3^{b}	4.0^{a}	5.6^{a}	S	3872.5^{ab}	2.0^{b}	3.5^{abc}	T
'IITA-TMS-IBA 011371'	4702.5^{ab}	4.0^{a}	8.1^{a}	7382.5^{ab}	4.0^{a}	7.4^{bc}	6073.8^{a}	4.0^{a}	6.1^{a}	5485.0^{b}	4.0^{a}	5.5^{ab}	S	4257.5^{a}	2.0^{b}	2.8^{cb}	T
'IITA-TMS-IBA 070593'	5004.0^{ab}	3.0^{a}	5.0^{b}	8667.8^{a}	4.5^{a}	8.6^{ab}	5747.5^{a}	4.0^{a}	5.8^{a}	7778.8^{a}	4.5^{a}	7.8^{a}	S	4372.5^{a}	2.5^{ab}	4.4^{a}	S
'IITA-TMS-IBA 070539'	5307.5^{ab}	3.0^{a}	5.4^{ab}	6697.5^{ab}	4.0^{a}	6.7^{c}	3997.0^{bc}	3.0^{a}	3.9^{b}	5948.8^{b}	4.5^{a}	6.0^{a}	S	3891.3^{ab}	3.0^{ab}	4.0^{ab}	S
'NR 07/0220'	3247.5^{b}	3.0^{a}	3.3^{b}	3565.0^{b}	4.0^{a}	3.6^{d}	2470.0^{c}	3.0^{a}	2.3^{c}	4127.0^{c}	3.5^{a}	2.7^{b}	S	2315.0^{b}	2.0^{b}	2.5^{c}	T
'IITA-TMS-IBA 30572' (control)	5418.3^{ab}	4.0^{a}	5.5^{ab}	7126.3^{ab}	5.0^{a}	7.2^{bc}	5490.0^{ab}	4.0^{a}	5.5^{a}	6736.3^{ab}	5.0^{a}	6.8^{a}	S	4295.0^{a}	3.5^{a}	4.3^{a}	S
LSD ($P \leqslant 0.05$)	2580.6	1.8	2.7	4660.8	1.7	1.5	1723.6	1.6	1.6	1342.2	1.3	2.8		1660.8	1.2	1.2	

[1]n = 8: means of four replications × two experiments. Non- inoculated control pots recorded no galling; LSD: Least significant difference. Values within a column followed by a different letter are significantly different ($P \leqslant 0.05$).

[2]Total Density (TD) = root and soil densities combined from 10 g roots and 250 g soil.

[3]Gall Index (GI) = (1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = >100 galls) (after Taylor & Sasser, 1978).

[4]RF = Nematode Reproduction Factor (Oostenbrink, 1966).

[5]Degree of Resistance (DoR) based on Canto-Saenz (1983) host suitability designation (Susceptible (S): RF > 1, GI > 2, significant growth/yield loss. Tolerance (T): RF > 1, GI ⩽ 2, No significant growth/yield loss; Resistance (R): RF ⩽ 1, GI ⩽ 2, no significant growth/yield loss; Hypersusceptible (H): RF ⩽ 1, GI > 2, significant growth/yield loss).

per cultivar per treatment. Cassava stems *ca* 15 cm long were planted at an angle into 10 l pots filled with steam-sterilised sandy-loam topsoil, maintained for 3 weeks for roots to develop, before inoculating a suspension of approximately 1000 each of *M. enterolobii*, *M. incognita*, *M. javanica* or *M. arenaria* eggs, or a combination of all four species at 250 each, in 10 l pots. Inoculum was delivered into a furrow made with a trowel around each stem and then the soil replaced after inoculation. A non-inoculated control received the same volume of water but no nematodes. All plants were then irrigated daily with 500 ml tap water. Mean daily temperatures were 26-32°C.

Nematode inoculum

A pure population each of *M. enterolobii*, *M. incognita*, *M. javanica* and *M. arenaria*, originally isolated from infected tomato (*Solanum lycopersicum*) plants (dos Santos *et al.*, 2019), were maintained in the screenhouse at IITA on tomato plants. Galled, infected roots of tomato were gently removed from pots and rinsed under running water to remove soil debris, chopped into 2-3 cm pieces and nematode eggs extracted using the Hussey & Barker (1973) sodium hypochlorite method. The eggs were collected on a 25 μm sieve, which were rinsed into a beaker, reduced to 30 ml and the egg suspension density quantified using 3 × 1 ml aliquots in a Doncaster (1962) ringed counting dish under the stereomicroscope (×40). The nematodes were inoculated in suspension of tap water at the rate of 1000 eggs pot^{-1}.

Nematode evaluation

At harvest, plants were carefully removed and roots tapped free of soil. Nematode eggs and second-stage infective juveniles (J2) were extracted and their density calculated from roots as above, using a 10 g sub-sample after weighing, chopping and thoroughly mixing all roots for each plant. The total number of nematodes per root system was then calculated. Nematodes were extracted from 250 g soil sub-samples using the Whitehead & Hemming (1965) tray method following thorough mixing of the soil from each pot, and using a double-ply extractor sandwiched between two plastic sieves with 250 ml water after spreading out the soil in the sieve. Nematode extracts were removed after 24 h, allowed to settle for 5 h and the volume adjusted to 30 ml by siphoning off the excess (Caveness, 1975). The nematode density was assessed under the microscope (3 × 1 ml aliquots) and the total number of soil nematodes per pot calculated. Total number of nematodes per pot from soil and feeder roots data was used to calculate the nematode reproduction factor (RF) (Oostenbrink, 1966):

$$\mathrm{RF} = \left((P_{\mathrm{f\,w10}} \times W_{\mathrm{rp}}) + (P_{\mathrm{f\,250\,g}} \times M_{\mathrm{sp}})\right)/P_{\mathrm{i}}$$

$P_{\mathrm{f\,w10}}$ = Final number of *Meloidogyne* eggs in 10 g feeder roots divided by 10
W_{rp} = Total weight of fresh feeder roots in pot
$P_{\mathrm{f\,250\,g}}$ = Final number of *Meloidogyne* J2 and eggs in 250 g of soil divided by 250
M_{sp} = Total mass of soil in pot
P_{i} = Inoculation density (initial number of *Meloidogyne* eggs)

Plant growth and nematode damage evaluation

Four months after planting (MAP) at harvest, plant height, stem girth, aerial fresh weight and feeder root weight per plant were determined. The number of galls on 5 cm of feeder roots per plant, removed randomly at harvest, was counted and galling index (GI) per 5 cm plant root assessed using the 1-5 scale of Taylor & Sasser (1978) (1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = >100 galls). Cultivars were tested and categorised into resistance levels using nematode RF, GI and average crop yield (Afolami, 2000; Afolami *et al.*, 2004). (Resistance: RF $\leqslant$ 1, GI $\leqslant$ 2, no significant growth/yield loss; Tolerance: RF > 1, GI $\leqslant$ 2, no significant growth/yield loss; Susceptible: RF > 1, GI > 2, significant growth/yield loss; Hypersusceptible: RF $\leqslant$ 1, GI > 2, significant growth/yield loss).

Statistical analysis

Data were subjected to a factorial analysis of variance (ANOVA) using SAS 9.4 (2012) statistical package and means were separated using least significant difference (LSD) at $P \leqslant 0.05$. Analysis of data for plant growth data was separated for each experiment as ANOVA showed significant ($P \leqslant 0.05$) differences (interaction) between the two experiments but not for the nematode density, damage and resistance rating data.

Results

All cultivars evaluated reacted to *M. enterolobii*, *M. incognita*, *M. javanica* and *M. arenaria* infection, with GI

ranging from 2 to 5 (Table 1). Significant ($P \leqslant 0.05$) differences in their abilities to support the reproduction of the four species were observed among most cultivars. All the cultivars were rated susceptible to *M. enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and the combination of species, with the exception of 'IITA-TMS-IBA011368', 'IITA-TMS-IBA011371' and 'NR 07/0220', which were rated tolerant to *M. arenaria* with a GI less than or equal to 2 and a RF greater than 1, but their growth and plant weight were mostly not significantly reduced (Table 2). The highest number of root galls were observed in plants infected with the combination of the four species, while the lowest was recorded in plants infected with *M. arenaria* (Table 1). The highest number of galls in both trials was recorded in 'IITA-TMS-IBA070593' (Table 1).

Stunted aerial growth was observed on most plants infected with *M. enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and the combined species at harvest (Table 3a, b). Crop growth was significantly ($P \leqslant 0.05$) suppressed on inoculated plants for most cultivars, except for some cultivars infected with *M. enterolobii* and *M. arenaria*. Significant ($P \leqslant 0.05$) reduction was also recorded in stem girth of most infected plants compared with the controls. Generally, plants infected with *M. incognita* or the combined species resulted in the most drastic suppression of plant growth and development, while *M. arenaria*-infected plants recorded the least suppression in both trials (Tables 3, 4; Fig. 1).

Discussion

The current study demonstrates the high susceptibility of elite, biofortified cassava cultivars to four tropical species of *Meloidogyne*. Of particular note is the raised level of damage, following the combined inoculation with all four species, compared with single species infection. Furthermore, the study demonstrates the susceptibility of cassava to *M. enterolobii*, and this species additionally poses a threat to cassava production and has been recorded recently as occurring on cassava (Oyetunde *et al.*, 2021). The key four species of this tropical group are *M. arenaria*, *M. enterolobii*, *M. incognita* and *M. javanica*. These tropical RKN species are highly polyphagous and demonstrate substantial variation in virulence and aggression (Trudgill & Blok, 2001). Infection by these four species alone likely amounts to an insurmountable yet undetermined level of loss to agricultural productivity across crops, unparalleled by any other pest or pathogen group in terms of reduced yield and post-harvest losses in the tropics (Trudgill *et al.*, 2000; Trudgill & Blok, 2001; Coyne *et al.*, 2018; Sikora *et al.*, 2018). Although not well recognised as pests of cassava, exceptionally high losses have been associated with RKN infection (Coyne & Affokpon, 2018), indicating the importance of these overlooked pests on an otherwise 'hardy' crop. Much damage probably goes unnoticed though, as cassava roots are naturally uneven meaning that low levels of galling damage could be readily overlooked. Infected roots can also deteriorate and decompose before harvest, leaving no observable symptoms to link RKN infection to reduced yields. Losses thus become attributed to other reasons, such as low soil fertility or rainfall (Coyne & Affokpon, 2018; Coyne *et al.*, 2018). Despite relatively low initial inoculum levels (P_i) of *Meloidogyne* spp. in the field in Nigeria, the growth and yield of improved, elite cassava cultivars were significantly ($P \leqslant 0.05$) reduced (Akinsanya & Afolami, 2019). Studies also demonstrated the potential damage of *M. incognita* to novel biofortified cultivars in pots (Akinsanya *et al.*, 2020a) and to *Meloidogyne* spp. in the field (Akinsanya *et al.*, 2020b). How these nutritionally improved and elite cultivars would react to other species of tropical RKN was unknown and therefore of much interest.

Although our current study was conducted under controlled conditions in pots and for 4 months of growth only, significant ($P \leqslant 0.05$) suppression in most growth parameters measured was observed, with the highest root galling and damage in plants following inoculation with a combination of the four species. While pot studies are not ideal to assess yield impact on cassava, the damage to roots and plant growth in the current study provides a suitable indication of potential field damage by the four species. The study also further demonstrates the susceptibility of cassava to *M. enterolobii*, which was only recently recorded infecting cassava in Africa (Oyetunde *et al.*, 2021). The current study now clearly demonstrates its ability to infect and cause damage to cassava. Given that *M. enterolobii* is prevalent in Nigeria, at least on vegetable crops (dos Santos *et al.*, 2019), it is highly likely that *M. enterolobii* is infecting cassava and other crops in the field, either in isolation or in combination, but being overlooked. As individual species inoculations, *M. incognita* and *M. javanica* were most damaging, with the least damage observed in plants inoculated with *M. arenaria* only. Three cultivars were rated as tolerant to *M. arenaria*, with a GI less than or equal to 2 and a RF greater than 1, but their growth and plant weight were mostly not significantly reduced, relative to the non-inoculated control (Afolami,

Table 2. Resistance evaluation of biofortified cassava cultivars following inoculation with 1000 eggs of *Meloidogyne enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and their combination (250 eggs of each species) in 10 l pots[1].

Cultivar	*M. enterolobii*			*M. incognita*			*M. javanica*			*Meloidogyne* spp.			DoR[5]	*M. arenaria*			DoR[5]
	GI[2]	RF[3]	Yield diff. (g) (Plt wgt)[4]	GI[2]	RF[3]	Yield diff. (g) (Plt wgt)[4]	GI[2]	RF[3]	Yield diff. (g) (Plt wgt)[4]	GI[2]	RF[3]	Yield diff. (g) (Plt wgt)[4]		GI[2]	RF[3]	Yield diff. (g) (Plt wgt)[4]	
'IITA-TMS-IBA 011368'	3.0	5.6	−87.7	4.0	6.4	−98.8	3.0	6.2	−62.5	4.0	8.0	−150.6	S	2.0	3.6	12.6	T
'IITA-TMS-IBA 011412'	3.0	5.9	−234.0	5.0	9.5	−283.7	4.0	3.9	−126.9	4.0	5.6	−284.0	S	2.0	3.5	−80.6	S
'IITA-TMS-IBA 011371'	4.0	8.1	−60.7	4.0	7.4	−74.0	4.0	6.1	−52.6	4.0	5.5	−69.6	S	2.0	2.8	18.7	T
'IITA-TMS-IBA 070593'	3.0	5.0	−147.6	4.0	8.6	−179.3	4.0	5.8	−148.6	5.0	7.8	−177.4	S	3.0	4.4	−69.3	S
'IITA-TMS-IBA 070539'	3.0	5.4	−134.0	4.0	6.7	−82.5	3.0	3.9	−81.3	4.0	6.0	−113.5	S	3.0	4.0	−31.0	S
'NR 07/0220'	3.0	3.3	−40.7	4.0	3.6	−64.8	4.0	2.3	−29.2	3.0	2.7	−30.9	S	3.0	2.5	8.6	T
'IITA-TMS-IBA 30572' (control)	4.0	5.5	−122.7	5.0	7.2	−188.3	4.0	5.5	−149.7	5.0	6.8	−186.7	S	4.0	4.3	−83.5	S

[1]n = 8: means of four replications × two experiments.
[2]Gall Index (GI) = (1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = >100 galls) (after Taylor & Sasser, 1978).
[3]RF = Nematode Reproduction Factor (Oostenbrink, 1966).
[4]Yield difference between inoculated and control fresh plant weight.
[5]Degree of Resistance (DoR) based on Afolami (2000) and Afolami *et al.* (2004). (Susceptible (S): RF > 1, GI > 2, significant growth/yield loss; Tolerance (T): RF > 1, GI ⩽ 2, No significant growth/yield loss; Resistance (R): RF ⩽ 1, GI ⩽ 2, no significant growth/yield loss; Hypersusceptible (H): RF ⩽ 1, GI > 2, significant growth/yield loss).

Table 3. Growth and development of six biofortified cassava cultivars in pots in the screenhouse following inoculation with four species of *Meloidogyne* and their combination: first trial[1].

	Control[3]	Treatment[2]					
		M. enterolobii	*M. incognita*	*M. javanica*	*M. arenaria*	*Meloidogyne* spp.	LSD ($P \leqslant 0.05$)
'IITA-TMS-IBA011368'							
Plant height (cm)	147.7^{a}	93.8^{ab}	58.3^{b}	84.5^{ab}	139.5^{a}	41.0^{b}	66.7
Stem girth (cm)	1.3^{a}	0.9^{ab}	0.5^{bc}	0.7^{ab}	1.1^{ab}	0.4^{c}	0.6
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
'IITA-TMS-IBA011412'							
Plant height (cm)	182.0^{a}	122.5^{ab}	83.8^{b}	163.0^{ab}	173.8^{ab}	90.5^{b}	92.6
Stem girth (cm)	1.3^{a}	0.9^{ab}	0.7^{b}	1.3^{a}	1.1^{ab}	0.6^{b}	0.6
Fresh shoot weight (g)	437.2^{a}	184.4^{bc}	119.8^{c}	273.0^{ab}	321.3^{ab}	137.1^{bc}	137.1
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
'IITA-TMS-IBA01371'							
Plant height (cm)	160.5^{a}	119.5^{ab}	93.0^{ab}	100.5^{ab}	124.0^{ab}	71.0^{b}	60.6
Stem girth (cm)	1.1^{a}	0.7^{b}	0.8^{b}	1.1^{a}	1.2^{a}	0.7^{b}	0.5
Fresh shoot weight (g)	248.8^{a}	199.7^{bc}	182.6^{bc}	234.0^{ab}	233.9^{ab}	163.3^{c}	48.2
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
'IITA-TMS-IBA070593'							
Plant height (cm)	110.5^{a}	100.3^{a}	69.0^{b}	80.0^{ab}	74.5^{b}	85.0^{ab}	79.8
Stem girth (cm)	1.0^{a}	1.0^{a}	0.7^{b}	0.7^{b}	0.7^{b}	0.9^{ab}	0.5
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
Fresh shoot weight (g)	317.6^{a}	198.5^{bc}	141.0^{c}	152.6^{bc}	232.1^{b}	131.6^{c}	131.6
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
'IITA-TMS-IBA070539'							
Plant height (cm)	130.5^{a}	120.5^{ab}	72.0^{b}	88.5^{ab}	81.0^{ab}	71.5^{b}	61.6
Stem girth (cm)	1.2^{a}	0.8	0.8^{b}	0.9^{ab}	0.9^{ab}	0.5^{b}	0.5
Fresh shoot weight (g)	261.1^{a}	105.6^{bc}	97.4^{c}	152.8^{b}	234.4^{ab}	135.3^{b}	48.4
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
'NR 07/0220'							
Plant height (cm)	75.0^{ab}	43.0^{c}	64.3^{bc}	66.5^{bc}	90.5^{a}	37.5^{c}	82.0
Stem girth (cm)	0.7^{ab}	0.6^{ab}	0.6^{ab}	0.9^{a}	0.8^{ab}	0.5^{b}	0.7
Fresh shoot weight (g)	145.4^{ab}	108.4^{b}	99.3^{b}	108.3^{b}	160.7^{a}	119.5^{b}	122.3
Fresh shoot weight (g)	249.1^{ab}	141.6^{ab}	133.9^{ab}	180.3^{ab}	266.9^{a}	97.9^{b}	123.7
'IITA-TMS-IBA30572' (control)							
Plant height (cm)	213.3^{a}	163.5^{b}	116.8^{c}	136.0^{bc}	172.0^{b}	98.0^{c}	101.3
Stem girth (cm)	1.3^{a}	0.9^{ab}	0.5^{b}	0.5^{b}	0.9^{ab}	0.5^{b}	0.5
Fresh shoot weight (g)	321.5^{a}	162.7^{bc}	135.0^{c}	158.9^{bc}	231.5^{b}	141.3^{c}	94.8

[1]n = 4: means of four replications; LSD: Least Significant Difference ($P \leqslant 0.05$); for each treatment group values within a row followed by a different letter are significantly ($P \leqslant 0.05$) different.
[2]Treatment with 1000 eggs of *M. enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and 250 eggs of each species in the combined.
[3]Control: No treatment applied.

Table 4. Growth and development of six biofortified cassava cultivars in pots in the screenhouse following inoculation with four species of *Meloidogyne* and their combination: second trial.

	Control[3]	Treatment[2]					
		M. enterolobii	*M. incognita*	*M. javanica*	*M. arenaria*	*Meloidogyne* spp.	LSD ($P \leqslant 0.05$)
'IITA-TMS-IBA011368'							
Plant height (cm)	132.5^{a}	108.0^{ab}	75.50^{bc}	100.5^{ab}	136.0^{a}	57.8^{c}	40.0
Stem girth (cm)	1.4^{a}	1.2^{ab}	0.6^{b}	1.1^{ab}	1.1^{ab}	0.4^{b}	0.4
Fresh shoot weight (g)	251.2^{a}	175.3^{bc}	168.9^{bc}	195.0^{b}	258.5^{a}	101.2^{c}	62.0
'IITA-TMS-IBA011412'							
Plant height (cm)	200.8^{ab}	127.0^{bc}	92.5^{c}	178.5^{b}	231.8^{a}	81.5^{c}	52.4
Stem girth (cm)	1.4^{a}	0.8^{b}	0.6^{b}	1.2^{ab}	1.2^{ab}	0.6^{b}	0.3
Fresh shoot weight (g)	390.0^{a}	174.8^{bc}	139.6^{c}	300.4^{b}	344.5^{ab}	122.1^{c}	58.0
'IITA-TMS-IBA01371'							
Plant height (cm)	185.0^{a}	94.0	110.8^{c}	101.5^{c}	153.8^{b}	97.5^{c}	64.8
Stem girth (cm)	1.3^{a}	0.6^{b}	0.7^{b}	0.9^{ab}	1.3^{a}	1.1^{ab}	0.4
Fresh shoot weight (g)	271.70^{a}	199.5^{b}	190.0^{b}	181.4^{b}	249.3^{ab}	218.0^{b}	81.6
'IITA-TMS-IBA070593'							
Plant height (cm)	133.5^{a}	110.5^{ab}	90.0^{b}	94.8^{b}	102.5^{ab}	95.0^{b}	62.5
Stem girth (cm)	1.1^{a}	0.7^{b}	0.7^{b}	0.7^{b}	0.9^{ab}	0.7^{b}	0.4
Fresh shoot weight (g)	331.0^{a}	155.0^{bc}	136.1^{c}	198.9^{bc}	277.9^{b}	162.3^{bc}	71.1
'IITA-TMS-IBA070539'							
Plant height (cm)	149.8^{a}	125.2^{ab}	92.5^{b}	119.0^{ab}	141.9^{a}	92.5^{b}	36.4
Stem girth (cm)	1.1^{a}	0.9^{ab}	0.7^{b}	0.9^{ab}	1.0^{a}	0.7^{b}	0.4
Fresh shoot weight (g)	245.1^{a}	132.6^{b}	126.0^{b}	190.9^{ab}	209.9^{ab}	144.0^{b}	71.2
'NR 07/0220'							
Plant height (cm)	83.0^{ab}	78.0^{ab}	72.3^{ab}	74.8^{ab}	103.8^{a}	55.3^{b}	38.4
Stem girth (cm)	0.8^{a}	0.7^{ab}	0.6^{b}	0.9^{a}	0.7^{ab}	0.7^{ab}	0.4
Fresh shoot weight (g)	169.4^{a}	125.0^{ab}	86.0^{b}	148.1^{ab}	167.6^{a}	133.5^{ab}	49.8
'IITA-TMS-IBA30572' (control)							
Plant height (cm)	245.3^{a}	207.5^{ab}	117.6^{c}	169.3^{bc}	233.1^{a}	126.9^{c}	90.6
Stem girth (cm)	1.5^{a}	0.8^{ab}	0.5^{b}	0.5^{b}	0.8^{ab}	0.5^{b}	0.6
Fresh shoot weight (g)	312.6^{a}	226.1^{b}	122.1^{c}	175.8^{bc}	235.6^{b}	119.5^{c}	100.6

[1]n = 4: means of four replications; LSD: Least Significant Difference ($P \leqslant 0.05$); for each treatment group values within a row followed by a different letter are significantly ($P \leqslant 0.05$) different.
[2]Treatment with 1000 eggs of *M. enterolobii*, *M. incognita*, *M. javanica*, *M. arenaria* and 250 eggs of each species in the combination.
[3]Control: No treatment applied.

2000; Afolami *et al.*, 2004). Tolerance in this context implied that *M. arenaria* reproduced with gall formation in the roots of these biofortified cultivars (RF > 1, GI $\leqslant$ 2) but did not cause significant ($P \leqslant 0.05$) plant damage at harvest. Rather, the infected plants reacted with raised plant growth compared with non-inoculated control plants (Afolami, 2000; Afolami *et al.*, 2004). The susceptible cultivars differed from the tolerant cultivars by the extent of the yield losses ($P \leqslant 0.05$) experienced by *M. incognita*, *M. javanica*, *M. enterolobii* and a combination of the four species.

Our study shows that all four prevalent tropical *Meloidogyne* species were able to infect and cause damage to cassava, especially in combination. The cassava cultivars used in this study have been developed to improve food security and reduce malnutrition, but, given their high sus-

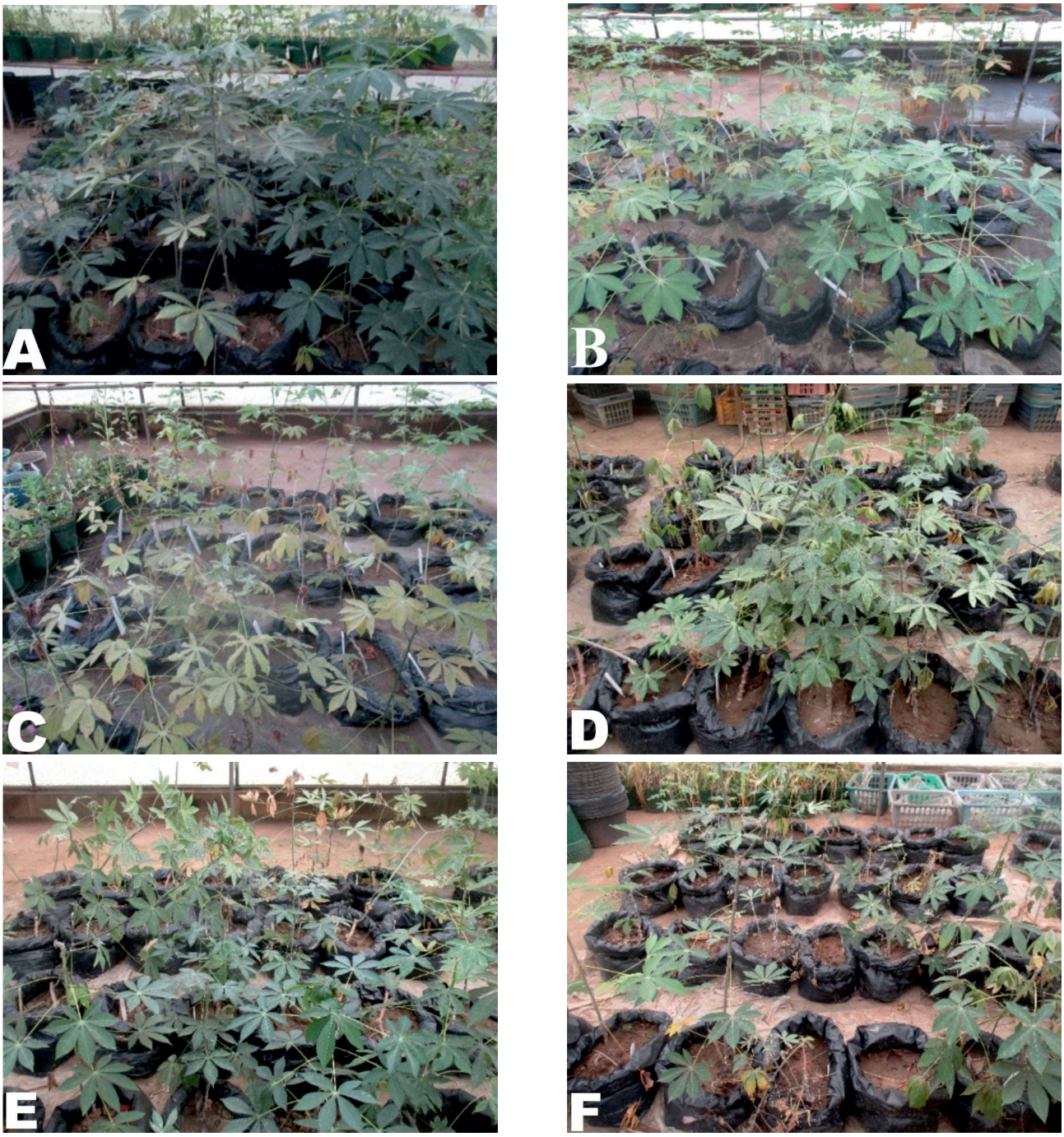

Fig. 1. Growth of all cassava cultivars in 10 l pots at 3 months after inoculation with four species of *Meloidogyne* and their combination. A: All biofortified cassava cultivars + control cultivar + zero inoculation; B: All biofortified cassava cultivars + control cultivar + 1000 eggs of *M. enterolobii*; C: All biofortified cassava cultivars + control cultivar + 1000 eggs of *M. incognita*; D: All biofortified cassava cultivars + control cultivar + 1000 eggs of *M. javanica*; E: All biofortified cassava cultivars + control cultivar + 1000 eggs of *M. arenaria*; F: All biofortified cassava cultivars + control cultivar + 250 eggs each of *M. enterolobii*, *M. incognita*, *M. javanica*, and *M. arenaria*.

ceptibility to the prevalent RKN species, their potential to achieve this will be undermined. A previous study on the same cultivars also showed that infection with RKN can negatively affect the nutritional quality (Akinsanya *et al.*, 2020b) in addition to yields. An effective nematode control strategy such as breeding for resistance to RKN is therefore necessary to safeguard these special cassava cultivars against these nematode pests. Cassava cultivars with resistance against RKN have been identified (Coyne *et al.*, 2004; Udo *et al.*, 2008; Abidemi, 2014) and so it should be possible to channel this resistance into new improved, biofortified cultivars to overcome the threat of RKN.

Acknowledgements

We thank the CGIAR research programme for Roots, Tubers and Bananas (CRP-RTB), Nematology unit and Cassava breeding unit, IITA-Ibadan, Nigeria.

References

Abidemi, M.O. (2014). *Plant-parasitic nematodes associated with cassava in southwestern Nigeria and control of* Meloidogyne incognita *with* Tithonia diversifolia *and plastic mulch*. Ph.D. Thesis, University of Ibadan, Ibadan, Nigeria, pp. 185-200.

Afolami, S.O. (2000). Suggestions for the improvement of current methods of studying and reporting resistance to root-knot nematodes. *International Journal of Nematology* 10, 94-100.

Afolami, S.O., Atungwu, J.J., Orisajo, S.B. & Odeyemi, S. (2004). Going beyond Gall Index in studying and reporting resistance to root-knot nematodes. *Nigerian Journal of Plant Protection* 21, 25-32.

Afuape, S.O. (2009). Sweet potato research at the National Root Crops Research Institute (NRCRI), Umudike, Abia State. In: Akoroda, M.O. & Egeonu, I. (Eds). *Proceedings of the 1st national sweet potato conference, 16-18th September, 2008*. Ibadan, Nigeria, University of Ibadan, pp. 13-14.

Akinsanya, A.K. & Afolami, S.O. (2018). Effect of seven elite cassava (*Manihot esculenta* Crantz) varieties to infection by *Meloidogyne* spp. and other nematodes in the field. *Nematropica* 48, 50-58.

Akinsanya, A.K. & Afolami, S.O. (2019). Screenhouse response of seven elite cassava (*Manihot esculenta* Crantz) varieties to *Meloidogyne incognita* infection. *Nematropica* 49, 91-98.

Akinsanya, A.K. & Coyne, D. (2021). The resilient cassava: undermined by root-knot nematodes. In: Sikora, R.A., Desaeger, J. & Molendijk, L. (Eds). *Integrated nematode management: state-of-the-art and visions for the future*. Wallingford, UK, CAB International, pp. 381-387. DOI: 10.1079/9781789247541.0053

Akinsanya, A.K., Afolami, S.O., Kulakow, P. & Coyne, D.L. (2020a). The root-knot nematode, *Meloidogyne incognita*, profoundly affects the production of popular biofortified cassava cultivars. *Nematology* 22, 667-676. DOI: 10.1163/15685411-00003331

Akinsanya, A.K., Afolami, S.O., Kulakow, P., Parkes, E. & Coyne, D. (2020b). Popular biofortified cassava cultivars are heavily impacted by plant parasitic nematodes, especially *Meloidogyne* spp. *Plants* 9, 802. DOI: 10.3390/plants9060802

Busani, B. (2011). *Nigeria: not everyone pleased with new Vitamin A-fortified cassava*. Available online at www.globalissues.org/news/2011/12/30/12339 (accessed 13 January 2012).

Canto-Saenz, M. (1983). The nature of resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood. In: Sasser, J.N. (Ed.). *Proceedings of the third research & planning conference on root-knot nematodes* Meloidogyne *spp. International* Meloidogyne *project*. Raleigh, NC, USA, North Carolina State University Graphics, pp. 160-165.

Caveness, F.E. (1975). A simple siphon method of separating nematodes from excess water. *Nematropica* 5, 30-32.

Caveness, F.E. (1982). Root-knot nematodes as parasites of cassava. *IITA Research Briefs* 3, 2-3.

Coyne, D.L. & Affokpon, A. (2018). Nematode parasites of tropical root and tuber crops. In: Sikora, R.A., Coyne, D.L., Hallman, J. & Timper, P. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*, 3rd edition. Wallingford, UK, CAB International, pp. 252-289.

Coyne, D.L., Talwana, H.A.L. & Maslen, N.R. (2003). Plant parasitic nematodes associated with root and tuber crops in Uganda. *African Crop Protection* 9, 87-98.

Coyne, D.L., Khizzah, W. & Whyte, J. (2004). Root-knot nematode damage to cassava in Kenya. *Roots* 9, 3-5.

Coyne, D.L., Tchabi, A., Baimey, H., Labuschagne, N. & Rotifa, A. (2005). Distribution and prevalence of nematodes (*Scutellonema bradys* and *Meloidogyne* spp.) on marketed yam (*Dioscorea* spp.) in West Africa. *Field Crops Research* 96, 142-150.

Coyne, D.L., Cortada, L., Dalzell, J.J., Claudius-Cole, A.O., Haukeland, S., Luambano, N. & Talwana, H. (2018). Plant-parasitic nematodes and food security in Sub-Saharan Africa. *Annual Review of Phytopathology* 56, 381-403. DOI: 10.1146/annurev-phyto-080417-045833

Doncaster, C.C. (1962). A counting dish for nematodes. *Nematologica* 7, 334-336. DOI: 10.1163/187529262X00657

dos Santos, M.F.A., da Silva Mattos, V., Monteiro, J.M.S., Almeida, M.R.A., Jorge, A.S., Jr, Cares, J.E., Castagnone-Sereno, P., Coyne, D. & Carneiro, R.M.D.G. (2019). Diversity of *Meloidogyne* spp. from peri-urban areas of sub-Saharan Africa and their genetic similarity with populations from the Latin America. *Physiological and Molecular Plant Pathology* 105, 110-118. DOI: 10.1016/j.pmpp.2018.08.004

FAO (2018). *Food and agriculture data*. Available online: http://Faostat.fao.org. Accessed: July 21 2019.

Gapasin, R.M. (1980). Reaction of golden yellow cassava to *Meloidogyne* spp. inoculation. *Annals of Tropical Research* 33, 92-100.

HarvestPlus (2014). Available online: https://www.harvestplus.org/crop/vitamin-a-cassava/. Accessed: 12 June 2021.

Hussey, R.S. & Barker, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Disease Reporter* 57, 1925-1928.

Latham, M.C. (1979). *Human nutrition in tropical Africa*. *FAO Food and Nutrition Series No.16*. Rome, Italy, FAO.

Levitt, T. (2011). Can GM-free biofortified crops succeed after golden rice controversy? https://theecologist.org/2011/dec/12/can-gm-free-biofortified-crops-succeed-after-golden-rice-controversy. Accessed: 16 December 2011.

Montagnac, J.A., Davis, C.R. & Tanumihardjo, S.A. (2009). Nutritional value of cassava for use as a staple food and recent advances for improvement. *Comprehensive Reviews in Food Science and Food Safety* 8, 181-194. DOI: 10.1111/j.1541-4337.2009.00077.x

Okigbo, B.N. (1980). Nutritional implications of projects giving high priority to the production of staples of low nutritive quality. In the case for cassava (*Manihot esculenta* Crantz) in the humid tropics of West Africa. *Food Nutrition Bulletin* 2, 1-10.

Oostenbrink, M. (1966). *Major characteristics of the relationship between nematodes and plants, vol. 66*. The Netherlands, Mededelingen Landbouwhogeschool Wageningen, pp. 44-46.

Oyetunde, A.K., Kolombia, Y.A., Adewuyi, O., Afolami, S.O. & Coyne, D.L. (2022). First report of *Meloidogyne enterolobii* infecting cassava (*Manihot esculenta*) resulting in root galling damage in Africa. *Plant Disease* 106, 1533. DOI: 10.1094/PDIS-08-21-1777-PDN

Pagan, C., Coyne, D., Carneiro, R., Kariuki, G., Luambano, N., Affokpon, A. & Williamson, V.M. (2015). Mitochondrial haplotype-based identification of ethanol-preserved root-knot nematodes from Africa. *Phytopathology* 105, 350-357. DOI: 10.1094/PHYTO-08-14-0225-R

Rosa, J.M.O., de Oliveira, S.A., Jordão, A.L., Siviero, A. & de Oliveira, C.M.G. (2014). Nematoides fitoparasitas associados à mandioca na Amazônia Brasileira. *Acta Amazonica* 44, 271-277.

Saltzman, A., Birol, E., Bouis, H.E., Boy, E., De Moura, F.F., Islam, Y. & Pfeiffer, W.H. (2013). Biofortification: progress toward a more nourishing future. *Global Food Security* 2, 9-17. DOI: 10.1016/j.gfs.2012.12.003

SAS Institute (2013). *SAS user's guide: statistics, version 9.4*. Cary, NC, USA, SAS Institute.

Sikora, R.A., Coyne, D.L., Hallman, J. & Timper, P. (2018). Reflections on nematology in subtropical and tropical agriculture. In: Sikora, R.A., Coyne, D.L., Hallman, J. & Timper, P. (Eds). *Plant parasitic nematodes in subtropical and tropical agriculture*, 3rd edition. Wallingford, UK, CAB International, pp. 1-19.

Tanumihardjo, S.A., Bouis, H., Hotz, C., Meenakshi, J.V. & McClafferty, B. (2008). Bio-fortification of staple crops: an emerging strategy to combat hidden hunger. *Comprehensive Reviews in Food Science and Food Safety* 7, 329-334.

Taylor, A.L. & Sasser, J.N. (1978). *Biology, identification and control of root-knot nematodes (*Meloidogyne *species)*. Raleigh, NC, USA, North Carolina State University Graphics, Department of Plant Pathology and USAID.

Théberge, R.L. (1985). *Common African pests and diseases of cassava, yam, sweet potato and cocoyam*. Ibadan, Nigeria, International Institute of Tropical Agriculture, pp. 26-29.

Trudgill, D.L. & Blok, V.C. (2001). Apomictic, polyphagous root-knot nematodes: exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology* 39, 53-77. DOI: 10.1146/annurev.phyto.39.1.53

Trudgill, D.L., Bala, G., Blok, V.C., Daudi, A., Davies, K.G., Gowan, S.R., Fargette, M., Madulu, J.D., Mateille, T., Mwageni, W. *et al.* (2000). The importance of tropical root-knot nematodes (*Meloidogyne* spp.) and factors affecting the utility of *Pasteuria penetrans* as a biocontrol agent. *Nematology* 2, 823-845. DOI: 10.1163/156854100750112789

Udo, I.A., Ogbaji, P.O. & Ukeh, D.A. (2008). The response of some cassava (*Manihot esculenta* Crantz) cultivars to infection by *Meloidogyne incognita* race 1 in Calabar, Nigeria. *Agricultural Journal* 3, 25-30.

Whitehead, A.G. & Hemming, J.R. (1965). A comparison of some qualitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 5, 25-38. DOI: 10.1111/j.1744-7348.1965.tb07864.x