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Research Application Summary

Population build-up and damage of *Scutellonema bradys* and *Meloidogyne* spp. on local yam cultivars under field and storage conditions in Benin

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Abstract

Plant-parasitic nematodes are some of the most damaging pests in yam production and storage in West Africa. Four local yam cultivars (cv. Aïmon, Baniouré Kpika, Klatchi and Môrôkô) were assesed for their resistance to plant-parasitic nematodes in both fields and storage. Nematode population densities and damage and tuber yields were collected during the field study while nematode populations densities and damage and tuber weight losses were assessed over five month storage period. At harvest, *Meloidogyne* spp. and *S. bradys* population densities were significantly lower in tubers of cv. Klatchi, Môrôkô and Baniouré Kpika than that of cv. Aïmon. Average tuber yields varied between 1325 g/m² (cv. Baniouré Kpika) and 1678 g/m² (cv. Môrôkô). During storage, *Meloidogyne* spp. population densities in tubers were reduced by up to 90% after 3-month storage period and 100% after 5-month storage period. In contrast, a high build-up of *S. bradys* populations was observed in tubers over a 5-month storage period with reproductive factors varying from 4.22 (cv. Aïmon) to 14.04 (cv. Klatchi). Tubers weight losses varied from 51.64% (cv. Môrôkô) to 58.23% (cv. Klatchi) over 5-month storage period. This study pointed out the need for developing integrated nematode management technology to provide quality yam tubers to consumers.

Key words : *Dioscorea* spp., nematode management., post-harvest loss, plant-parasitic nematodes, West Africa

Résumé

Les nématodes phyto-parasites sont parmi les ravageurs les plus nuisibles à la production et au stockage de l'igname en Afrique de l'Ouest. Quatre cultivars locaux d'ignames (cv. Aïmon, Baniouré Kpika, Klatchi et Môrôkô) ont été évalués pour leur résistance aux nématodes phytoparasites dans les champs et lors du stockage. Les densités de population de nématodes, les dommages et les rendements des tubercules ont été collectés pendant l'étude au champ, tandis que les densités de population de nématodes, les dommages et les pertes de poids des tubercules ont été évalués pendant une période de stockage de cinq mois. A la récolte, les densités de population de Meloidogyne spp. et de S. bradys étaient significativement plus faibles dans les tubercules des cv. Klatchi, Môrôkô et Baniouré Kpika que celle du cv. Aïmon. Les rendements moyens des tubercules ont varié entre 1325 g/m² (cv. Baniouré Kpika) et 1678 g/m² (cv. Môrôkô). Pendant le stockage, les densités de population de Meloidogyne spp. dans les tubercules ont été réduites jusqu'à 90% après une période de stockage de 3 mois et de 100% après une période de stockage de 5 mois. En revanche, une forte accumulation de populations de S. bradys a été observée dans les tubercules au cours d'une période de stockage de 5 mois avec des facteurs de reproduction variant de 4,22 (cv. Aïmon) à 14,04 (cv. Klatchi). Les pertes de poids des tubercules ont varié de 51,64 % (cv. Môrôkô) à 58,23 % (cv. Klatchi) sur une période de stockage de 5 mois. Cette étude a souligné la nécessité de développer une technologie de gestion intégrée des nématodes pour fournir des tubercules d'igname de qualité aux consommateurs.

Mots clés: *Dioscorea* spp., gestion des nématodes, perte post-récolte, nématodes phytoparasites, Afrique de l'Ouest.

Introduction

Sub-Saharan Africa is facing many challenges including improvement of agricultural productivity to support its rapidly growing population (Coyne *et al*., 2018). Yam (*Dioscorea* spp.), due to its high production and consumption in West Africa, is one of the strategic crops for food security for more than 60 million people (Adifon *et al*., 2019). In Benin, yam is one of the most important food crops with annual production of 2,944,944 tonnes based on 2018 estimates (FAOSTAT, 2020). While there has been an increase in the production of this crop in Benin over the past decades largely due to the increase in planting area (Adifon *et al*., 2019), yam production faces various constraints such as the decline in the level of soil fertility and attack by pests and diseases.

Previous studies have reported that plant-parasitic nematodes represent a major constraint in yam production and storage (Coyne *et al*., 2006). According to Coyne and Affokpon (2018), *Scutellonema bradys*, *Meloidogyne* spp., *Pratylenchus coffeae* and *P. sudanensis* are considered to be the most economically damaging to yam crop They affect negatively tuber yield and quality in fields as well as in storage. Surveys in yam production areas in Benin revealed the predominance of *Meloidogyne* spp. and *S. bradys* in the proportions of 45% and 44%, respectively (Affokpon *et al*., 2017). *Scutellonema bradys* is responsible for the dry rot and cracks observed on the layer of yam tubers while *Meloidogyne* spp. causes galls on tubers (Coyne and Affokpon, 2018). Moreover, upon infestation, these nematodes weaken the resistance of yam varieties to other pathogens, predisposing them to secondary infections and rapid deterioration. Thus, dry rot of tubers is usually followed by bacterial or fungal wet rot during storage, causing in some cases complete loss of tuber stocks (Coyne and Affokpon, 2018). Therefore, these nematodes represent a serious threat not only to yam producers but also to traders and processors.

Several control methods have been developed against yam parasitic nematodes in the recent years. These include the rational use of chemical nematicides (Opperman *et al*., 2016), yam seed treatment with hot water (Coyne *et al*., 2010), organic and mineral amendments (Baimey *et al*., 2006 ; Morais *et al*., 2016), use of cover crops (Claudius-Cole, 2005; Carmo *et al*., 2014), biological agents (Tchabi *et al*., 2016) and resistant varieties (Coyne and Affokpon, 2018). However, adoption of most of these management strategies is still low due to either their high cost which most smallholder farmers fail to afford, their feasibility for use by the smallholder farmers or their harmful effects on the environment and human health. Likewise, no improved yam variety has been able so far to show complete resistance to key yam parasitic nematodes.

Meanwhile, tuber yield losses continue to increase thus exacerbating food insecurity and poverty among smallholder farmers in yam belt countries. In such situation, developing an integrated and cost-effective management strategy for these parasitic nematodes remains a key challenge while identifying local cultivars that can produce better in nematode-infested fields and store for long period is an urgent need. The current study was primarily undertaken to assess nematode population densities and damage of *S. bradys* and *Meloidogyne* spp. to four yam cultivars in fields and storage conditions in Benin.

Materials and methods

Experimental site. The study was carried out in the experimental site (6°37'30.8"N and 2°13'56.9"E) of the Nematology Research Unit at Sékou, Municipality of Allada in Benin. The field trial was conducted from May 2018 to January 2019 followed by the post-harvest experiment from January to June 2019. The experimental site is located in the sub-humid savannah region with a sub-equatorial climate characterized by two wet seasons from mid-March to mid-July and mid-September to mid-November, alternating with two dry seasons. The annual average rainfall is between 1000 and 1200 mm and the yearly mean temperature between 25 and 30 ^{0}C (Affokpon *et al*., 2011).

Planting material. Four local yam cultivars were assessed: Aïmon (produced in Sub-humid and Guinea Savannah zones), Banioure Kpika (produced in Guinean and Sudan Savannah zones), Klatchi (produced in Sub-humid Savannah zones) and Môrôkô (produced in Sub-humid Savannah and Sudan Savannah zones). Cultivars were selected based on the importance of their production and their high market valueregions of origin. Seed yam, used as planting material, was produced from minisetts in plant-parasitic nematode-free field.

Nematode inoculum. *Scutellonema bradys* and *Meloidogyne* spp., the most damaging parasitic nematodes of yam in Benin as reported by Affokpon *et al*. (2017), were targeted in this study. Prior to planting, initial nematode population densities (Pi) in mounds were determined as 10 *Meloidogyne* spp. and 0 *S. bradys* per 250 cm^3 soil using centrifugation method (Affokpon *et al*., 2011). Due to low nematode Pi, nematodes were artificially inoculated into individual mounds. For *Meloidogyne*, each mound was artificially inoculated with approximately 1900 second stage juveniles and 620 eggs of mixed populations of *M. incognita*, *M. javanica* and *M. enterolobii* by introducing known weights of infested lettuce roots. In the case of *S. bradys*,18 juveniles and adults were also used for artificially inoculation per mound using infested yam peels (Pi) by introducing known weights of infested yam peels close to the base of the plants.

Field experiment details. The field trial was set-up in a randomized complete block design with four replicates. Each replicate consisted of four treatments representing the four yam cultivars. Individual plots, consisting of had four rows of four mounds, were spaced at 1.5 m apart and individual mounds spaced at 1.2 m apart. A week prior to seed yam planting, nematode inocula, described above, were applied by digging a hole at the top of each mound to a depth of 20 cm. Each mound was planted with a single seed yam following the experimental design.

Estimation of nematode population densities at harvest. Nematode population densities were estimated at harvest from tubers and soil samples. To determine nematode population densities in tubers, three tubers were randomly selected per plot. Then, peels were removed from a 5 cm x 5 cm area on four sides of each tuber (Method modified from Coyne *et al.,* 2006) and throughly mixed before 25 g peels were used for nematode extraction. For soil nematodes, a composite soil sample of 250 cm^3 was constituted per plot from soil cores collected in the four central mounds in the area adjacent to the tubers. Nematode extraction from peel or soil samples was done by centrifugation technique as described by Affokpon *et al.* (2011). Thereafter, nematodes were morphologycally identified and counted under a microscope (magnification x20).

Assessment of nematode damage at harvest. Before removing peels, tuber samples were subjected to visual assessment of nematode symptoms. Gall and dry rot symptoms were rated for *Meloidogyne* spp. and *S. bradys*, respectively, on a 1 – 5 scale: 1 = clean tuber; 2 = 1-25% of tuber skin showing targeted symptoms (low level of damage); 3 = 26-50% of tuber skin showing targeted symptoms (low to moderate level of damage); 4 = 51-75% of tuber skin showing targeted symptoms (moderate to severe level of damage); and 5= 76-100% tuber skin showing the targeted symptoms (high level of damage) (Claudius-Cole, 2005).

Estimation of tuber yields at harvest. Tubers were harvested in the four central mounds per plot at 9 weeks after planting. Tuber yields were then determined as the ratio between tuber weight and subsequent area, and expressed as g/m^2.

Storage experiment. At harvested, three tubers were randomly collected from individual plots and further stored in yam barn for five months to assess nematode population build-up and tuber damage. Nematode population densities in tubers were determined at three and five months after harvest (MAH) following the procedure described above for tuber nematode assessment at harvest. At each nematode assessment period, tubers were weighed to estimate the percentage weight loss. Data analysis. Statistical analysis was performed using R software version 4.0.2 (R Core Team, 2015). To meet a normal distribution, data from nematode counts was transformed to log10(x+1) and percentage of tuber weight losses to Arcsin $(x/100)^{1/2}$ (Gomez and Gomez, 1984 ; Dagnelie, 2003). All data were subjected to analysis of variance (one-way or factorial ANOVA where appropriate). Treatment means that were significantly different were separated using Fisher's Least Significant Difference test at $P \leq 0.05$.

Results and discussion

***Meloidogyne* spp. population densities and galling severity at harvest.** At harvest, *Meloidogyne* spp. population density in tubers was significantly higher ($P < 0.05$) for cultivar Aïmon (92 nematodes / 25 g of tuber peels) than for the other three yam cultivars (Table 1). In contrast, nematodes were detected only in soil of cv. Môrôkô at very low density (2 juveniles / 250 cm3 soil). Galling severity of tubers was similar ($P > 0.05$) for all four cultivars (Table 1).

***Scutellonema bradys* population densities and tuber dry rot severity at harvest.** *Scutellonema bradys* population densities in tubers varied significantly ($P < 0.05$) between cultivars (Table 2). Nematodes recovered from tubers of Klatchi, Baniouré Kpika and Môrôkô were significantly lower (less than 50%) than from Aïmon. No *S. bradys* was recovered from the soil samples. Tuber dry rot severity was similar for all cultivars (Table 2).

Table 1. *Meloidogyne* spp. population densities and tuber gall index at harvest

Yam cultivars	Mean *Meloidogyne* spp. population density at harvest[a]		Tuber gall index[b] (1-5)
	Tubers (25 g tuber peel)	Soil (250 cm^3)	
Aïmon	92 b	0 a	1,33 a
Baniouré Kpika	81 a	0 a	1,67 a
Klatchi	78 a	0 a	1,00 a
Môrôkô	78 a	2 a	1,33 a
Fisher	4,431	1,00	0 ,407
Probabilité	0,041	0,441	0,752

Data are means of four replicates. Means in columns followed by different letters are significantly different ($P \leq 0.05$) based on Fisher LSD test using one-way ANOVA.
[a]Statistical analysis was undertaken on log10(x+1) transformed data.
[b]Gall index was assessed on a scale of 1-5 where 1 = clean tuber and 5 = 76-100% tuber skin showing galls symptoms (high level damage) (Claudius-Cole, 2005).

Table 2. *Scutellonema bradys* population densities and tuber dry rot severity at harvest

Yam cultivars	Mean *S. bradys* population density at harvest[a]		Tuber dry rot severity[b] (1-5)
	Tubers (25 g tuber peel)	Soil (250 cm^3)	
Aïmon	180 b	0 a	1,33 a
Baniouré Kpika	71 a	0 a	1,50 a
Klatchi	56 a	0 a	1,33 a
Môrôkô	79 a	0 a	2,17 a
Fisher	5,582	-	1,889
Probabilité	0,0231	-	0,210

Data are means of four replicates. Means in columns followed by different letters are significantly different ($P \leq 0.05$) based on Fisher LSD test using one-way ANOVA.
[a]Statistical analysis was undertaken on log10(x+1) transformed data.
[b]Dry rot severity was assessed on a scale of 1-5 where 1 = clean tuber and 5 = 76-100% tuber layer showing dry rot symptoms (high level damage) (Claudius-Cole, 2005).

The present study showed that none of four cultivars was resistant to *Scutellonema bradys* or *Meloidogyne* spp. Coyne and Affokpon (2018) reported the absence of yam varieties fully resistant to these nematodes among cultivated yam species. Nevertheless, several authors have underlined the great variability between yam cultivars in their degree of susceptibility to these nematodes during yam nematode survey and pot screening for resistance (Coyne *et al.,* 2006; Ettien *et al.,* 2013). This observation was confirmed in our study where the cultivars Baniouré Kpika, Klatchi and Môrôkô showed better resistance to *Meloidogyne* spp. and *S. bradys* than Aïmon.

Tuber yields. Nine months after planting, average tuber yields varied between 1325 g/m² (cv. Baniouré kpika) to 1678 g/m² (cv. Môrôkô) with no significant differences between yam cultivars (P> 0.05) (data not shown). Tuber yields observed in our study were similar to those reported by Coffi (2019). This result confirmed that under favourable conditions, the impact of nematodes on yam production might be rather qualitative and not quantitative (Coyne *et al.,* 2012).

***Meloidogyne* spp. population density in tubers during storage.** All cultivars recorded a significant reduction (between 77 and 90%) in *Meloidogyne* population densities over a 3-month storage period (Figure 2). At 5 MAH, no *Meloidogyne* spp. was recovered from tubers. The drastic reduction of *Meloidogyne* spp. at 3 MAH and their subsequent death at 5 MAH, regardless of cultivars, might be due to a gradual halt in the development of the nematodes. This observation has also been reported by Mudiope *et al.* (2012) who found a gradual reduction in densities of *Meloidogyne* spp. after harvest until the death of all nematodes after four month storage. Considering that *Meloidogyne* species are sedentary endoparasites, they could be killed under the action of tuber secretions which might isolate the parasites in a necrotic area inside the tubers.

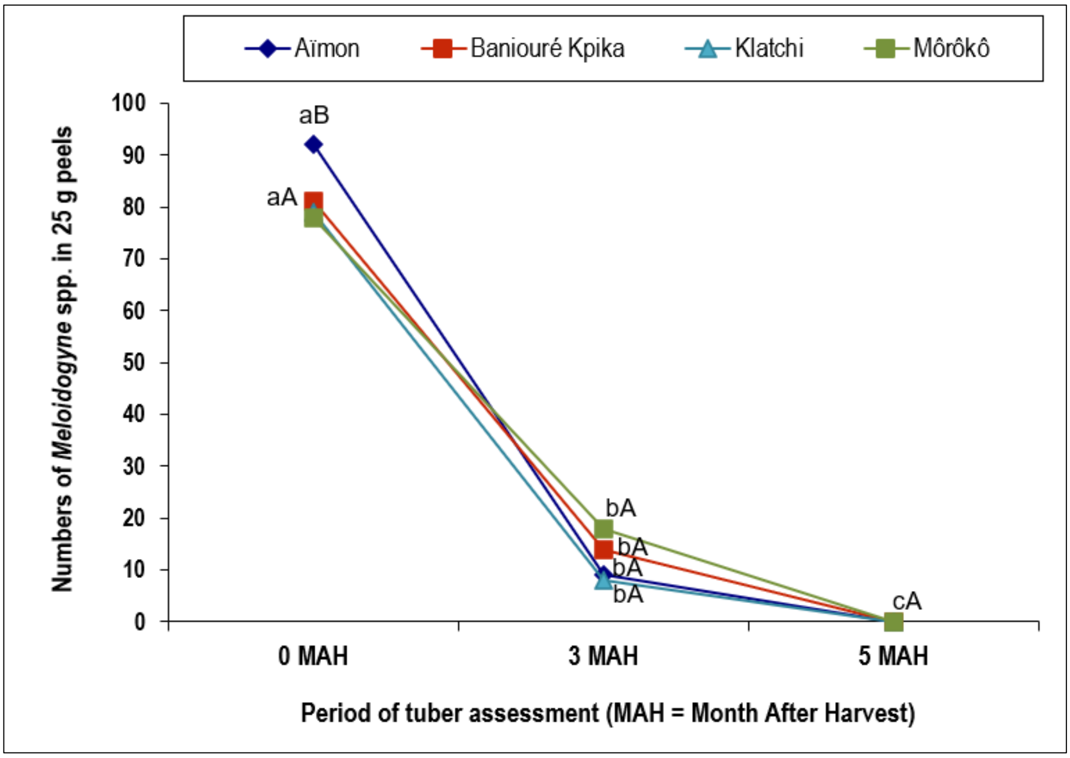

Figure 2. *Meloidogyne* spp. population densities in tubers of four yam cultivars over a 5-month storage period

Data are means of four replicates. Statistical analysis was undertaken on log10(x+1) transformed data, with back-transformed data being presented.

For a given yam cultivar, markers with the same lower case letters indicate no significant differences (P > 0.05) between assessment periods after Fisher's LSD.
For a given assessment period, markers with the same high case letters indicate no significant differences (P > 0.05) between yam cultivars after Fisher's LSD.

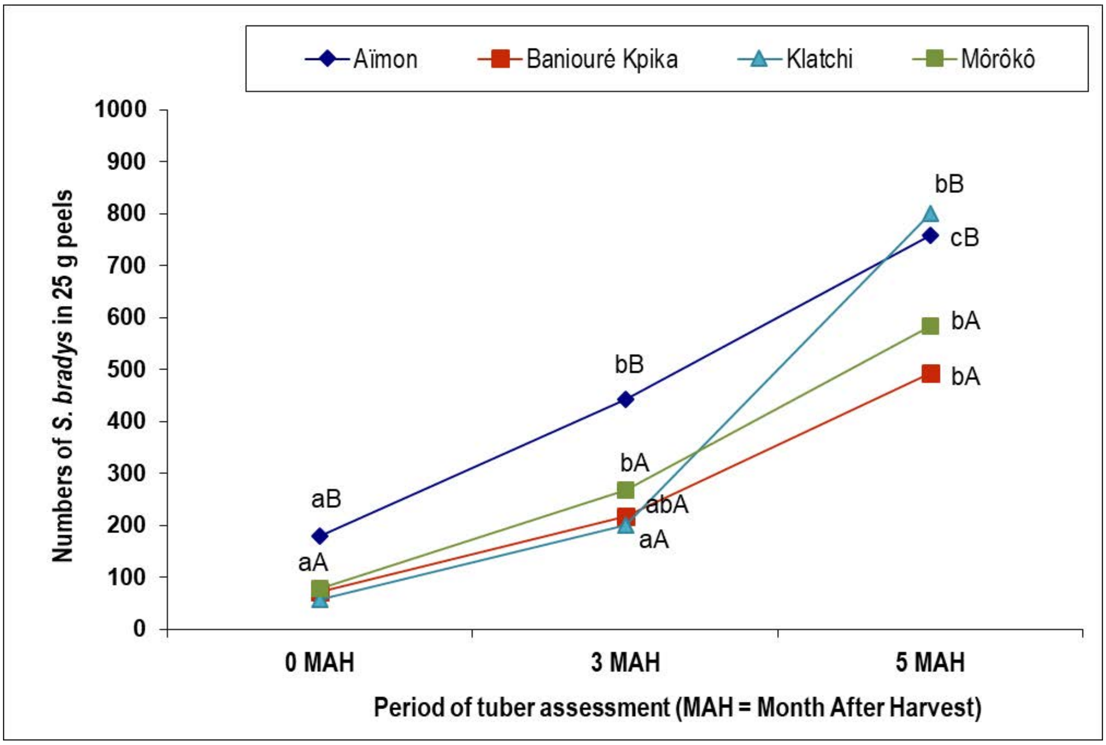

Figure 2. *Meloidogyne* spp. population densities in tubers of four yam cultivars over a 5-month storage period

Data are means of four replicates. Statistical analysis was undertaken on log10(x+1) transformed data, with back-transformed data being presented.
For a given yam cultivar, markers with the same lower case letters indicate no significant differences (P > 0.05) between assessment periods after Fisher's LSD.
For a given assessment period, markers with the same high case letters indicate no significant differences (P > 0.05) between yam cultivars after Fisher's LSD.

***Scutellonema bradys* population density in tubers during storage.** Regardless of the cultivar, *S. bradys* population density significantly increased (P <0.05) over the storage period (Figure 3). However, nematode reproductive factor varied between cultivars with cv. Klatchi showing the highest rate (14.04) and cv. Aïmon the lowest (4.22) after five month storage. For a given period of assesment, nematode population densities varied significantly (P <0.05) between yam cultivars.

After five month storage, tubers from Klatchi and Aïmon had the highest nematode population densities (800 and 759 nematodes per 25 g peels, respectively). The significant increase in the number of *S. bradys* observed in tubers during storage proved that these yam cultivars did not prevent the nematodes from multiplication during storage. *Scutellonema bradys* was known to have the ability to feed and reproduce in yam during storage (Coyne and Affokpon, 2018).

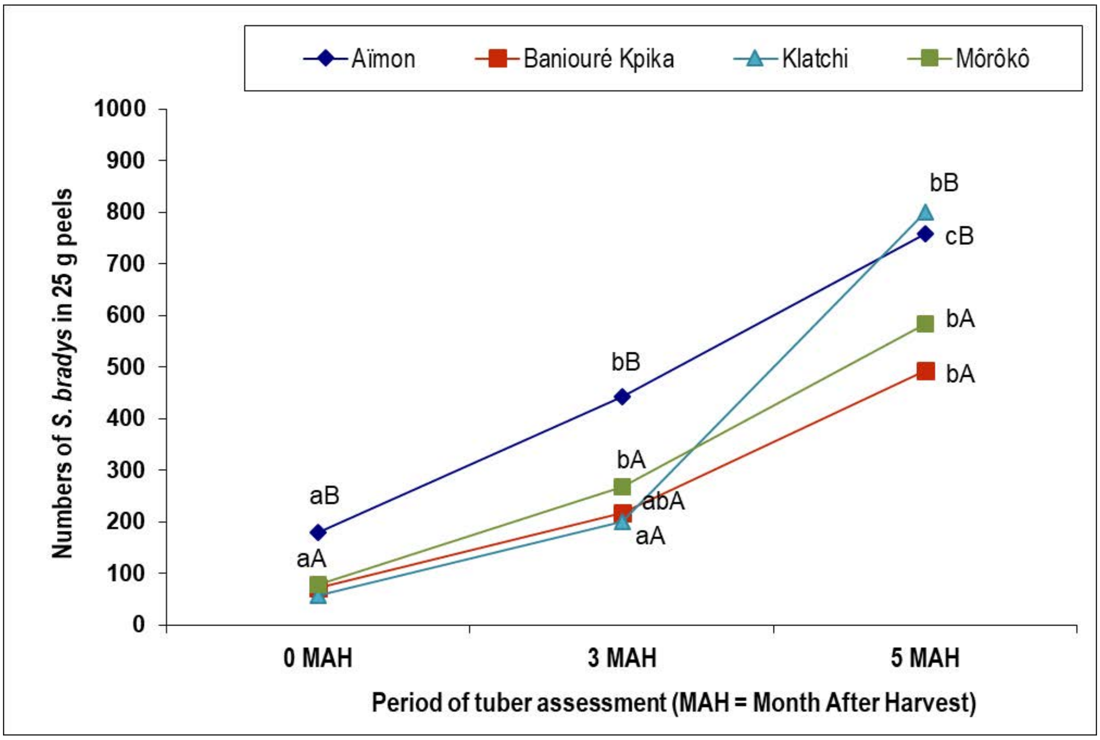

Figure 3. *Scutellonema bradys* population densities in tubers of four yam cultivars over a 5-month storage period

Data are means of four replicates. Statistical analysis was undertaken on log10(x+1) transformed data, with back-transformed data being presented.
For a given yam cultivar, markers with the same lower case letters indicate no signi!cant differences (P > 0.05) between assessment periods after Fisher's LSD.
For a given assessment period, markers with the same high case letters indicate no significant differences (P > 0.05) between yam cultivars.

Tuber weight losses during storage. Losses of tuber weight varied between cultivars and between storage periods (data not shown). Tuber weight reductions were more marked at 5 month storage with Klatchi recording the highest percentage (average of 51.64%). Yam tubers naturally lose water during storage due to metabolic processes (breathing and sweating). In our study, market weight losses were recorded in some cultivars such as Klatchi, suggesting that these metabolic processes would be accentuated due to nematode infestations as explained by Baimey *et al*. (2009).

Conclusion

The current study confirms that identifying yam cultivars fully resistant to key plant-parasitic nematodes remains a major challenge. In addition to their direct effects on yam during growth, nematodes contribute marketly to heavy tuber losses during storage. The management of nematodes on yam should be therefore a priority in terms of improving productivity, but also in terms of improving the quality and the storage duration of yam tubers produced in nematode infested fields. Nevertheless, variability in nematode susceptibility levels oberved between the four cultivars offers the advantage for their use in breeding programs.

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