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African Nightshade and African Spinach Decrease Root-Knot Nematode and Potato Cyst Nematode Soil Infestation in Kenya

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Abstract

Plant-parasitic nematodes, particularly root-knot nematodes (RKN: *Meloidogyne* spp.) and cyst nematodes (CN: *Globodera* and *Heterodera* spp.) cause severe yield reduction in most cultivated crops and are of high economic importance. African nightshade (*Solanum* spp.) and African spinach (*Amaranthus* spp.) are important African indigenous vegetables (AIV) and are rich sources of nutrition and income. However, their host status to plant-parasitic nematodes remains largely speculative. Therefore, a survey was conducted which revealed that *S. villosum* exhibited high root galling, whereas on *S. scabrum*, *A. cruentus*, and *A. dubius* root galling was rare or very low. Additionally, soil collected from the rhizosphere of *S. villosum* and *S. scabrum* contained few cysts of potato cyst nematodes (PCN), and no developing PCN females were observed on the roots of growing plants. Therefore, we studied the dynamics of RKN and PCN on *A. dubius*, *A. cruentus*, *S. scabrum*, and *S. villosum* over 2 years in a field experiment. The effects of AIV crop species on RKN and PCN soil infestation were evaluated using susceptible *S. lycopersicum* or *S. tuberosum*. After first, second, and third cultivation of *A. dubius*, *A. cruentus*, and *S. scabrum*, RKN infestation of the soil decreased by more than 85%, whereas *S. scabrum* and *S. villosum* decreased PCN densities by more than 80%. When cropping susceptible crops, after three seasons of successive cultivation of these AIV, galling index and number of developing PCN females measured on susceptible crops decreased by more than 75%. Wilting and RKN-PCN coinfection incidences also decreased significantly. Here, we present data that support the development of a novel cropping system including African spinach and African nightshade, which reveals a high potential to manage RKN and PCN in an environmentally friendly, effective, and productive way.

Plant-parasitic nematodes, particularly tropical root-knot nematodes (RKN: *Meloidogyne* spp.) and cyst nematodes (CN: *Globodera* and *Heterodera* spp.) are plant pathogens of high economic importance causing severe yield losses in most cultivated crops. The life cycle of RKN and CN includes phases of survival in the soil, invasion of plant roots, and development inside root tissues. On susceptible host plants, rapid multiplication of nematodes inside root tissues leads to the development of disease symptoms such as root galling and cyst formation, respectively (Bartlem et al. 2013; Huang 1985; Perry 1989; Sijmons et al. 1991). This is associated with the formation of specific feeding cells from which they withdraw nutrients for the entire parasitic phase. As nematode-induced disease symptoms may impair water and nutrient uptake by the plant (Dropkin 1972; Jones 1981), yield losses of up to 30% have been reported on several crops such as potato, tomato, eggplant, and melon (Nicol et al. 2011). Yield loss caused by RKN and CN compromise the sustainability of crop production and is an obstacle for attaining food security.

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RKN and CN are obligate root parasites that have evolved highly sophisticated parasitic relationships with their host plants which are based on the formation of specific feeding sites (Hussey and Grundler 1998). The biology of RKN and CN is similar, consisting of developmental stages, egg, four juvenile stages, and the adult stage. However, potato cyst nematodes (PCN) tend to be much more host specific and require host stimulus for egg hatching (Perry and Clark 1977). The parasitic stage of RKN and PCN is entirely dependent on a suitable host plant and is highly vulnerable to the risk of starvation in the absence of a suitable host plant.

Numerous factors have contributed to the widespread occurrence of RKN and PCN in smallholder cropping systems. In Africa, these systems are complicated and often characterized by a simultaneous cultivation of crop species that supports development of RKN and PCN. This is aggravated by lack of awareness and proper nematode diagnostics. Thus, most farmers are unprepared and ill-equipped to respond effectively to the RKN and PCN problem. Consequently, RKN and PCN population densities have increased and their spread facilitated through the distribution of contaminated planting material, irrigation water, rainfall runoff, soil attached to farming implements, animal hooves, and footwear. In addition, intercontinental exchange of propagating material and trade has facilitated the global spread of highly damaging nematode species. This is well illustrated by the introduction of *G. rostochiensis* and *G. pallida* into Kenya (Mburu et al. 2018; Mwangi et al. 2015). Human-aided distribution of nematodes is further supported by the wide spread of RKN such as *M. arenaria*, *M. incognita*, and *M. javanica* in Africa and across the world (Onkendi et al. 2014; Wesemael et al. 2011). In addition, reports of some RKN species such as *M. enterolobii* are also on the rise (Chitambo et al. 2016; Coyne et al. 2018; Onkendi et al. 2014). The occurrence of PCN in smallholder farms is worrisome because RKN is already a heavy burden (Coyne et al. 2018). Accordingly, the presence of RKN and PCN threatens low-income farming systems which are essential for food production and livelihood.

Considering the above-mentioned situation, diminishing the yield loss caused by RKN and PCN is urgently required. The use of nematicides to control plant-parasitic nematodes has been gradually restricted due to undesirable effects on health and the environment

(Zasada et al. 2010). Nevertheless, several techniques, such as soil tillage, plant-derived nematicidal compounds, sanitation, heat-based methods, biological control, green manure, trap crops, cover crops, and host resistance, are available to support the management of RKN and PCN (Bélair et al. 2016; Collange et al. 2011; Pickup 2016; Trudgill et al. 2014; Zasada et al. 2010). However, implementing these control methods alone is often not sufficient. RKN are capable of multiplying on resistant tomato and pepper varieties (Djian-Caporalino et al. 2011; Kiewnick et al. 2009), and certain populations of PCN are capable of multiplying on resistant potato varieties (Fournet et al. 2018). Recently, biological control products have been released to combat nematode problems, but their effects are not always reliable and consistent (Cray et al. 2016; Mwaura et al. 2017; Ward et al. 2012). Innovative strategies to control RKN and PCN are therefore urgently required.

In some African countries, there is renewed interest in African indigenous vegetables (AIV) because of their role in food and nutrition security. AIV such as African spinach (Amaranthaceae: *Amaranthus* spp.) and African nightshade (Solanaceae: *Solanum* spp.) are produced by farmers for food, nutrition, and livelihood security (Cernansky 2015; Dinssa et al. 2016; Gruber 2017; Moyo et al. 2017; Neugart et al. 2017; Ukam et al. 2016). The host status of African spinach and African nightshade to RKN and PCN varies in response to infestation by a range of species and environmental conditions. Several studies demonstrated that *Amaranthus* species such as *A. cruentus* are poor hosts for RKN (Ferris et al. 1993; Nchore et al. 2013; Rodríguez Kábana et al. 1988). A screening of non-tuber-bearing Solanaceous plants showed that *S. nigrum* species were resistant to PCN (Scholte 2000). Meanwhile, some studies indicate that species of African nightshade and African spinach might act as alternative hosts for RKN and PCN (Boydston et al. 2010; Kokalis-Burelle and Rosskopf 2012; Rott et al. 2011). This created a conundrum regarding the precise host status of *Amaranthus* spp. and *Solanum* spp. to RKN and PCN, particularly under African conditions.

Here, we performed a field survey and detailed field trials to study the impact of nematodes on cultivation of AIV. The objectives of the current work were (1) to determine if *Solanum* spp. and *Amaranthus s*pp. are hosts for RKN and PCN, (2) to determine the identity of RKN and PCN parasitizing *Solanum* spp. and *Amaranthus* spp., (3) to determine the population dynamics of RKN and PCN on *Solanum* spp. and *Amaranthus* spp., and (4) to determine the potential of *Solanum* spp. and *Amaranthus* spp. to manage RKN and PCN.

Materials and Methods

Plant-parasitic nematode survey of AIV in Kenya. African nightshade and African spinach are among the key AIV that have been targeted for promotion in Africa for smallholder farmer agroecosystems. We therefore conducted a survey during the period of June and August 2015 to study RKN and PCN root symptoms and soil infestation. Soil and root samples were collected from a total of 25 farms. At each farm, approximately 0.2 ha of land used for vegetable production was sampled. The following numbers of farms were visited in different counties: 4 in Kiambu County, 3 in Nyandarua County, 4 in Machakos County, 6 in Kakamega County, 5 in Murang'a County, and 3 in Busia County. The following crops were sampled: African nightshade (*S. villosum* and *S. scabrum*), African spinach (*A. dubius* and *A. cruentus*), potato (*S. tuberosum*), and tomato (*S. lycopersicum*). Root and soil samples collected from different counties were analyzed for occurrence of RKN and PCN. Crop damage levels were also determined.

RKN and PCN associated with each crop species were determined by uprooting the entire plants. Twelve plants were examined for each crop species at each farm. From this material, 12 samples of roots and

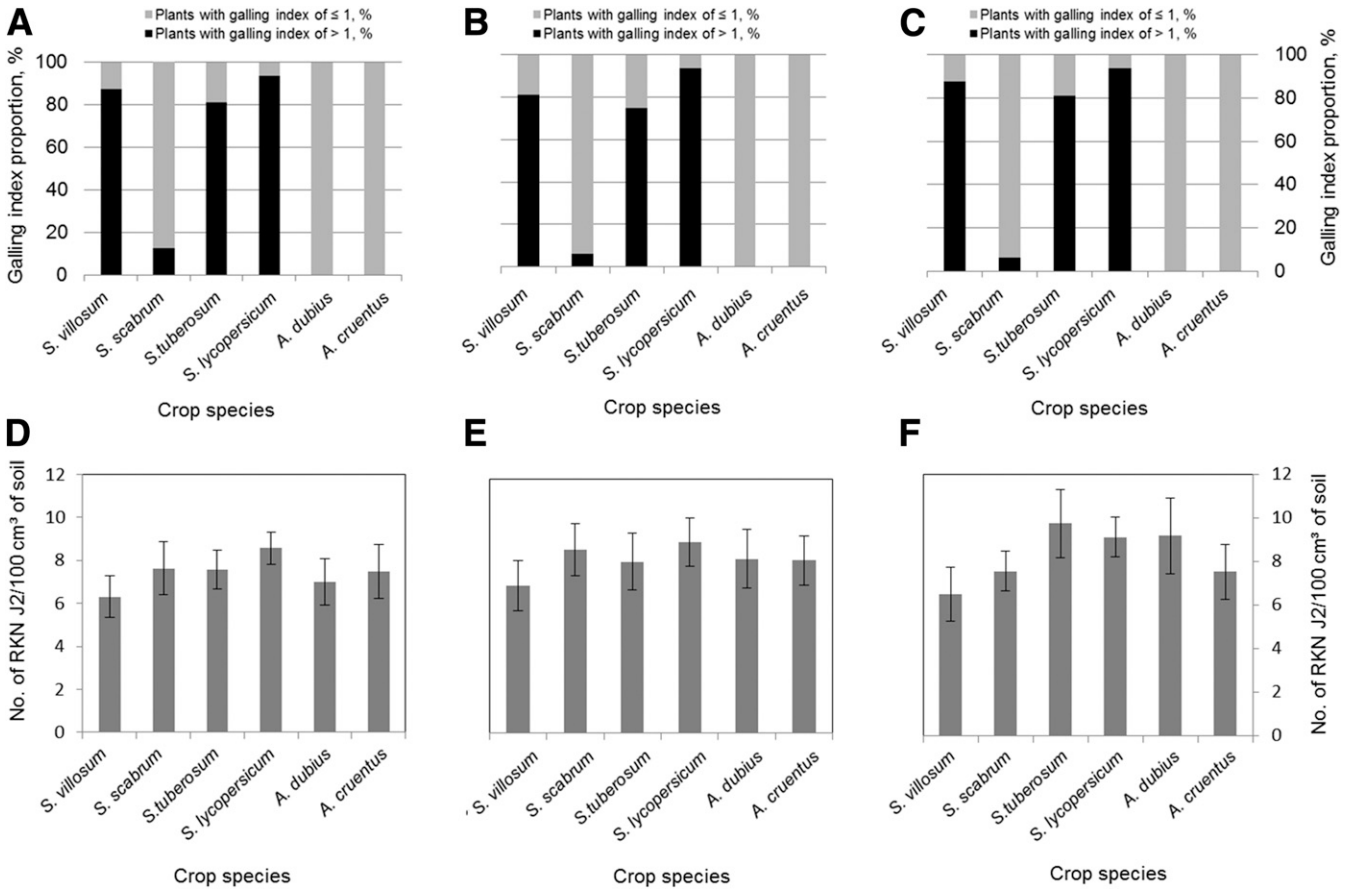

Fig. 1. Root galling proportion: (**A**) Murang'a County, (**B**) Machakos County, and (**C**) Kakamega County. The corresponding root-knot nematode (RKN) soil infestation levels of second-stage infective juveniles (J2) isolated from rhizosphere of different crops: (**D**) Murang'a County, (**E**) Machakos County, and (**F**) Kakamega County. Values of the bars with different letters are significantly different at $P \leq 0.05$. A - Amaranthus, S - Solanum, RKN - root-knot nematodes, J2 - second-stage infective juveniles.

adhering soil were collected at about a 15-cm depth. RKN infestation was assessed as number of galls per plant and using a rating scale of 0 to 5, where 0 = no galls; 1 = 1 to 2; 2 = 3 to 10; 3 = 11 to 30; 4 = 31 to 100; and 5 = more than 100 galls (Taylor and Sasser 1978). PCN infestation was assessed by counting the number of females developed on the roots. Soil samples were mixed thoroughly and sieved before collecting five 100 cm^3 subsamples for nematode extraction. RKN second-stage infective juveniles (J2) were extracted immediately, while for PCN, the soil was air-dried before cyst extraction. For RKN J2 extraction, a modified Baermann technique was used. RKN J2 were distinguished from other plant-parasitic nematodes by their typical morphology (Jepson 1987). RKN J2 were counted in 5 cm^3 counting chambers under a 50× magnification stereo microscope (Leica MZ12, Nussloch, Germany). PCN were extracted using a Fenwick can. Briefly, individual subsamples of 100 cm^3 of soil were rinsed, and cysts collected on the second sieve (250 μm) were transferred to a filter paper. After drying, cysts were counted using a magnification lens. Ten cysts from different crop species were crushed separately in water, and three aliquots of each egg suspension were enumerated under a dissecting microscope at 25–50× magnification. Viability of eggs per cyst was assessed visually according to a standard protocol (Anonymous 2017).

Samples for RKN morphological analysis were analyzed within 72 h after collection. Identity of RKN females was assessed using perineal patterns (Eisenback et al. 1980). Perineal patterns were prepared from 20 females per county. For PCN cysts, cyst shape and color were used to discriminate PCN from other cyst nematodes. Mature females of RKN and PCN stored in absolute ethanol (99%) were used for molecular analysis. In order to confirm the morphological results, NADH dehydrogenase subunit 5 (NAD5) and Cytochrome c oxidase I (COX1) were amplified and sequenced to determine species identity of RKN and PCN. Amplification and sequencing of RKN and PCN were carried out on 15 samples per crop. Briefly, genomic DNA was extracted from females. A single adult female nematode was immersed in 60 μl of sterile water and was thoroughly crushed using a sterile toothpick. Thereafter, DNA was extracted using worm lysis buffer (WLB; 10 mM Tris HCL, pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$, 1 mM DDT, 0.45% Tween 20) and proteinase K. PCR amplification was carried out using Taq DNA polymerase (Qiagen, Germany), with 3 μl of extracted nematode genomic DNA and 0.5 mM of each primer. Primers NAD5F2 (TATTTTTTGTTTGAGATATATTAG) and NAD5R1 (CGTGAATCTTGATTTTCCATTTTT) were used to amplify the NAD5 gene (Janssen et al. 2016). COI gene was amplified using primers JB3 (TTTTTTGGGCATCCTGAGGTTTAT) and JB4.5 (TAAAGAAAGAACATAATGAAAATG) (Derycke et al. 2010). The PCR amplification conditions were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, with a final extension at 72°C for 7 min. The PCR product was visualized on 1% gel stained with GelGreen (Biotium, U.S.A.). Each PCR amplicon was purified and subsequently submitted for direct Sanger sequencing (GATC Biotech, Germany).

Impact of AIV cultivation on population dynamics of RKN and PCN and subsequent nematode management in tomato and potato. The field trials were carried out at an experimental station at Kenya Agricultural & Livestock Research Organization (KALRO 1.1518°S; 36.6852°E) from 2015 to 2017. This site has a climate classified as warm and temperate. The climate at KALRO is considered to be Cfb according to the last revision of Köppen-

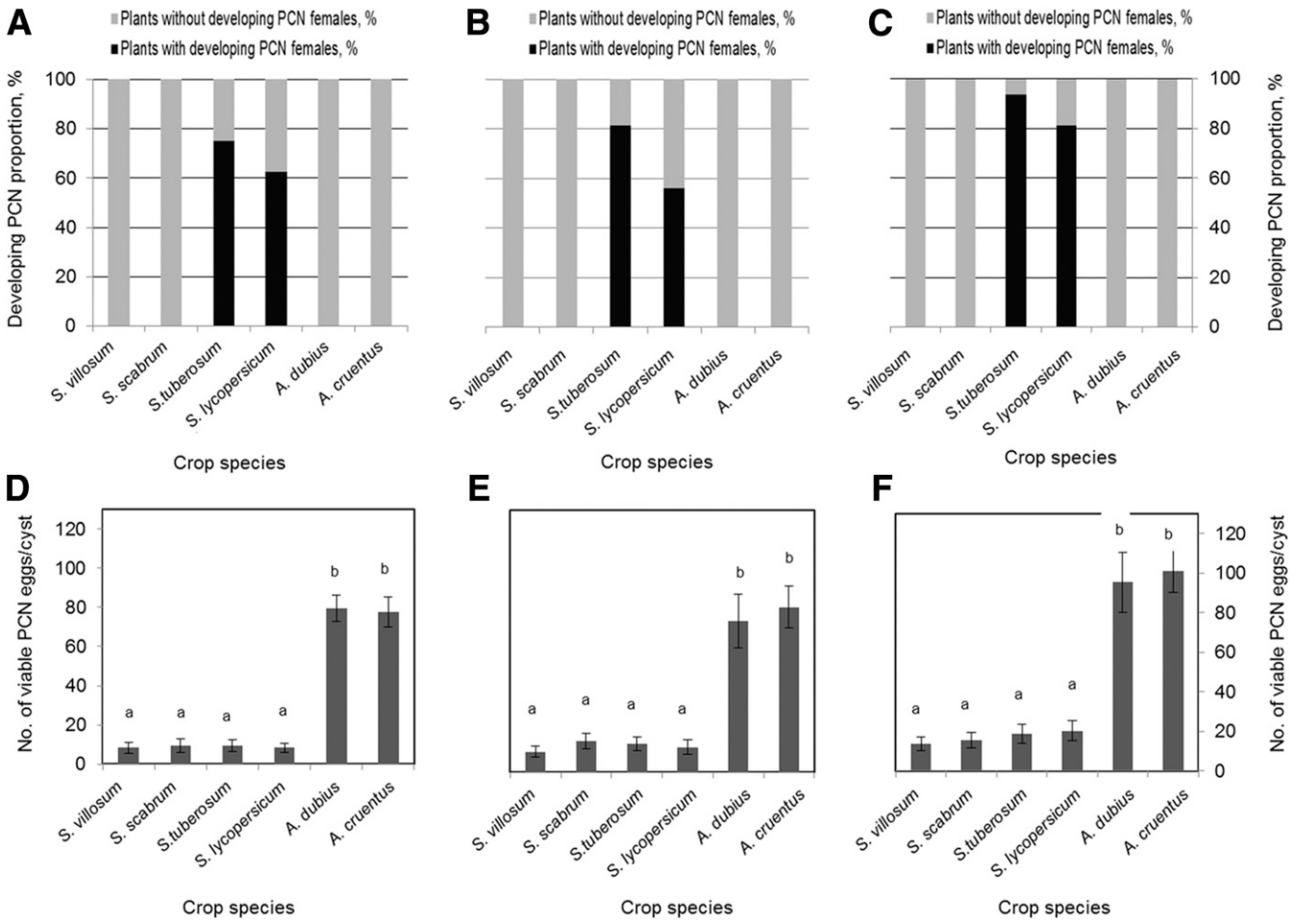

Fig. 2. The proportion of plants with developing potato cyst nematodes (PCN) females: (**A**) Kiambu County, (**B**) Murang'a County, and (**C**) Nyandarua County. The corresponding PCN soil infestation levels isolated from rhizosphere of different crops: (**D**) Murang'a County, (**E**) Kiambu County, and (**F**) Nyandarua County. Values of the bars with different letters are significantly different at $P \leq 0.05$. A - Amaranthus, S - Solanum, PCN - potato cyst nematodes.

Geiger climate classification (Kottek et al. 2006). The average temperature is 15.3°C and the average annual rainfall is 1,263 mm. The sites had natural infestations of PCN and RKN. At the RKN site the following species were present: *M. incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii*, and *M. hapla* as well as an associated *Meloidogyne* species. At the PCN site, *G. rostochiensis* and *G. pallida* were present, as well as an associated *Globodera* species. Both field trials had similar experimental parameters and were conducted across the following seasons: first growing season August to November 2015, second growing season February to May 2016, third growing season August to November 2016, and fourth growing season March to June 2017. Sampling was conducted at preplanting, 6 weeks after planting, and 12 weeks after planting.

The experiment was a randomized complete block design with main plots measuring 10 × 10 m. The main plots were subdivided into subplots of 3 × 3 m. The plots were maintained and used in each growing season. AIV and tomato seeds were sourced from Simlaw Seeds Company Ltd. (Nairobi, Kenya). Seed potatoes were sourced from the seed production unit of KALRO (Tigoni, Kenya). AIV and tomato seeds were sown and raised in a nursery bed for 1 month before being transplanted in the field at a planting density of 14 plants/m^2. Chitted potato tubers were planted at 10 plants/m^2. Well-decomposed cow manure was incorporated at a rate of 4 kg/m^2 before planting. The seedlings were irrigated after transplanting to enhance their establishment. Thereafter, the crop was managed in accordance to the normal farmer's practices. During the dry spell, supplemental irrigation was applied.

The experiment consisted of two phases. In the first phase, the impact of AIV on the population dynamics of RKN and PCN was considered. African nightshade (*S. scabrum* and *S. villosum*) and African spinach (*A. dubius* and *A. cruentus*) were selected for inclusion in the experiment because of their widespread cultivation in the region. The crops were grown for three successive seasons in main plots (i.e., first, second, and third growing seasons). In the second phase of the experiment, the effect of cultivating AIV for three successive seasons on RKN and PCN management was assessed by the cultivation of susceptible crops (*S. lycopersicum* cv. Moneymaker and *S. tuberosum* cv. Shangi) in the fourth growing season. The following cropping sequences were adopted to assess effects of AIV cropping system on RKN: (1) 3 seasons *A. dubius*-followed by-*S. lycopersicum*, (2) 3 seasons *S. villosum*-followed by-*S. lycopersicum*, and (3) 3 seasons *S. scabrum*-followed by-*S. lycopersicum*. The following cropping sequences were adopted to assess the effects of AIV cropping system on PCN: (1) 3 seasons fallow-followed by-*S. tuberosum*, (2) 3 seasons *A. dubius*-followed by-*S. tuberosum*, (3) 3 seasons *S. villosum*-followed by-*S. tuberosum*, and (4) 3 seasons *S. scabrum*-followed by-*S. tuberosum*. Plots that were previously under *A. cruentus* were not included in the second phase. Collection of data on J2 soil population density, galling index, and number of viable cysts was determined as described above. Visual assessment

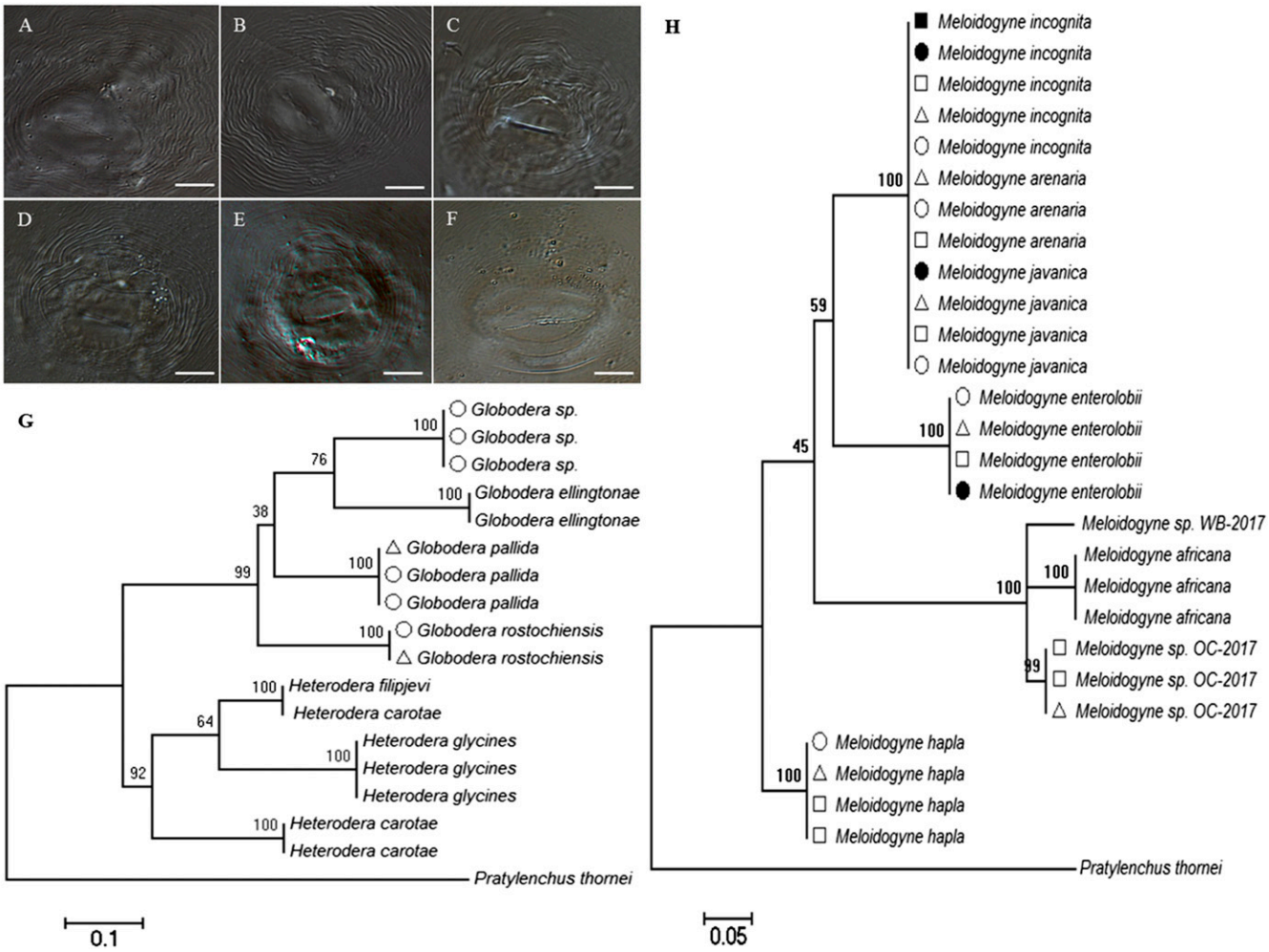

Fig. 3. Determination of root-knot nematodes (RKN) and potato cyst nematode (PCN). (**A-F**) Perineal pattern of RKN isolated from different crop species: (**A-C**) *Meloidogyne incognita*, *M. javanica*, and *M. arenaria*, (**D**) *M. hapla*, (**E**) *M. enterolobii*, and (**F**) *Meloidogyne* sp. (**G**) Phylogenetic tree based on mitochondrial cytochrome oxidase I (COI) sequences of *Globodera* and *Heterodera* spp. (**H**) Phylogenetic tree based on mitochondrial COI sequences of RKN. Values above branches are Maximum Likelihood bootstrap values. For details on phylogenetic reconstruction see Materials and Methods. The following symbols represent the host plant from which the adult nematodes were extracted: ○ *S. tuberosum*, □ *S. villosum*, ■ *A. dubius*, ● *S. scabrum*, and Δ *S. lycopersicum*. A - Amaranthus, S - Solanum. Scale bar = 25 μm.

on plant health was also performed and recorded as slightly wilted, wilted, severely wilted, or nearly dead. To confirm the presence of bacterial wilt (*Ralstonia solanacearum*) on the wilted plants, the crown was cut and placed in water. Bacteria oozed from the exposed vascular elements of wilted plants in 8–12 min, forming milky strands flowing into water and confirming the presence of *R. solanacearum* (Riley et al. 2002). The presence of galls and developing PCN females on the same plant was used to assess RKN-PCN coinfection. Plants were considered coinfected if RKN and PCN females were observed on the roots of the same plant. The number of flowers per plant as a measure for productivity was counted from the same treatments after assessing wilting and coinfection incidences.

Data and statistical analysis. During the survey, RKN crop damage was categorized as the proportion of plants with a galling index ≤1 and >1. For PCN, crop damage was expressed as the proportion of plants with developing PCN females and those without. RKN and PCN crop damage was then expressed as a percentage of the total number of plants sampled per individual crop species. Visited farms were analyzed at the county level.

In the controlled experiment, wilting incidences were calculated for each treatment as the proportion of wilted plants expressed as a percentage of total number of plants sampled. RKN-PCN coinfection incidence was calculated as the proportion of plant roots simultaneously infected by both RKN and PCN expressed as a percentage of a total number of plants sampled. Nematode density data were $\log_{10}(x + 1)$ transformed before analysis in order to meet normality and constant variance assumptions. Repeated measures analysis of variance was used to test the effect of AIV on abundance of J2 of RKN and PCN viable eggs and galling index. Analyses of variance (ANOVA) were conducted to assess the impact of AIV on developing PCN female nematodes, wilting incidences, RKN galling index, and number of flowers on subsequent susceptible crop. A P value ≤0.05 was considered statistically significant. All statistical analyses were performed using SigmaPlot v. 12.5 (Systat Software, San Jose, CA, U.S.A.).

Nematode DNA sequences were first queried via Standard Nucleotide BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to examine whether the sequence would match any species in the database (Altschul et al. 1990). ClustalW (https://www.ebi.ac.uk/Tools/msa/clustalo/) was used for a detailed comparison of obtained DNA sequences with related reference sequences of related species. Phylogenetic analyses were conducted using *MEGA* version 6, and maximum likelihood analyses were conducted with 5,000 bootstrap replicates under the GTR + I + G model according to Tamura et al. (2013).

Results

Plant-parasitic nematode survey of AIV in Kenya. Consistently, *S. lycopersicum*, *S. villosum*, and *S. tuberosum* plants had galling indices of >1, whereas *A. dubius*, *A. cruentus*, and *S. scabrum* plants were associated with galling indices of ≤1 across the counties studied (Fig. 1A–C). A further examination of the soil showed no statistical differences in RKN J2 population densities (Fig. 1D–F). There was a low number of RKN J2 in soil extracted from the root rhizosphere of *A. dubius*, *A. cruentus*, and *S. scabrum* despite consistent galling indices of ≤1 across the counties. Similarly across the counties, *S. tuberosum* and *S. lycopersicum* were associated with developing PCN females on their roots. In contrast, no developing PCN females were recorded on *A. dubius*, *A. cruentus*, *S. scabrum*, and *S. villosum* (Fig. 2A–C). There was no statistical difference in the number of viable PCN eggs/cyst collected from *S. tuberosum*, *S. lycopersicum*, *S. scabrum*, and *S. villosum* (Fig. 2D–F). Although no developing PCN females were observed on *A. dubius* and *A. cruentus*, the number of viable PCN eggs/cyst extracted was high.

Some RKN species could be clearly identified based on female perineal pattern, but morphological differentiation was not possible between *M. javanica*, *M. arenaria*, and *M. incognita*. RKN female perineal patterns from pure cultured samples ranged from the general lateral ridges that divide the dorsal and ventral striae observed on *M. javanica* to high, squarish dorsal arch that is normally observed on *M. incognita* (Fig. 3A–C). *M. hapla* female patterns were characterized by flattened ovoidal shape and subcuticular punctations in the smooth tail terminal area, and the lateral ridges were absent (Fig. 3D). Female perineal patterns of *M. enterolobii* were round to dorso-ventrally ovoid. Lateral lines were not distinguishable

Table 1. Root-knot nematode (RKN) and potato cyst nematode (PCN) species identified from different crops in five counties in Kenya using mtDNA-based technique

	African spinach		Tomato	Potato	African nightshade		Sequences (NAD5/COI)[a]
Crop species	*A. dubius*	*A. cruentus*	*S. lycopersicum*[b]	*S. tuberosum*[c]	*S. scabrum*	*S. villosum*[b]	
County			Nematode species[d]				Accession numbers
Kakamega	×	×	Ma, Mi, and Mj	-	×	Ma, Mi, and Mj	MH399836, MH399835, MH399834, MH399833, MH399843, MH399842, MH399841, MH399825
Kiambu	Mi	Mi	**Ma**, **Me**, Mh, **Mi**, Mj, Msp., and Gr	**Ma**, Me, Mh, Mi, Mj, **Gr**, Gp, and Gsp.	Me and Mj	Ma, Me, **Mh**, Mi, **Mj**, and Msp.	MH005023, MH005027, MH005026, MH005025, MH399805, MH399832, MH399802, MH399817, MH399820, MH399823, MH399822, MF322782
Machakos	×	×	**Ma**, Me, **Mi**, and Mj	-	Me and Mi	Ma, **Me**, **Mi**, and **Mj**	MH399837, MH399845, MH399844, MH399824
Murang'a	×	Mi	Ma, Me, Mh, Mi, Mj, and Gr	Ma, Me, **Mh**, **Mi**, Mj, and Gr	Mj	Ma, **Me**, Mh, **Mi**, and Mj	MH399832, MH399831, MH399829, MH399828, MH399839, MH399838, MH399803, MH399801, MH399816, MF773722
Nyandarua	×	×	**Ma**, **Mh**, Mi, **Mj**, and Gr	**Ma**, Mh, **Mi**, **Mj**, Gr, and Gp	×	Ma, Mh, Mi, and Mj	MH399827, MH005024, MH399830, MH399800, MH399815, MH399818

[a] NAD5 = NADH dehydrogenase subunit 5. COI = Cytochrome c oxidase I.
[b] RKN multiple species infection were detected.
[c] RKN-PCN coinfection were detected.
[d] Ma = *Meloidogyne arenaria*, Me = *M. enterolobii*, Mh = *M. hapla*, Mi = *M. incognita*, Mj = *M. javanica*, Msp. = *Meloidogyne* sp., Gr = *Globodera rostochiensis*, Gp = *G. pallida*, Gsp. = *Globodera* sp. Species in bold were detected in combination from a single plant. × = no RKN or PCN were detected from the roots. - = no crop was observed.

(Fig. 3E). Some of the perineal patterns of a sample of RKN females from *S. villosum* and *S. lycopersicum* did not conform to the normal description of other RKN. These perineal patterns were characterized by very fine striae and very low dorsal arch (Fig. 3F); it could not be assigned to a described species. The spherical brown cysts isolated from the soil and pale yellow females observed on the roots were identified as *G. rostochiensis* or *G. pallida.* In some samples, cysts were light brown to brown in color and subspherical, which did not conform to the normal description of *G. rostochiensis* or *G. pallida*, indicating the occurrence of an associated *Globodera* sp.

DNA sequence blasting and sequence alignment of COI gene identified the following RKN species (Table 1; Fig. 3H); *M. hapla* parasitizing *S. lycopersicum*, *S. tuberosum*, *S. villosum*, and *S. scabrum* (accession numbers KX137039, MH399800–MH399802), *M. enterolobii* parasitizing *S. lycopersicum*, *S. tuberosum*, *S. villosum*, and *S. scabrum* (accession numbers KT936633, MH399803–MH399805), and an associated *Meloidogyne* sp. parasitizing *S. lycopersicum* and *S. villosum* (accession number MF351699). This region failed to differentiate *M. javanica*, *M. incognita*, and *M. arenaria*. Therefore, DNA sequence blasting and sequence alignment of NAD5 gene was used to differentiate these species. The sequence alignment of the NAD5 gene identified *M. javanica* parasitizing *S. lycopersicum*, *S. tuberosum*, *S. villosum*, and *S. scabrum* (accession numbers KY436071, MH399831–MH399837), *M. arenaria* parasitizing *S. lycopersicum*, *S. tuberosum*, *S. villosum*, and *S. scabrum* (accession numbers MH399824–MH399830), and *M. incognita* parasitizing *S. lycopersicum*, *S. tuberosum*, *S. villosum*, *S. scabrum*, *A. dubius*, and *A. cruentus* (accession numbers MH005027, MH399838–MH399845). Phylogenetic analysis based on the COI gene sequence revealed an associated *Meloidogyne* sp. closely related to *M. africana* (Fig. 3H).

Three PCN species were identified based on COI DNA analysis (Table 1; Fig. 3G): *G. rostochiensis* (accession numbers MF773722, MH399815–MH399817), *G. pallida* (accession numbers MH399818–MH399820), and an associated *Globodera* sp. (accession numbers MG438286, MH399821–MH399823), which is closely related to *G. ellingtonae*.

Impact of AIV cultivation on population dynamics of RKN and PCN and subsequent nematode management in tomato and potato. Our survey results showed that RKN parasitism was very low on *A. dubius*, *A. cruentus*, and *S. scabrum*. A field trial was conducted at a site that had natural soil infestation of *M. incognita*, *M. javanica*, *M. hapla*, *M. enterolobii*, and an associated *Meloidogyne* sp. At the beginning of the experiment, no significant differences in RKN population densities existed among the plots assigned to different crop treatments (Fig. 4A). By the end of the first season, population densities of RKN were significantly increased under *S. villosum* and were significantly reduced under *A. dubius*, *A. cruentus*, and *S. scabrum* (Fig. 4A). In seasons 2 and 3, these dynamics continued. However, the successive cultivation of susceptible *S. villosum* promoted RKN soil infestation and root galling (Fig. 4A and B) and severe wilting and root galling (Supplementary Fig. S1D and E). The two species of African spinach achieved a similar RKN suppressive effect. Therefore, plots under *A. cruentus* were not considered in the next experiment. After three successive seasons of cultivating AIV, a RKN-susceptible *S. lycopersicum* cv. Moneymaker planted under AIV resulted in different galling indices and number of flowers at 6 weeks after planting. The galling indices were lower in *S. scabrum* and *A. dubius*, and the number of flowers were higher compared with *S. villosum* (Fig. 4C).

In our survey, no adult PCN females were observed on *S. scabrum* and *S. villosum*, and very few viable PCN eggs were found in cysts extracted from the soil surrounding the roots of these plants. A field

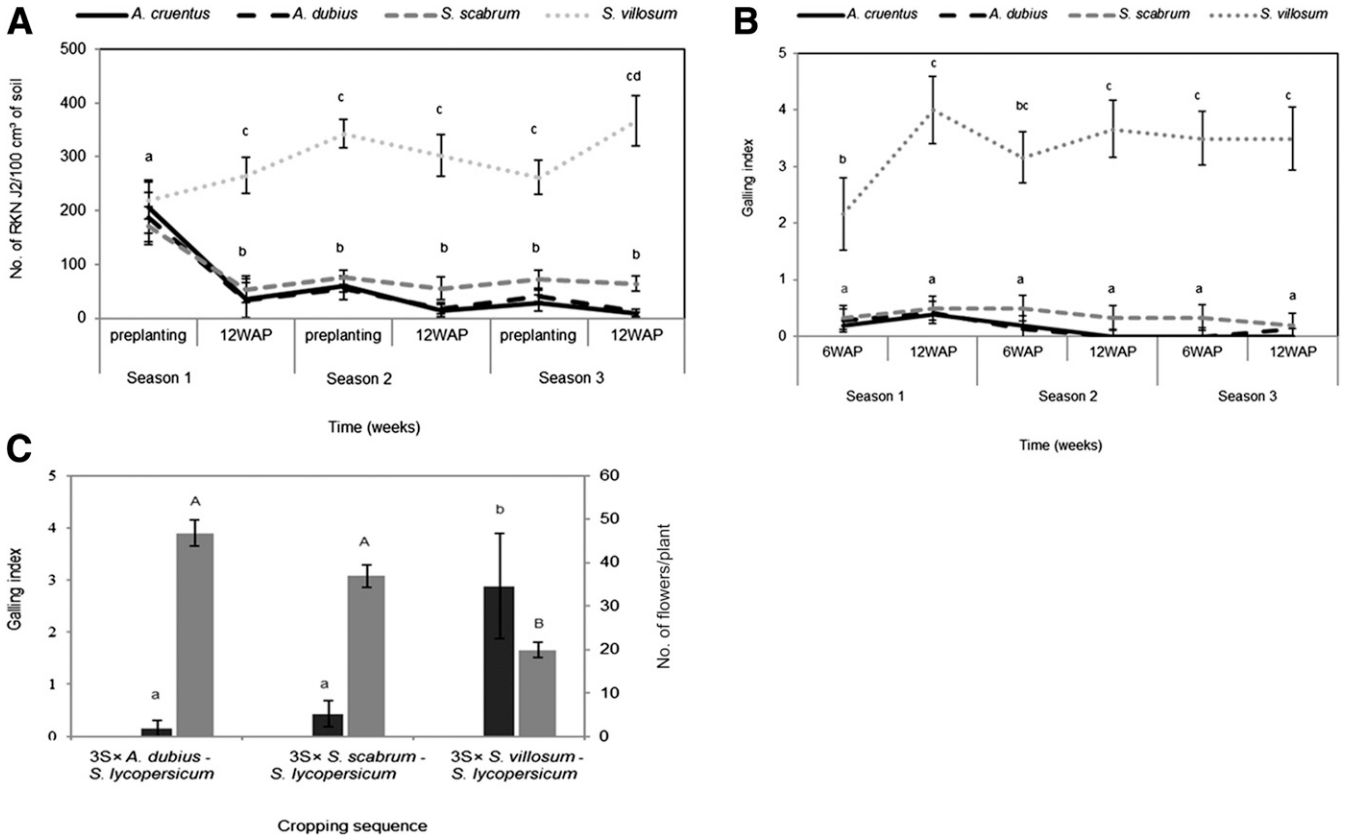

Fig. 4. Impact of African indigenous vegetables (AIV) cultivation on population dynamics of root-knot nematodes (RKN) and subsequent nematode management in tomato. (**A**) RKN population densities under AIV cultivation for three successive seasons; (**B**) Root galling on AIV crops; (**C**) Galling index and the number of flowers on *Solanum lycopersicum* at 6 weeks after planting following three successive (3S) cultivations of AIV. Values of the bars with different letters are significantly different at $P \leq 0.05$ using Tukey posthoc multiple comparisons test. Error bars represent standard deviation of mean. In section C, dark bars represent primary axis and light bars represent secondary axis. A - Amaranthus, S - Solanum.

trial was conducted at a site that had natural soil infestation of *G. rostochiensis*, *G. pallida*, and an associated *Globodera* sp. The same site also had a natural infestation of RKN. At the beginning of the experiment (season 1), there were no significant differences in PCN population densities among the different AIV crops. By the end of season 1, population densities of PCN (measured as viable eggs/cyst) were significantly lower on *S. scabrum* and *S. villosum* compared with *A. dubius* and *A. cruentus* or fallow (Fig. 5A). In seasons 2 and 3, these dynamics continued. After three successive seasons of cultivation of AIV, a PCN-susceptible *S. tuberosum* cv. Shangi had different responses in wilting incidences, number of PCN females, and number of flowers at 6 weeks after planting. The incidence of wilting on *S. tuberosum* was significantly reduced when planted after *S. scabrum* and *S. villosum* (Fig. 5B), but wilted and stunted *S. tuberosum* plants were observed under fallow and *A. dubius*. The number of PCN females was lower in *S. scabrum* and *S. villosum* compared with *A. dubius* and fallow (Fig. 5C). The effect of AIV on RKN-PCN coinfection also varied. Potato plants with both root galls and PCN females were observed. RKN-PCN coinfection on *S. tuberosum* was significantly reduced when planted after *S. scabrum*, *S. villosum*, and *A. dubius*, and the number of flowers was higher in *S. tuberosum* planted after *S. scabrum* (Fig. 5D).

Discussion

Parasitism of crops by RKN and PCN is a major constraint for food production. In Africa, smallholder cropping systems are complicated and often characterized by simultaneous cultivation of crop species that support development of RKN and PCN. Hence, the current farming system increases the economic impact of these nematodes.

AIV including African nightshade (*S. scabrum* and *S. villosum*) and African spinach (*A. dubius* and *A. cruentus*) are neglected and underutilized crops, but have been a part of farming practices and nutrition in traditional societies in Africa. However, there is a lack of information on their host status to RKN and PCN, and so far no study has focused on the impact of these crops on RKN and PCN dynamics. Here, we demonstrate that reintroduction of African nightshade and African spinach into cropping systems can be used to reduce RKN and PCN populations and yield effects on following susceptible crop species.

Implementation of an effective management strategy to control plant-parasitic nematodes requires accurate nematode species identification and their respective host plants (Taylor and Sasser 1978). Thus, in this study, we first characterized the different RKN and PCN infecting *S. scabrum*, *S. villosum*, *S. lycopersicum*, *S. tuberosum*, *A. dubius*, and *A. cruentus*. We employed both morphological and molecular approaches to identify the RKN and PCN species. Current morphological identification procedures were able to differentiate some, but not all, of the RKN. Despite morphological identification failing to give a clear resolution to separate tropical RKN species such as *M. javanica*, *M. arenaria*, and *M. incognita*, the other RKN, *M. hapla*, *M. enterolobii*, and an associated *Meloidogyne* sp. were clearly separated from each other by using perineal patterns (Eisenback et al. 1980). The widely used barcode gene COI reliably differentiated *M. hapla*, *M. enterolobii*, and *Meloidogyne* sp. from the other tropical RKN. The recently identified NAD5 gene fragment DNA marker (Janssen et al. 2016) allowed a reliable identification of the most common tropical RKN *M. javanica*, *M. arenaria*, and *M. incognita*. The phylogenetic position of an associated *Meloidogyne*

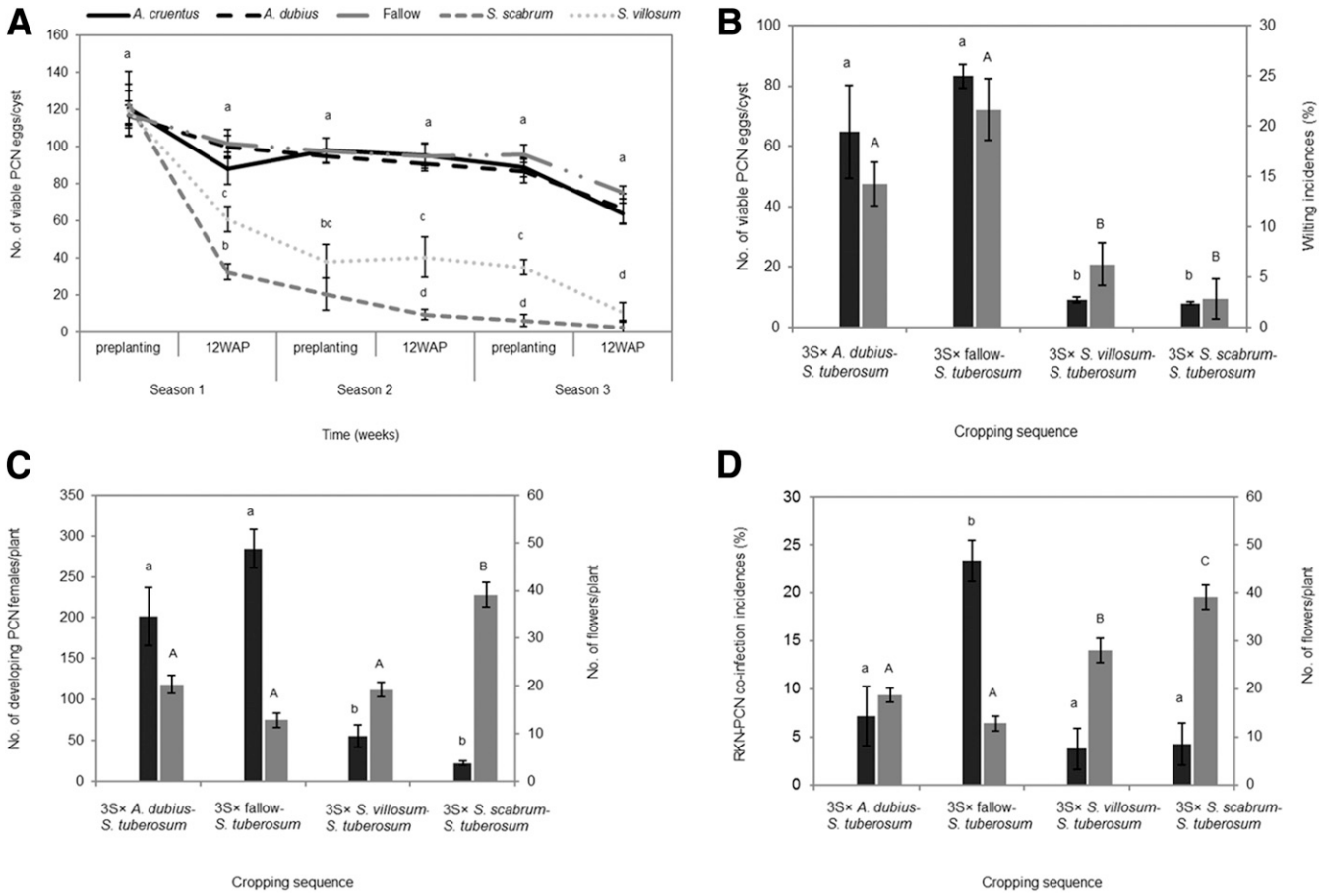

Fig. 5. Impact of African indigenous vegetables (AIV) cultivation on population dynamics of potato cyst nematodes (PCN) and subsequent nematode management in potato. (**A**) PCN population densities under AIV cultivation for three successive seasons; (**B**) PCN population densities and wilting incidences on *Solanum tuberosum* at 6 weeks after planting following three successive (3S) cultivations of AIV; (**C**) Number of developed PCN females and the number of flowers on *S. tuberosum* at 6 weeks after planting following three successive (3S) cultivations of AIV; (**D**). Coinfection incidences and the number of flowers on *S. tuberosum* at 6 weeks after planting following three successive (3S) cultivations of AIV. Values of the bars with different letters are significantly different at $P \leq 0.05$ using Tukey posthoc multiple comparisons test. Error bars represent standard deviation of mean. In section B, C, and D, dark bars represent primary axis and light bars represent secondary axis. A - Amaranthus, S - Solanum.

sp. indicates a closer relationship with *M. africana*, which was previously reported on coffee (Janssen et al. 2017). Furthermore, COI gene sequence identified *G. rostochiensis* and *G. pallida* and reliably differentiates the associated *Globodera* sp. from the other PCN. The presence of *G. rostochiensis* and *G. pallida* parasitizing *S. tuberosum* was recently reported in Kenya (Mburu et al. 2018; Mwangi et al. 2015). The phylogenetic position of the associated *Globodera* sp. indicates a closer relationship with *G. ellingtonae*, which was previously reported on potato (Handoo et al. 2012). Remarkably, most RKN and PCN lineages identified in the current study have a global distribution favoring the hypothesis that spread was aided by humans through agriculture (Castagnone-Sereno et al. 2013).

We detected nematode species such as *M. hapla*, *G. rostochiensis*, and *G. pallida*, which are usually found in temperate climates, in a moderate tropical climate. It shows that these nematode species have the ability to successfully compete with tropical species. It underlines that temperate nematode species have to be considered as pathogens in tropical and subtropical management systems. Temperate RKN such as *M. hapla* have already been reported in subtropical conditions (Chitambo et al. 2018; Meressa et al. 2014), indicating the ability of these nematodes to adapt their temperature or climate preferences. This underpins the need for a proper nematode diagnosis. It also highlights that crops have to be resistant against several RKN and PCN species for nematode management under the current situation in many parts of Africa.

RKN were mainly associated with *S. villosum*, *S. lycopersicum*, and *S. tuberosum*, indicating that RKN are capable of causing damage on these crop species. In fact, they had been previously reported as good hosts for RKN (Nchore et al. 2013; Onkendi et al. 2014; Sikora and Fernandez 2005). By contrast, *A. dubius* and *A. cruentus* showed resistance to the studied RKN species, and only *M. incognita* were able to induce very few galls on these species. In the literature, the host status of *Amaranthus* spp. to RKN is not clear. Previously, it was shown that several *Amaranthus* species were resistant to RKN (Babatola and Awoderu 1986; Reddy et al. 1980). Later, Ferris et al. (1993) found that *A. caudatus*, *A. hypochondriacus*, and *A. cruentus* were nonhosts to *M. chitwoodi*, and *A. retroflexus* was rated as a poor host for *M. chitwoodi*. In contrast, a recent study indicated that *A. tricolor* supports *M. incognita* reproduction (Vaingankar et al. 2018). This suggests that the genus *Amaranthus* is highly diverse and is composed of many species and possibly varieties that vary in response to *Meloidogyne* infection. In principle, there are three types of plant responses to *Meloidogyne* infection: (i) susceptible – indicated by nematode development and plant damage; (ii) resistant – causing low root galling in *A. dubius* and *A. cruentus* resulting in low nematode reproduction; and (iii) tolerant – showing low reduction of root and shoot traits but strongly supporting nematode development. The latter was described in a recent study which demonstrated that *A. tricolor* genotype IC-0598184 performed well after infection by *M incognita* (Vaingankar et al. 2018). The fact that *A. dubius and A cruentus* were resistant to RKN identified in this study make them ideal candidates for RKN management.

PCN identified in this study were only associated with *S. lycopersicum* and *S. tuberosum*, but not *S. scabrum* and *S. villosum*, indicating resistance in these crops. *S. scabrum* and *S. villosum* belongs to the Solanaceae family, and Scholte (2000) reported the ability of non-tuber-bearing Solanaceae plants to stimulate PCN hatching. In contrast, non-Solanaceae plants such as *A. dubius* and *A. cruentus* do not have an effect on PCN hatching. Thus, after successive cultivation of African nightshade, the number of developing PCN observed on *S. tuberosum* was reduced. Dandurand et al. (2013) used a resistant trap crop, *Solanum sisymbriifolium* to control PCN, and this approach decreased PCN cyst infestation in the soil by more than 90%. Related nightshade belonging to the non-tuber-bearing species in the *Solanum* genus have been demonstrated to stimulate PCN egg hatch and to prevent further development of PCN. We found a similar effect of the analyzed nightshades in our study.

Simultaneous occurrence of two or more different nematode species renders host resistance deployed against one species ineffective, because another species is not affected by the resistance response. It is known that tomato cultivars carrying *Mi-1.2* gene introgressed from *Solanum peruvianum* are resistant to *M. incognita*, *M. javanica*, and *M. aranaria*, but not *M. enterolobii* (Kiewnick et al. 2009). In Africa, multiple species of RKN infections have been reported (Chitambo et al. 2018; Kolombia et al. 2017), indicating that multiple nematode infections are ubiquitous in Africa, but too often ignored. Our results indicate that *A. dubius* and *A. cruentus* are resistant to the studied RKN species including *M. enterolobii.* This species has been reported to overcome resistance of most cultivated crops carrying resistance genes against other RKN, including resistant cotton, sweet potato, tomatoes (*Mi-1* gene), soybean (*Mir1* gene), potato (*Mh* gene), sweet pepper (*Tabasco* gene), bell pepper (*N* gene), and cowpea (*Rk* gene) (Berthou et al. 2003; Brito et al. 2007; Castagnone-Sereno 2012; Cetintas et al. 2008; Yang and Eisenback 1983).

Our studies indicate that AIV resistant to RKN and/or PCN are ideal cover crops for management of both of the groups of nematodes or can be used as rotational crops, relay crops etc. as well. Integrating these crops in the smallholder cropping system as cover crops, rotational crops, or relay crops has several advantages including nematode control and dietary diversification. Elsewhere, cover crops are used in various production systems to provide many benefits such as pest and disease management, addition of organic matter to soil, and increased productivity of cash crops. For example, the use of cover crops in the Brassicaceae family such as oilseed radish, white mustard, and winter rapeseed decreased sugar beet cyst nematode population densities (Lelivelt and Hoogendoorn 1993; Wen et al. 2017).

In summary, we have shown that accurate diagnosis of RKN and PCN will help provide proper implementation of an effective management decision. We identified *A. dubius* and *S. scabrum* with the ability to suppress RKN species identified in this study, whereas *S. scabrum* and *S. villosum* suppressed the identified PCN species. *S. scabrum* suppressed both RKN and PCN identified in this study. According to our results, these crop species can be used to manage RKN and PCN. We recommend that growers intending to simultaneously control PCN and RKN should use *S. scabrum*. Although *S. villosum* was able to suppress PCN, it is highly susceptible to RKN and should not be used where RKN are detected. This finding is a major relief to the resource constrained smallholder farmers who are overburdened by plant-parasitic nematodes, pests, and diseases as well as nutritional challenges. The reintroduction of AIV species into the existing cropping systems may be a way of promoting agro-biodiversity to improve resilience to plant-parasitic nematodes, pests, and diseases as well as dietary diversification in Africa. Therefore, this approach can be used as a simple management strategy for RKN and PCN in an environmentally friendly, effective, and productive way.

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