# RESEARCH/INVESTIGACIÓN

# HIGH THROUGHPUT PROPAGATION MATERIALS FOR EVALUATING THE RESPONSE OF YAM TO NEMATODE INFESTATION UNDER CONTROLLED ENVIRONMENT

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## ABSTRACT

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Yam is a vegetatively propagated crop generally multiplied using a portion (sett) of the tuber, which represents 30% of the cost of production. This study evaluated four propagation materials of yam, (i) vine seedlings from aeroponic system (VS), (ii) seedlings from semi autotrophic hydroponics (SAH), (iii) mini-tubers, and (iv) minisetts for their suitability for evaluating resistance of yams to nematodes. Two recently released yam genotypes, TDr 95/19177 and TDr 89/02665, were challenged with *Meloidogyne incognita* and *Scutellonema bradys*. Plastic pots were arranged in a screenhouse following a completely randomized design with twelve replicates. Plants were inoculated six weeks after planting with 5,000 eggs of *M. incognita* or 5,000 mixed individuals of *S. bradys*. Data were collected during vegetative growth, at harvest, and during storage. Vine length, number of leaves, and number of vines were not significantly different at the vegetative growth stage ($P > 0.05$). At harvest, the nematodes had significant effects on vine length, fresh and dry shoot weight, and tuber diameter ($P < 0.05$). After storage, there were significant losses in tubers weight of 61.8% and 43.3%, respectively, for *S. bradys* and *M. incognita* inoculated plants ($P < 0.05$). Damage indexes for all the planting materials were not significantly different, however, nematode recovery was less in VS and SAH plants compared to minisetts and mini-tuber plants. Mini-tubers and minisetts are apparently more reliable as planting materials to be used when screening yam genotypes.

*Key words: Dioscorea rotundata*, host status, *Meloidogyne incognita*, planting materials, screening, *Scutellonema bradys*, soil worm, storage, yam growth

## RESUMEN

Fassinou, C. G., N. G. Maroya, A. O. Claudius-Cole, y M. O. Akoroda. 2024. Materiales de propagación de alto rendimiento para evaluar la respuesta del ñame a la infestación de nematodos en un ambiente controlado. Nematropica 54:131-148.

El ñame es un cultivo propagado vegetativamente que generalmente se multiplica utilizando una porción (set) del tubérculo, lo cual representa el 30% del costo de producción. Este estudio evaluó cuatro materiales de propagación de ñame: (i) esquejes tomados del tallo procedentes de un sistema aeropónico

(VS), (ii) plántulas de hidroponía semi-autotrófica (SAH), (iii) mini tubérculos y (iv) minisetts, para determinar su idoneidad en la evaluación de la resistencia de los ñames a los nematodos. Dos genotipos de ñame recientemente liberados, TDr 95/19177 y TDr 89/02665, fueron evaluados contra *Meloidogyne incognita* y *Scutellonema bradys*. Macetas de plástico se organizaron en un invernadero siguiendo un diseño completamente al azar con doce repeticiones. Las plantas fueron inoculadas seis semanas después de la siembra con 5000 huevos de *M. incognita* o 5000 mezcla de individuos de *S. bradys*. Se recolectaron datos durante el crecimiento vegetativo, en la cosecha y durante el almacenamiento. La longitud de tallos, el número de hojas y el número de tallos no mostraron diferencias significativas en la etapa de crecimiento ($p > 0.05$). En la cosecha, los nematodos tuvieron efectos significativos en la longitud de los tallos, el peso fresco y seco de los brotes, y el diámetro del tubérculo ($p < 0.05$). Después del almacenamiento, hubo pérdidas significativas en el peso de los tubérculos de 61,8% y del 43,3%, respectivamente, para las plantas inoculadas con *S. bradys* y *M. incognita* ($p < 0.05$). Los índices de daño para todos los materiales de siembra no fueron significativamente diferentes, sin embargo, la recuperación de nematodos fue menor en las plantas VS y SAH en comparación con las plantas procedentes de minisetts y mini-tubérculos. Los mini tubérculos y las minisetts son aparentemente más confiables como materiales de siembra cuando se evalúan genotipos de ñame.

*Palabras clave*: *Dioscorea rotundata*, estado de hospederos, *Meloidogyne incognita*, materiales de siembra, evaluación, *Scutellonema bradys*, nematodos del suelo, crecimiento de ñames, almacenamiento

## INTRODUCTION

Yam is the world's fourth most important tuber crop after potatoes, cassava, and sweet potatoes (Viruel *et al*., 2016; Padhan and Panda, 2020). In Africa, yam is the second most important source of carbohydrate after cassava (Bassey, 2017). It plays a crucial role in the food security and livelihood of at least 90 million people in West Africa (Hahn *et al*., 1987; Maroya *et al*., 2017). About 48 million tons of yam (93% of global production) are produced on four million hectares annually in this sub-region, mainly in five countries including Benin, Ivory Coast, Ghana, Nigeria, and Togo (Maroya *et al*., 2014). Yam is mostly cultivated for consumption purposes as a staple food but also plays a role in the medicinal, social, and cultural life of Africans (Amusa, 2000; Mignouna *et al*., 2008; Izekor and Olumese, 2010; Andres *et al*., 2017).

However, yam production is affected by many factors that can lead to reductions in economic value. Pests and diseases are the second major factor that cause reductions in yam quantity and quality (Osei *et al*., 2015; Kolombia, 2017). Plant-parasitic nematodes (PPNs) are considered to be one of the major pests that damage yams in the field and during storage (Ogaraku and Usman, 2008; Shehu *et al*., 2010; Ibitoye and Attah, 2012). The yam nematode (*Scutellonema bradys*), root-knot nematodes (*Meloidogyne* spp.), and root-lesion nematodes (*Pratylenchus* spp.) are the major PPNs associated with yams (Bridge *et al*., 2005; Coyne and Affokpon, 2018). *Scutellonema bradys* and *Meloidogyne* spp. were reported as the most important nematodes in West Africa (Bridge *et al*., 2005; Coyne *et al*., 2006). *Scutellonema bradys* causes cracking and dry rot disease on yam tubers, while *Meloidogyne* spp. induce galling, which result in deformed tubers (Nwauzor and Fawole, 1981; Moura, 1997; Bridge *et al*., 2005). The observed damage can lead to a loss of tuber yield ranging from 0-52% in the field and 80-100% in storage, indicating that damage is more severe during tuber storage. Damaged tubers become unusable for planting and unsuitable for sale (Smit, 1967; Bridge, 1982; Baimey *et al*., 2009). Nematode management methods include cultural practices such as intercropping, crop rotation, fallow-free of host plants, and the use of nematode-free and healthy planting material (Claudius-Cole *et al*., 2014); physical methods such as hot water treatment (HWT) of planting materials (Bridge *et al*., 2005); chemical methods such as the use of nematicides and herbicide (Zhang *et al*., 2010); biological methods such as the use of antagonistic plants (Osei *et al*., 2011); beneficial fungi and bacteria that are pathogen to plant-parasitic nematodes (Janssens *et al*., 2023); and the use of resistant cultivars (Onkendi *et al*., 2014).

The use of resistant cultivars and healthy planting material are among the most effective control methods for nematode management in yam production. Healthy, nematode-free planting material is by far the most appropriate means of preventing nematode damage on yam (Coyne and Affokpon, 2018). Yam is traditionally propagated using saved tubers selected from previous seasons (Aighewi *et al*., 2015). This method of propagation is characterized by low multiplication ratios (1:10) (Maroya *et al*., 2017) and requires up to 30% of the previous harvest to serve as propagules (Omotayo *et al*., 2018). The quality of the seed yam is also uncertain since the direct use of planting material from one field to another increases the risk of disease spread (Kolombia *et al*., 2016). Several modern approaches of rapidly obtaining healthy planting materials have been developed at the International Institute of Tropical Agriculture (IITA) and include *in vitro* tissue culture technique, temporary immersion bioreactor system, aeroponics, hydroponic, and semi autotrophic hydroponic (SAH) systems (IITA, 2000; Coyne *et al*., 2010; Maroya *et al*., 2014). These methods contribute to obtaining healthy planting material in a short time, making it crucial to identify the planting material, apart from the tuber, that is most appropriate for evaluating the host status of the crop to nematode damage, especially *S. bradys* and *M. incognita*. In line with this, the objectives of the study were to: (i) evaluate the effect of *S. bradys* and *M. incognita* on growth and yield parameters of two yam genotypes based on the type of planting material, (ii) assess the effect of both nematodes on yam tubers in storage, and (iii) determine the host status of yam planting materials to *M. incognita* and *S. bradys* infestations.

## MATERIALS AND METHODS

The screenhouse experiment was conducted for six months (November 2019 to May 2020) at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (latitude: 7°28'59"N; longitude: 3°54'21"E; 212 m a.s.l.). The experiment was established in late November during the dry season with temperatures ranging from 19°C to 39°C and from 18°C to 49°C inside and outside the screenhouse, respectively. Relative humidity varied from 17% to 83% and from 20% to 92% inside and outside the screenhouse, respectively. Harvested tubers were stored for three months (May to August, 2020) in a storeroom where the temperature ranged from 16.5°C to 31°C.

### *Experimental materials and design*

The experiment was conducted using two recently released yam varieties, TDr 95/19177 ('Kpamyo') and TDr 89/02665 ('Asiedu'). Each variety had four different planting materials: mini-tubers (6 months old), minisetts (6 months old), vine seedlings (28 days), and SAH seedlings (28 days). Plants were inoculated with either *S. bradys* or *M. incognita*, while the control was not inoculated. All the planting materials were provided by IITA from the yam aeroponic system unit and the SAH system unit. To prepare the planting medium, topsoil was collected and sterilized at 90°C for 2 hr using a electrical steam sterilizer. Plastic 10 L pots with dimensions of 23.5 cm in diameter and 26 cm in height were filled with 10 kg of sterilized soil and planted with one plant of the plant materials vine seedling, SAH seedling, mini-tuber, or minisett. Pots were arranged in a screenhouse following a completely randomized design with 12 replicates giving a total number of 288 pots used in this experiment.

### *Inoculum preparation and inoculation*

*Scutellonema bradys* infected yam tubers were used to prepare *S. bradys* inoculum. Infected yam tubers were cleaned and peeled. Then, peels were chopped into small pieces using a sharp knife. Nematodes were then extracted from the chopped infected yam peels following the pie-pan method (Hooper, 1990). The chopped infected yam peels were properly mixed to ensure homogeneity and samples of 20 g each were spread on a milk filter placed within a plastic sieve. A plastic dish was placed under each sieve containing the chopped yam peel and water was gently poured into the plastic dish through the gap between the sieve and the dish. The dish was labelled and kept for 48 hr to allow nematodes to move from the chopped yam peel through the milk filter into the water at the bottom of the plastic dishes. After the extraction period, the

sieves were carefully removed from the plastic dishes and the excess water in the sieves was drained into the plastic dishes before the milk filter with the chopped yam peel was discarded. Thereafter, the water (containing the nematodes) in the dishes was poured and rinsed into labelled plastic cups. The suspensions were left for 2 hr to allow *S. bradys* to settle and the supernatants were gently poured off.

Heavily galled roots of tomato plants served for *M. incognita* inoculum. The eggs of *M. incognita* were extracted from heavily galled roots of tomato (Hussey and Barker, 1973). Pots containing plants infected with *M. incognita* were watered to allow easy removal of the plants from the soil. With the aid of the hand trowel, the plants were gently removed without damaging roots. The galled roots of the plants were washed under running tap water to remove adhering soil, cut into small pieces using a pair of scissors, and then transferred into a conical flask. Using 0.5% sodium hypochlorite (NaOCl), a solution of 1,000 ml was prepared by adding 900 ml of water into a graduated cylinder containing 100 ml of NaOCl. The prepared solution was then added to the chopped galled roots in the conical flask. This was then shaken vigorously for about 4 min. to help to dislodge the nematode eggs from the gelatinous matrix. The mixture was poured into a stack of three sieves of 212, 90, and 25 µm of aperture size (the large aperture size on top and the smallest one at the bottom). The two upper sieves were rinsed with water to move the eggs into the bottom sieve. The bottom sieve was also rinsed gently to remove all traces of NaOCl solution, and then the concentrated water suspension with *M. incognita* eggs was poured into a beaker.

The population density of nematodes was estimated under a compound microscope. Three sub-samples of 1 ml of mixed water containing the nematodes were used to estimate nematode densities. Plants generated from the yam planting materials (mini-tubers, minisetts, vine seedlings, SAH seedlings) initially planted in pots were inoculated six or seven weeks after planting with the inoculum containing 5,000 eggs and juveniles of *M. incognita*, and 5,000 mixed stages of *S. bradys*. To apply the inoculum, each pot was first watered to make the soil moist before inoculation. A shallow trench was dug around the stem of each plant in the pot to a depth of 5 cm leaving some of the roots exposed. An estimated volume of 5.1 ml and 3.3 ml of *S. bradys* and *M. incognita* inoculum, respectively, was drawn and released into each trench using a syringe and the inoculum was continuously mixed before the next drawing to ensure homogeneity. The trench was immediately re-covered after inoculation and plants were left for 24 hr before watering. The inoculation of *S. bradys* was done 6 wk after planting, while the plants receiving *M. incognita* were inoculated 7 wk after planting to avoid contamination during inoculum preparation.

*Data collection*

During plant growth, data were collected every 2 wk on the main vine length using a measuring tape; number of vines, and leaves by manual counting; and, plant vigour scored 1 to 3 (1 = weak, 2 = moderate-vigorous, 3 = vigorous) (Asfaw, 2016). Data on leaf chlorophyll content was collected when all the plants had established their leaves using a Konica Minolta SPAD-502 Plus (Tokyo, Japan). The experiment was harvested 6 months after planting (136 days after inoculation), and data on plant senescence at harvest scored on a scale of 1 to 4 (1 = entire plant still green; 2 = 1/3 of leaves are yellowing; 3 = 2/3 of leaves are yellowing; 4 = the entire plant is yellowing). Number of tubers per plant was collected by manual counting. Fresh tuber weight, fresh root weight, and fresh and dry shoot weight were determined, while tuber length was measured using a measuring tape, and tuber diameter was measured using a Spurtar Vernier Caliper 0-150 mm (0-6 Inch) with a precision of 0.02 mm. Data on nematodes damage on tubers and roots such as tuber and root galling index, tuber dry rot index, and tuber cracking index were also collected by visual observation of symptoms severity on a scale of 1 to 5 where 1 = no damage (0%), 2 = mild damage (1-25%), 3 = moderate (26-50%) 4 = severe (51-75%), and 5 = highly severe (76-100%) (Coyne *et al*., 2014).

Both nematodes were extracted from soil, root, and tubers to estimate their final population densities. A modification of the Hussey and Barker (1973) procedure was used for *M. incognita* extraction. Samples of 10 g of roots and tuber peels were separately chopped and placed in a 0.5% sodium hypochlorite solution for two 5 sec bursts using a laboratory blender. The mixed

solution was additionally shaken for four min, to dislodge the nematode eggs from the gelatinous matrix, and poured over a stack of three sieves of 212, 90, and 25 µm. The NaOCl was washed off under running tap water and the different nematode stages were collected into a beaker. To extract *S. bradys*, the pie-pan method of Hooper (1990) was followed. Roots and tuber peels weighing 20 g were separately chopped and placed in a milk filter inside a sieve and tray for 48 hr. The suspensions was collected into a beaker and left for 2 hr to allow the nematodes to settle and the supernatant was gently poured off. The same procedure was used for nematode extraction from soil using 100 ml of soil per sample. Using a pipette, each extract was homogenized, and 1 ml was drawn and released into a counting dish. Nematodes were counted under a compound microscope with the aid of a tally counter. This was repeated three times to obtain an average.

*Post-harvest evaluation*

After harvest, tubers were kept for three months under an ambient environment (24-27°C and 70-80% RH) in a storeroom. Tuber weight loss was calculated as the difference between the initial weight of the tuber before storage (TWBS) and the weight of the tuber after storage (TWAS). The percentage weight loss (PWL) was then determined by dividing TWL by TWBS and multiplying by 100. TWL= TWBS – TWAS, where TWBS is the initial weight of tuber before storage and TWAS is the weight of the tuber after storage. PWL = (Tuber weight loss)/(Initial weight of tuber before storage)×100.

Nematode damage on tubers was again assessed after the storage period according to the rating scale by Coyne *et al*. (2014). The host status was determined at the planting material level based on damage index (DI) and reproductive factor (RF), using a modified rating scale adapted from Sasser *et al*. (1984) (Damage Index (DI) ≤ 2 and RF ≤ 1 = Resistant; DI ≤ 2 and RF > 1 = Tolerant; DI > 2 and RF > 1 = Susceptible; DI > 2 and RF ≤ 1 = Hyper-susceptible). The RF was calculated as RF = Pf/Pi; where Pf is the final nematode population density and Pi is the initial nematode population density per pot. Galling and dry rot of yam tubers, respectively, was considered as the damage index for *M. incognita* and *S. bradys* when rating the host status.

*Statistical analyses*

Data were analysed using descriptive statistics for all the treatments and analysis of variance (ANOVA) was executed in R statistical software 4.2.0 version using the agricolae package (R-Core Team, Vienna, Austria). The hierarchical organization of the means was done with the Fisher's Least Significant Difference (LSD) at $P \leq 0.05$. Data on nematode densities were square-root transformed before analysis. Pearson's correlation coefficients (r) were computed and used to assess the relationship between yam tuber yield and the yield components. The traits that had a significant relationship with tuber yield were used to conduct a multiple regression analysis to determine how much the variation in such traits contributed to the variation in tuber yield.

## RESULTS

*Growth parameters of yam materials*

At 10 wk after inoculation (WAI) vine length, number of leaves, and number of vines were not significantly different between nematode inoculated and non-inoculated controls for both varieties and planting materials ($P > 0.05$). However, leaf chlorophyll content was significantly affected by nematodes ($P = 0.0071$) and planting materials ($P = 0.0000$), but varieties were not significantly different from each other. Generally, leaves of non-inoculated plants were richer in chlorophyll (31.89 ± 1.95 µmol/m$^2$) compared to leaves of nematode-inoculated plants with mini-tubers and minisetts having higher chlorophyll content in comparison to plants from SAH and vine seedlings (Table 1). Plants from vine and SAH seedlings had increased vegetative growth from four to six weeks after planting, while most of the tubers (mini-tubers and minisetts) had not sprouted. However, after 6 wk, almost all mini-tubers and minisetts had sprouted and showed subsequent rapid growth greater than seedlings produced from vine and SAH materials. Non-inoculated plants had the highest vine length, followed by

Table 1. Effects of *Meloidogyne incognita* and *Scutellonema bradys* on chlorophyll content of leaves of plants from different yam planting materials at 10 weeks after inoculation.

| Variety | Planting material[y] | Chlorophyll content[x] | | | LSD |
|---|---|---|---|---|---|
| | | Control | *M. incognita* | *S. bradys* | |
| Asiedu | MT | 41.8 a A[z] | 38.4 a AB | 32.4 b B | 7.1 |
| | MS | 40.1 a A | 39.0 a A | 39.0 a A | 4.3 |
| | SAH | 25.3 b A | 23.7 b A | 18.1 c A | 11.0 |
| | VS | 27.1 b A | 16.1 b B | 21.4 c AB | 10.0 |
| LSD | | 5.8 | 10.9 | 6.2 | |
| Kpamyo | MT | 39.8 a A | 33.0 a A | 33.0 a A | 7.9 |
| | MS | 37.4 ab A | 33.7 a A | 33.1 a A | 5.9 |
| | SAH | 18.4 c A | 17.8 b A | 17.5 b A | 13.6 |
| | VS | 25.2 bc A | 20.3 b A | 23.8 ab A | 16.1 |
| LSD | | 12.8 | 10.3 | 9.5 | |
| Mean | | 31.9 A | 27.8 B | 27.3 B | 3.0 |
| SE | | 2.0 | 2.1 | 1.7 | |

[x]Values are means of 12 replicates.
[y]MT = Mini-tuber, MS = Minisett, SAH = Semi autotrophic hydroponic seeding, VS = Vine seedlings.
[z]Means with the same lowercase letter (s) within a column per genotype are not significantly different according to the LSD test ($P \leq 0.05$). Means with the same uppercase letter (s) within row are not significantly different according to the LSD test ($P \leq 0.05$).

plants inoculated with *M. incognita* (Fig. 1), while *S. bradys* inoculated plants had the lowest vine length ($P = 0.09$). In terms of survival, the number of surviving plants from SAH and vine materials decreased with time; SAH seedlings had the lowest percentage survival of 64.3%.

*Yield and yield-related parameters of the yam planting materials*

Of the 11 parameters measured, there were significant differences in seven of the parameters ($P < 0.05$; Table 2). Except for the number of vines and fresh tuber weight, all other parameters were significantly different among planting materials ($P < 0.05$). Nematodes significantly affected the vine length at harvest, fresh and dry shoot weight, and tuber diameter. The interaction among genotypes, planting materials, and nematodes was only significant for number of vines. This indicated that nematodes did not significantly affect tuber yield (measured as tuber weight) of the yam genotypes irrespective of the planting material used. In general, for most parameters measured, non-inoculated 'Kpamyo' mini-tuber plants performed better, while *S. bradys*-inoculated Asiedu SAH plants had the lowest yield.

*Correlation and regression analyses*

Fresh tuber yield was positively and significantly correlated with tuber diameter (r = 0.59) and number of leaves (r = 0.52). Yield was also positively correlated with tuber length (r = 0.47), vine length (r = 0.37), number of vines (r = 0.25), plant vigour (r = 0.39), nematode population density in tubers (r = 0.27), and chlorophyll content of leaves (r = 0.25); however, there was a positive and non-significant correlation with fresh shoot weight. A negative correlation was observed between fresh tuber yield and senescing at harvest (Table 3).

The multi-regression analysis of fresh tuber yield gave the linear equation below with $R^2 = 0.6207$, indicating that the variation of the fresh yam tuber yield was explained by these factors to 62.1%. FTY = – 0.1 VL + 6.6 NV + 7.8 PV + 0.2 NL – 0.1 CC + 2.7 TL + 1.5 TD – 0.0 NPT – 38.7 (FTY = fresh tuber yield, VL =: vine length, NV = number of vines, PV = plant vigour, NL = number of leaves, CC = chlorophyll content of leaves, TL = tuber length, TD = tuber diameter,; and NPT = nematode population in tubers).

The regression analysis revealed that vine length had a negative and significant effect ($P <$

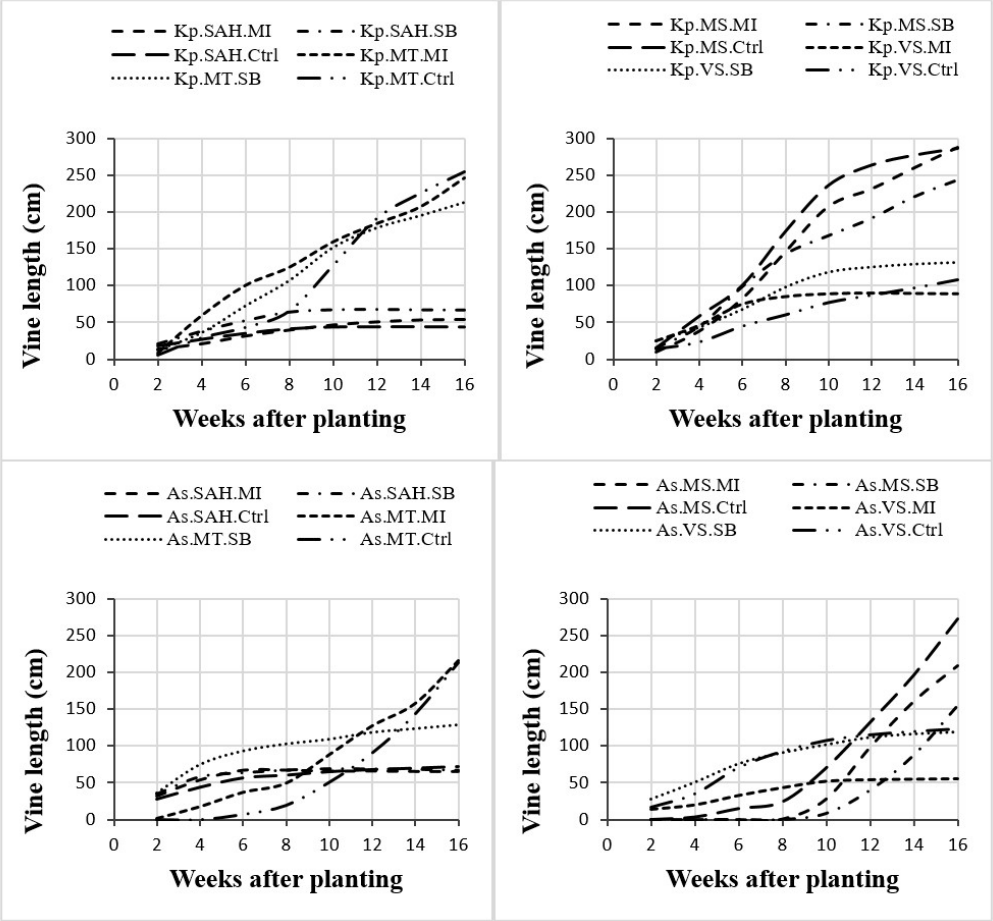


Figure 1. Vine length (cm) of yam planting materials during the first 16 weeks after planting. Values are means of 12 replicates. As = Asiedu, Kp = Kpamyo, MT = Mini-tuber, MS = Minisett, SAH = Semi autotrophic hydroponic seedlings, VS = Vine seedlings, Ctrl = Control, MI = *Meloidoygne incognita*, SB = *Scutellonema bradys*.

0.05) on the fresh tuber yield, while chlorophyll content of leaves and nematode population densities in tubers had negative and non-significant effects on the fresh tuber yield. However, the number of vines, tuber length and tuber diameter had a positive and significant effect on fresh tuber yield ($P < 0.05$), while plant vigour and the number of leaves exhibited a positive and non-significant effect on the fresh tuber yield ($P > 0.05$).

*Storage of yam materials*

Significant losses in tuber weight mainly occurred during the first two months of storage. Three months after storage, a highly significant loss was observed in the weight of the tubers among all variables, especially between nematode inoculated and non-inoculated plants ($P < 0.01$). Generally, the percentage of tuber weight loss in the stored tubers ranged from 16.2 to 82.0%. The lowest value was recorded in tubers from non-inoculated plants, while the highest percentage loss of tuber weight was observed in *S. bradys* inoculated plants. Thus, *S. bradys* was the more damaging nematode in storage causing an average loss of 61.8%, followed by *M. incognita*, which reduced tuber weight by 43.3%. The average percentage loss observed in the non-inoculated plants was 30.1%. Regardless of the nematode, mini-tuber and minisett-derived plants had the highest

Table 2. Mean squares from the ANOVA of yield-related parameters of yam genotypes (TDr 95/19177 'Kpamyo' and TDr 89/02665 'Asiedu'), planting materials (mini-tuber, minisett, vine seedlings from Aeroponics, and SAH seedlings), nematodes (*Meloidogyne incognita* and *Scutellonema bradys*), and their interactions (i.e., the interaction effects among yam genotypes, planting materials, and the two nematode species).

| Source of variation | Degree of freedom | Vine length at harvest (cm) | Plant vigor (1 to 3) | No. of vines | Fresh shoot weight (g) | Dry shoot weight (g) | No. of tubers | Fresh tuber weight (g) | Tuber length (cm) | Tuber diameter (cm) | Fresh root weight (g) | Senescing at harvest (1 to 4) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes (G) | 1 | 25618.9** | 0.0ns | 0.4ns | 1048.8* | 69.4* | 1.5** | 324.0ns | 43.5*** | 162.4* | 618.6* | 0.2ns |
| Planting Materials (PM) | 3 | 257571.7*** | 6.5*** | 0.2ns | 35631.7*** | 1587.2*** | 0.4* | 197.0ns | 39.8*** | 342.9*** | 8465.1*** | 20.5*** |
| Nematodes (N) | 2 | 23269.7*** | 0.1ns | 0.2ns | 2737.0*** | 185.3*** | 0.2ns | 95.8ns | 0.7ns | 113.7* | 212.9ns | 0.1ns |
| G x PM | 3 | 4181.4ns | 0.2ns | 0.2ns | 655.7* | 70.3*** | 0.1ns | 142.3ns | 34.2*** | 21.5ns | 310.4* | 1.3*** |
| G x N | 2 | 12274.8* | 0.0ns | 0.0ns | 48.3ns | 1.5ns | 0.0ns | 19.9ns | 0.3ns | 29.9ns | 32.4ns | 0.1ns |
| PM x N | 6 | 6758.5* | 0.1ns | 0.5** | 710.1** | 33.5** | 0.1ns | 322.3ns | 3.7ns | 56.6ns | 114.0ns | 0.4* |
| G x PM x N | 6 | 1935.7ns | 0.1ns | 0.3* | 193.4ns | 6.1ns | 0.1ns | 143.0ns | 1.7ns | 26.0ns | 43.4ns | 0.3ns |
| Error | 48 | 2420.7 | 0.2 | 0.1 | 177.1 | 8.8 | 0.2 | 265.0 | 2.6 | 24.7 | 73.3 | 0.2 |
| Mean | | 198.5 | 2.4 | 1.5 | 51.5 | 11.6 | 1.4 | 31.7 | 5.6 | 25.3 | 23.2 | 2.3 |
| SE | | 14.3 | 0.1 | 0.1 | 5.0 | 1.1 | 0.1 | 1.8 | 0.3 | 0.8 | 2.5 | 0.1 |

*, **, ***: significantly different at 0.05, 0.01, and 0.001 probability levels respectively; ns: non-significant

Table 3. Pearson's correlation coefficients (r) between yam tuber yield and its yield components.

| | VL[z] | NV | PV | NL | CC | FSW | NT | FTY | TL | TD | TG | TDR | FRW | RG | NPT | NPR | SH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VL | 1.00 | | | | | | | | | | | | | | | | |
| NV | 0.03 | 1.00 | | | | | | | | | | | | | | | |
| PV | 0.89*** | -0.01 | 1.00 | | | | | | | | | | | | | | |
| NL | 0.80*** | 0.27* | 0.69*** | 1.00 | | | | | | | | | | | | | |
| CC | 0.80*** | -0.05 | 0.90*** | 0.52*** | 1.00 | | | | | | | | | | | | |
| FSW | 0.85*** | -0.02 | 0.80** | 0.62*** | 0.82*** | 1.00 | | | | | | | | | | | |
| NT | 0.07 | 0.34** | 0.12 | 0.26* | 0.08 | 0.14 | 1.00 | | | | | | | | | | |
| FTY | 0.37** | 0.25* | 0.39** | 0.52*** | 0.25* | 0.13 | 0.01 | 1.00 | | | | | | | | | |
| TL | -0.15 | 0.04 | -0.10 | 0.01 | -0.16 | -0.33** | -0.36** | 0.47*** | 1.00 | | | | | | | | |
| TD | 0.79*** | 0.01 | 0.69*** | 0.67*** | 0.59*** | 0.65*** | -0.17 | 0.59*** | -0.02 | 1.00 | | | | | | | |
| TG | 0.32* | 0.01 | 0.32* | 0.20 | 0.23* | 0.21 | 0.15 | 0.09 | -0.13 | 0.26* | 1.00 | | | | | | |
| TDR | -0.02 | -0.07 | 0.03 | 0.12 | -0.08 | -0.07 | -0.05 | 0.19 | 0.25* | -0.09 | -0.31* | 1.00 | | | | | |
| FRW | 0.80*** | -0.04 | 0.75*** | 0.63*** | 0.75*** | 0.91*** | 0.23 * | 0.10 | -0.38** | 0.56*** | 0.32* | -0.05 | 1.00 | | | | |
| RG | 0.40** | -0.03 | 0.38** | 0.23* | 0.29* | 0.29* | 0.10 | 0.03 | -0.19 | 0.30* | 0.94*** | -0.28* | 0.42** | 1.00 | | | |
| NPT | 0.17 | 0.06 | 0.16 | 0.15 | 0.09 | 0.07 | 0.10 | 0.27* | -0.03 | 0.28* | 0.40** | 0.09 | 0.16 | 0.33** | 1.00 | | |
| NPR | 0.40** | -0.05 | 0.33** | 0.23* | 0.27* | 0.35** | 0.04 | 0.07 | -0.12 | 0.31* | 0.74*** | -0.19 | 0.39** | 0.81*** | 0.23 | 1.00 | |
| SH | -0.74*** | 0.08 | -0.81*** | -0.42*** | -0.86*** | -0.83*** | -0.11 | -0.11 | 0.38** | -0.54*** | -0.29* | 0.07 | -0.76*** | -0.38** | -0.03 | -0.35** | 1.00 |

[z]VL = Vine length, NV = Number of vines, PV = Plant vigour, NL = Number of leaves, CC = Chlorophyll content, FSW = Fresh shoot weight, NT = Number of tubers, FTY = Fresh tuber yield, TL = Tuber length, TD = Tuber diameter, TG = Tuber galling, TDR = Tuber dry rot); FRW = Fresh root weight, RG = Root galling, NPT = Nematode population in tuber, NPR = Nematode population in root, SH = Senescing at harvest.

*, **, ***: 5, 1, and 0.1% significant respectively.

followed by *M. incognita*, which reduced tuber weight by 43.3%. The average percentage loss observed in the non-inoculated plants was 30.1%. Regardless of the nematode, mini-tuber and minisett-derived plants had the highest percentage loss with 'Asiedu' tubers losing more weight in storage than 'Kpamyo' tubers (Table 4).

*Nematode damage and host status*

No nematodes were recovered from the non-inoculated plants 18 WAI. Low population densities of nematodes were extracted from roots, soil, and tubers of vine and SAH seedlings compared to mini-tuber and minisett. Generally, more nematodes were found in roots, followed by soil; very few nematodes were extracted from tubers at harvest. The total population density of *M. incognita* across genotypes and planting materials at harvest was almost five times the total population density of *S. bradys* (Fig. 2).

Symptoms caused by *M. incognita* included crazy root and galled tubers (Fig. 3a-c), whereas, *S. bradys* caused tuber cracking and dry rot damage (Fig. 3d-e). Non-inoculated plants had normal tubers (Fig. 3f). Tuber galling index before and after storage was significantly higher ($P$ <0.01) in *M. incognita*-inoculated plants compared to non-inoculated plants (Table 5). Mini-tuber- and minisett-derived tubers had heavy galling damage compared to tubers from SAH and vine seedlings. However, no significant effects of yam genotype were observed on the tuber galling index ($P > 0.05$). Tuber dry rot index was significantly higher before and after storage in all *S. bradys*-inoculated plants compared to control plants ($P < 0.01$). However, yam genotypes and planting materials were not significantly different in terms of dry rot index ($P > 0.05$). Tuber cracking was significantly affected by nematode, genotypes, and planting materials before and after storage ($P < 0.05$). Except for vine seedlings-derived plants of both genotypes, *S. bradys*-inoculated plants had the highest tuber cracking compared to non-inoculated plants.

Reproductive factor ranged from 0.1 to 0.9 for SAH and vine seedlings plants, and from 10.9 to 15.8 for mini-tubers and minisetts plants inoculated with *M. incognita* (Table 6). *Meloidogyne incognita* was able to reproduce at least 10 times in the mini-tubers and minisetts, while in SAH and vine seedlings, *M. incognita* population densities decreased compared to the initial population density. A similar trend was observed with *S. bradys* inoculated plants. In general, most plants from mini-tubers and minisetts of both genotypes were susceptible to *M. incognita* except minisetts of 'Asiedu', which was rated as tolerant. However, plants from SAH and vine seedlings of both genotypes were hyper-susceptible to *M. incognita* except vine seedlings of 'Asiedu', which was designated as resistant to *M. incognita*. When inoculated with *S. bradys*, all plants from SAH and vine seedlings of both genotypes were hyper-susceptible while plants from mini-tubers and minisetts, were tolerant except mini-tuber of 'Asiedu', which was susceptible.

## DISCUSSION

The effects of *M. incognita* and *S. bradys* on growth, yield and yield-related components of yam planting materials were investigated. In a screenhouse environment the nematodes resulted in only limited reductions and damage to yam roots and tubers, plant growth, yield, and yield-related components. These findings are consistent with the results reported by Claudius-Cole *et al*. (2020), who observed no significant difference in the number and weight of yam tubers among different yam accessions when comparing the effects of *M. incognita*, *S. bradys*, and *Pratylenchus brachyurus*. It is worth noting that the reduction in yam yield in terms of tuber weight may not always be attributed to nematode damage (Claudius-Cole *et al*., 2020). Overall, for most of the variables measured in this study, mini-tubers and minisetts performed better than SAH and vine seedlings in terms of growth and yield parameters of yam. These findings are consistent with Dama *et al*. (2019), who evaluated five different planting materials of white yam (including mini-tubers, minisetts, and vine seedlings) and found that tubers and minisetts were the best propagules for seed yam tuber production.

Understanding the magnitude and direction of the relationship between yield and its associated traits is crucial for identifying key characteristics that can be utilized for crop improvement (Virender *et al*., 2019). In this

Table 4. Effects of *Meloidogyne incognita* (*Mi*) and *Scutellonema bradys* (*Sb*) on yam tubers during storage.

| Variety | Planting material | Tuber weight loss during storage (g)[y] | | | | Percentage weight woss during storage (%)[y] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | *Mi* | *Sb* | LSD | Control | *Mi* | *Sb* | LSD |
| Asiedu | MT | 14.3 a A[z] | 13.8 a A | 26.8 a A | 20.4 | 45.1 a B | 40.8 ab B | 75.5 a A | 27.2 |
| | MS | 8.7 ab A | 10.2 a A | 12.9 a A | 10.8 | 28.3 ab B | 67.2 a A | 82.0 a A | 17.0 |
| | SAH | 8.5 b B | 10.1 a B | 20.9 a A | 8.7 | 30.7 ab B | 27.5 b B | 71.9 a A | 11.6 |
| | VS | 6.0 b A | 9.1 a A | 19.9 a A | 28.7 | 16.2 b B | 47.3 ab AB | 53.6 a A | 35.5 |
| LSD | | 5.6 | 9.5 | 25.0 | | 18.6 | 33.2 | 28.7 | |
| Kpamyo | MT | 11.0 a B | 37.3 a A | 21.4 a AB | 22.2 | 33.0 a B | 50.3 a AB | 63.2 ab A | 29.1 |
| | MS | 13.9 a AB | 9.8 ab B | 21.6a A | 11.0 | 29.5 a B | 40.8 a B | 74.7 a A | 25.5 |
| | SAH | 9.3 a A | 5.3 b A | 22.9 a A | 23.5 | 28.0 a A | 38.4 a A | 37.5 b A | 28.2 |
| | VS | 8.6 a A | 17.8 ab A | 17.9 a A | 24.0 | 30.3 a A | 33.9 a A | 36.0 b A | 24.1 |
| LSD | | 9.5 | 29.5 | 19.9 | | 18.0 | 29.0 | 29.9 | |
| Mean | | 10.0 B | 14.2 B | 20.5 A | 6.4 | 30.1 C | 43.3 B | 61.8 A | 10.1 |
| SE | | 0.9 | 2.8 | 2.6 | | 2.3 | 3.8 | 4.4 | |

[y]Values are means of 12 replicates.

[z]Means with the same lowercase letter (s) within column per genotype are not significantly different according to the LSD test ($P \leq 0.05$); means with the same uppercase letter (s) within row are not significantly different according to the LSD test ($P \leq 0.05$).

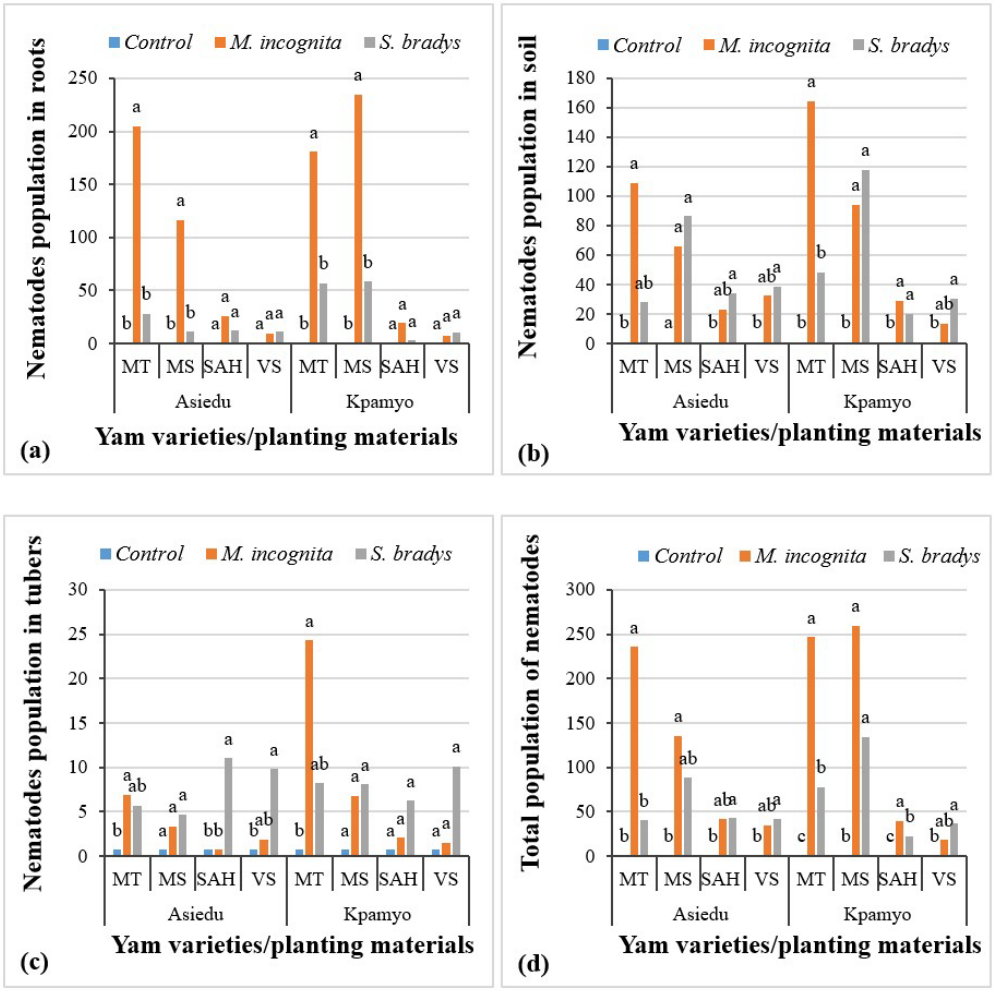


Figure 2. Nematode population densities in soil, roots, and yam tubers from different planting materials at 18 weeks after inoculation. Values were square-root transformed before analysis and are means of 12 replicates. Total population density of nematodes was the summation of total nematodes in soil, roots, and tubers. Bars with the same letter (s) within each planting material are not significantly different according to the LSD test ($P \leq 0.05$). MT = Mini-tuber, MS = Minisett, SAH = Semi autotrophic hydroponic seedlings, VS = Vine seedlings.

study, a positive correlation was observed between fresh tuber yield and several attributes, including number of leaves, tuber diameter, and tuber length, which is consistent with the results of Agbaje *et al*. (2003). These results showed that any positive increase in such traits will contribute to an increase in tuber yield. Results from multi-regression analyses indicated that vine length, number of vines, plant vigour, number of leaves, chlorophyll content, tuber length, and tuber diameter as yield components among the materials studied would be useful as selection indices for yield improvement.

There was weight reduction in the stored yam tubers, and damage by *M. incognita* and *S. bradys* on tubers also increased in the two genotypes during the storage period. During the three months of storage, *M. incognita* and *S. bradys* infections of tubers exacerbated tuber weight loss, with the *S. bradys*-inoculated accessions losing almost twice the weight loss of non-inoculated plants. Though population densities of *S. bradys* were lower compared to those of *M. incognita*, *S. bradys* induced significantly higher weight loss of yam tubers during storage. This was partly due to the dry rot disease in which the normal yam tissue was

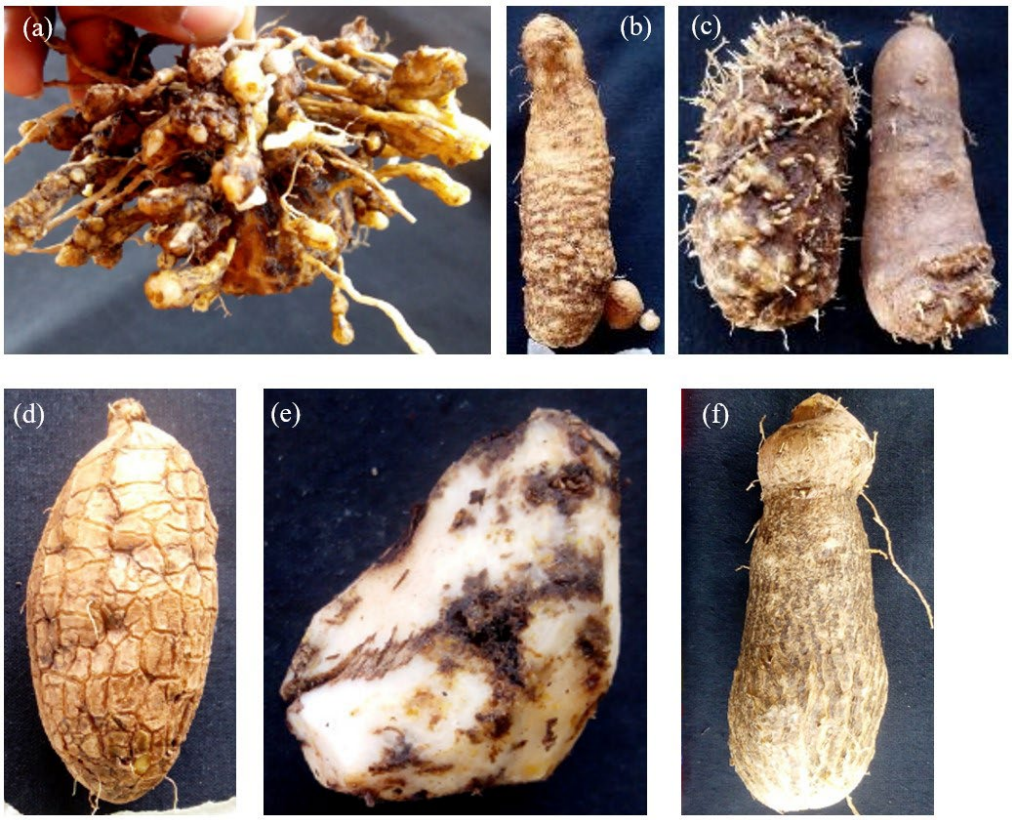


Figure 3. Yam tubers showing symptoms and damage by *Scutellonema bradys* (Sb) and *Meloidogyne incognita* (Mi). (a) mini-tuber of 'Kpamyo' showing crazy root on tuber caused by Mi; (b) Heavily galled and deformed tuber of semi autotrophic hydroponics (SAH) 'Kpamyo' due to Mi; (c) Deformed tubers of SAH 'Asiedu' with crazy root and galling damage due to Mi; (d) Tuber cracking caused by Sb; (e) micro tubers of 'Asiedu' with dry rot damage by Sb; (f) Non inoculated yam tuber from aeroponic system 'Asiedu'.

disrupted as a result of nematode feeding and movement in between cells. *Scutellonema bradys* also causes tuber cracking, which additionally enhances moisture loss from the tubers. The percentage tuber weight losses observed in the current study were higher than those reported by Baimey *et al*. (2006), who observed a maximum weight loss of up to 52% after five months of storage. The relatively higher percentage weight loss observed in the current study is possibly due to a combined effect of the nematodes and the type of planting material as mini-tuber- and minisett-derived tubers lost more weight compared to tubers from SAH and vine seedlings. It is noteworthy that the severity of damage by both nematodes increased during storage.

This study also revealed that all planting materials evaluated exhibited differences in their reactions to the two nematodes based on tuber damage and RF values. Host status ratings of the yam materials in this study partially agreed with those of earlier experiments carried out in nursery bags with the two nematodes using only vine seedlings (Kolombia, 2017). Mini-tuber- and minisett-derived plants were susceptible to *M. incognita*, while they were tolerant to *S. bradys*; plants from SAH and vine seedlings were rated as hyper-susceptible to both nematodes. Generally, neither of the yam genotypes were found to be resistant to *M. incognita* except plants from vine seedlings of 'Asiedu'. This may be explained by the small root system of this type of planting material where the nematode inoculated onto plants could not find any roots to feed on and consequently died.

This study provides relevant information on the response of two yam genotypes to *S. bradys* and *M. incognita* in a screenhouse environment and in storage at Ibadan, Nigeria. Of the four planting materials evaluated in this study,

Table 5. Damage indexes of *Meloidogyne incognita* (*Mi*) and *Scutellonema bradys* (*Sb*) on yam tubers before and after storage.

| Varieties | Planting material[y] | Period of Storage | Damage Index[x] | | | LSD | Tuber Cracking Index[x] | | | LSD |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Con. | *Mi* | *Sb* | | Con. | *Mi* | *Sb* | |
| Asiedu | MT | Before | 1.0 b[z] | 2.6 a | 2.4 a | 1.2 | 1.4 b | 1.4 b | 2.8 a | 1.1 |
| | | After | 1.0 b | 2.4 a | 2.5 a | 1.2 | 1.2 b | 1.4 b | 2.4 a | 0.5 |
| | MS | Before | 1.0 b | 2.3 a | 2.0 ab | 1.2 | 1.3 a | 1.6 a | 2.0 a | 1.4 |
| | | After | 1.0 a | 1.8 a | 1.8 a | 1.3 | 1.2 a | 1.3 a | 1.6 a | 0.8 |
| | SAH | Before | 1.0 b | 1.4 ab | 2.7 a | 1.3 | 1.3 b | 1.7 b | 3.1 a | 1.1 |
| | | After | 1.0 b | 2.3 a | 2.7 a | 1.1 | 1.0 b | 1.6 b | 2.3 a | 0.7 |
| | VS | Before | 1.0 b | 1.5 ab | 1.8 a | 0.7 | 2.8 a | 3.0 a | 2.5 a | 1.0 |
| | | After | 1.0 a | 1.8 a | 2.7 a | 2.1 | 2.4 a | 2.3 a | 2.5 a | 1.4 |
| Kpamyo | MT | Before | 1.0 c | 3.2 a | 1.8 b | 0.6 | 1.4 a | 1.7 a | 1.8 a | 1.0 |
| | | After | 1.0 b | 3.3 a | 1.2 b | 1.3 | 1.3 a | 1.5 a | 1.9 a | 0.7 |
| | MS | Before | 1.0 b | 2.5 a | 2.3 a | 1.0 | 1.3 a | 2.0 a | 2.1 a | 1.7 |
| | | After | 1.0 b | 2.8 a | 1.0 b | 0.4 | 1.4 ab | 1.3 b | 2.0 a | 0.6 |
| | SAH | Before | 1.0 b | 1.5 a | 1.8 a | 0.4 | 2.7 a | 2.9 a | 3.3 a | 1.2 |
| | | After | 1.0 c | 2.1 b | 2.5 a | 0.2 | 1.9 a | 2.6 a | 2.1 a | 1.0 |
| | VS | Before | 1.0 b | 1.4 b | 1.9 a | 0.4 | 2.6 b | 4.1 a | 2.7 b | 0.6 |
| | | After | 1.0 b | 2.3 a | 2.7 a | 0.9 | 1.0 b | 3.7 a | 2.5 ab | 1.5 |

[x]Values are means of 12 replicates

[y]MT = Mini-tuber, MS = Minisett, SAH = Semi autotrophic hydroponic seedlings, VS = Vine seedlings.

[z]Means with the same letter (s) across rows per parameter are not significantly different according to the LSD test ($P \leq 0.05$).

Table 6. Host status after three months of storage based on yam tuber damage index and reproductive factor of *Meloidogyne incognita (Mi)* and *Scutellonema bradys (Sb)*.

| Varieties | Planting material[w] | Damage Index (DI)[x] | | Reproductive Factor (RF)[y] | | Host status[z] | |
|---|---|---|---|---|---|---|---|
| | | *Mi* | *Sb* | *Mi* | *Sb* | *Mi* | *Sb* |
| Asiedu | MT | 2.4 | 2.5 | 10.9 | 5.6 | Susceptible | Susceptible |
| | MS | 1.8 | 1.8 | 11.0 | 2.4 | Tolerant | Tolerant |
| | SAH | 2.3 | 2.7 | 0.1 | 0.4 | Hyper-susceptible | Hyper-susceptible |
| | VS | 1.8 | 2.7 | 0.3 | 0.7 | Resistant | Hyper-susceptible |
| Kpamyo | MT | 3.3 | 1.2 | 11.8 | 4.1 | Susceptible | Tolerant |
| | MS | 2.8 | 1.0 | 15.8 | 2.1 | Susceptible | Tolerant |
| | SAH | 2.1 | 2.5 | 0.1 | 0.5 | Hyper-susceptible | Hyper-susceptible |
| | VS | 2.3 | 2.7 | 0.9 | 0.7 | Hyper-susceptible | Hyper-susceptible |

[w]MT = Mini-tuber, MS = Minisett, SAH = Semi autotrophic hydroponic seeding, VS = Vine seedlings.

[x]Damage Index rating scale by Coyne *et al.* (2014).

[y]RF (Reproductive Factor) = Final population (Pf) / Initial population.

[z]Host status = DI ≤ 2 and RF ≤ 1 = Resistant; DI ≤ 2 and RF > 1 = Tolerant; DI > 2 and RF > 1 = Susceptible; DI > 2 and RF ≤ 1 = Hyper-susceptible (adapted from Sasser *et al.*, 1984).

SAH and vine seedlings exhibited inconsistent responses to both nematodes. This provides evidence that the SAH and vine seedlings require further study and optimization for use in screening yam genotypes for reaction to nematodes. Mini-tubers and minisetts were more reliable as planting materials to be used when screening yam genotypes. Irrespective of genotype and planting material used, growth parameters measured were not significantly different, except for leaf chlorophyll content, which was significantly affected by both nematode species and the planting material used. At harvest, most yield-related parameters varied among planting materials, except for tuber yield which was not impacted by nematode infection or planting material. However, significant losses due to nematodes were observed during tuber storage with *S. bradys* impacting storage more, despite having a lower population density in tubers. Additionally, tuber weight loss was influenced by nematode inoculation, with the highest loss recorded in *S. bradys*-inoculated plants. To mitigate nematode damage in yam cultivation, farmers should consider implementing effective storage management strategies as the nematode damage on tubers is more severe during storage than growth.

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