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ORIGINAL ARTICLE

# Effect of single or dual inoculation of the arbuscular mycorrhizal fungus *Glomus mosseae* and root-nodulating rhizobacteria on reproduction of the burrowing nematode *Radopholus similis* on non-leguminous and leguminous banana intercrops

**Lieselot Van der Veken[1] · Ma.Teodora N. Cabasan[2] · Annemie Elsen[3,4] · Rony Swennen[5,6] · Dirk De Waele[6,7]**

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**Abstract**
The bio-protective effect of either single or dual mycorrhizal (AMF) and rhizobial colonisation of the roots of non-leguminous and leguminous banana intercrops differing in host response to *Radopholus similis* on the reproduction of this important migratory endoparasitic nematode was examined. Included in the study were sorgho-Sudan grass (good *R. similis* host), sweet potato and common bean (intermediate hosts), soybean and sunn hemp (poor hosts), and marigold (non-host). Significant plant growth-promoting effect of single AMF and rhizobial colonisation in the good and intermediate *R. similis* hosts sorgho-Sudangrass (AMF) and common bean (AMF and rhizobium), respectively, was observed whereas this plant growth-promoting effect was absent in the other intercrops with the exception of sunn hemp with significant plant growth-promoting effect of AMF colonisation on fresh root weight. An additive plant growth-promoting effect of dual AMF and rhizobial colonisation (on fresh shoot weight) was only observed in the poor *R. similis* host soybean. Single AMF and rhizobial colonisation also resulted in a significant bio-protective effect against *R. similis* in sorgho-Sudangrass (AMF), sweet potato cv. Inzovu (AMF) and common bean (AMF and rhizobium). The growth-promoting and bio-protective effects of AMF colonisation were clearly present in the good and intermediate *R. similis* hosts with moderate to high relative mycorrhizal dependency (RMD) values ranging from 47% (sorgho-Sudangrass) to 65% (common bean) but absent in the intermediate *R. similis* host sweet potato, which had a negative RMD value, and in the poor and non- *R. similis* hosts. Overall, no suppressive effect of *R. similis* infection on AMF and rhizobial colonisation was observed except in soybean and sunn hemp in which AMF colonisation was significantly reduced.

**Keywords** Banana intercrops · Biological control agent · *Bradyrhizobium japonicum* · *Glomus mossea* · Migratory endoparasitic nematode · *Musa* · Plant growth-promoting fungi · *Rhizobium etli* · Root-nodulation rhizobacteria

✉ Ma.Teodora N. Cabasan
mtncabasan@usm.edu.ph

[1] Pro Terra Agro, Bijlokstraat 144, 3020 Herent, Belgium

[2] Department of Biological Sciences, College of Science and Mathematics, University of Southern Mindanao, 9407 Kabacan, Cotabato, Philippines

[3] Soil Service of Belgium, W. de Croylaan 48, 3001 Heverlee, Belgium

[4] Department of Biology, Faculty of Sciences, Ghent University, K.L. Ledeganckstraat 35, 9000 Gent, Belgium

[5] Laboratory of Tropical Crop Improvement, Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven, Willem de Croylaan 42, 3001 Heverlee, Belgium

[6] International Institute of Tropical Agriculture (IITA), Arusha, Tanzania

[7] Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

## Introduction

Plant-parasitic nematodes are among the most important soil-borne pathogens of agricultural crops and can cause serious crop losses worldwide (Koenning et al. 1999; Nicol et al. 2011; Bernard et al. 2017). On banana (*Musa* spp.), *Radopholus similis* Cobb 1893, the burrowing nematode, is considered the most widespread and most damaging plant-parasitic nematode, causing severe yield losses in most tropical and subtropical regions of the world (Sikora et al. 2018). This endoparasite migrates through the cortical parenchyma of the root system of the host plant destroys the cells on which it feeds and leaves behind cavities (Haegeman et al. 2010; Duncan and Moens 2013). The most typical symptom caused by *R. similis* is root-lesion: purplish-black necrotic area which usually extends throughout the cortex but not in the stele (Speijer and De Waele 1997). *Radopholus similis* infection can cause root rot, fewer and smaller leaves, premature defoliation, lengthening of the vegetative growth duration, lower bunch weight, toppling of mature plants, and shortening of the plantation life span (Speijer and De Waele 1997; Talwana et al. 2003). In commercial tropical banana plantations, control of *R. similis* is based on two to four nematicide treatments each year (Haegeman et al. 2010). However, banana production takes place in homestead and community gardens, and smallholder fields (Lescot 2013) in which control of *R. similis* is mostly not practiced. In these small-scale production systems, two main banana-based cropping systems can be identified: sole cropping of banana or intercropping. Intercropping is the practice of growing two or more crops simultaneously for a substantial part of their cropping cycles. Banana are often grown together with both non-leguminous and leguminous crops. Important non-leguminous banana intercrops are coffee (*Coffea* sp.), cacao (*Theobroma cacao* L.), cassava (*Manihot esculenta* Crantz), cocoyam (*Colocasia esculenta* L.), maize (*Zea mays* L.), potato (*Solanum tuberosum* L.), sweet potato (*Ipomoea batatas* L.), sorghum (*Sorghum bicolor* L.) and yam (*Dioscorea alata* L.) (Liu et al. 1999). Important leguminous banana intercrops are cowpea (*Vigna unguiculata* L.), common bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* L. Merr.) (Okigbo and Greenland 1976; Aiyelaagbe and Jolaoso 1994; Liu et al. 1999). Among the many advantages of intercropping over conventional monocropping are that the increased plant diversity in the field may increase the incidence of natural enemies of soil-borne pathogens, reduce pathogen pressure and enhance crop production (Poveda et al. 2008). In these low-input cropping systems, biological control agents (BCA's), beneficial soil-borne micro-organisms that interact with roots and improve plant health (Molinari and Leonetti 2019; Tian et al. 2020) offer a promising alternative for the use of pesticides. Potential BCA's are arbuscular mycorrhizal fungi (AMF) and root-nodulating rhizobacteria.

AMF belong to the BCA group of plant growth-promoting fungi (PGPF; Kumar 2016; Verma et al. 2019; Tian et al. 2020). AMF are obligate root symbionts estimated to colonise more than 80% of all land plant species. They improve plant growth and health by growing a mycelium that emerges from the root system, penetrates the soil and increases the uptake of nutrients and water by their host. In return, AMF receive photosynthetic products from their host (Chen et al. 2018). AMF can also enhance the tolerance and resistance of their hosts to abiotic (*e.g.,* drought, aluminium toxicity, phosphorus deficiency) and biotic (*e.g.,* soil-borne diseases) stresses (Jeffries et al. 2003; Smith et al. 2010; Barea et al. 2013; Pozo et al. 2013; Bücking and Kafle 2015; Berruti et al. 2016; Chen et al. 2018; Molinari and Leonetti 2019; Verma et al. 2019; Tian et al. 2020). AMF have been reported to stimulate banana root and shoot growth (Umesh et al. 1988; Jaizme-Vega et al. 1997; Jaizme-Vega and Pinochet 1997; Pinochet et al. 1997; Gañán et al. 2011; Koffi et al. 2013).

AMF and plant-parasitic nematodes commonly co-inhabit the rhizosphere of their host plants. Numerous records of bio-protection by AMF against sedentary endoparasitic nematodes, mainly root-knot nematodes (*Meloidogyne* spp.), have been published whereas fewer records of bio-protection by AMF against migratory endoparasitic nematodes exist. Bio-protective effects of AMF against sedentary endoparasitic nematodes mainly involve the suppression of root galling and root-knot nematode reproduction in a wide variety of crops (*e.g.,* banana, grapevine, olive, tomato) (Siddiqui and Mahmood 1995; Jaizme-Vega et al. 1997; Calvet et al. 2001; Diedhiou et al. 2003; Hol and Cook 2005; Castillo et al. 2006; Vos et al. 2012a,b,c,d, 2013). Siddiqui and Mahmood (1995) reported bio-protective effects of *Glomus* spp. against *R. similis* and *Tylenchulus semipenetrans* Cobb, 1913, a semi-endoparasitic nematode, in citrus (*Citrus limon* L.) and against *R. similis* in banana. In addition, the bio-protective effect of AMF against the major migratory endoparasitic nematodes of banana (including *R. similis*, *Pratylenchus coffeae* Zimmermann, 1898 and *Pratylenchus goodeyi* Sher & Allen, 1953) has been demonstrated repeatedly (Umesh et al. 1988; Jaizme-Vega and Pinochet 1997; Jaizme-Vega et al. 1997; Elsen et al. 2003a,b, 2008, 2009; Koffi et al. 2013).

Root-nodulating rhizobacteria (rhizobia) belong to another group of BCA's, the plant growth-promoting rhizobacteria (PGPR; Reddy 2014; Kumar 2016; Verma et al. 2019; Tian et al. 2020) which are also able to enhance the tolerance of their hosts to abiotic and biotic stresses (Reddy 2014; Choudhary et al. 2016; Shaik et al. 2016; Vejan et al. 2016; Muthukumar et al. 2017; Verma et al. 2019; Tian

et al. 2020). Analogously to AMF, rhizobia receive photosynthetates from their host in return for assimilated nutrients (fixed atmospheric nitrogen) and are mainly known for their plant growth-promoting effects (see *e.g.,* Franche et al. 2009; Chen 2018; Wang 2019).

Research on the effect of plant-parasitic nematodes on root nodulation by rhizobia and vice versa has mainly dealt with the interaction between the soybean cyst nematode *Heterodera glycines* Ichinohe, 1952 and soybean, and between the most common root-knot nematode species and a variety of leguminous crops. In most instances, root nodulation by rhizobia suppressed the reproduction (and in the case of *Meloidogyne* spp. also root galling) of the plant-parasitic nematodes and improved plant growth (Huang 1987; Sharma and Tiagi 1990; Fazal et al. 1992; Khan et al. 2018) while infection by the plant-parasitic nematodes reduced either the number of nodules, their functionality or induced premature senescence of the nodules (Taha and Raski 1969; Mani and Sethi 1984; Huang 1987; Upadhyay and Dwivedi 1987; Nejad and Khan 1997; Vovlas et al. 1998; Khan et al. 2002, 2018; Desaeger et al. 2005; Wood et al. 2018).

In many instances, discrepancies in the efficacy of biological control observed under controlled conditions *vs* applied production conditions (*e.g.,* in the field) have been observed (Guetsky et al. 2001, 2002; Meyer and Roberts 2002). For BCA's applied to the phyllosphere, for instance, Guetsky et al. (2001) suggested that, *inter alia*, environmental conditions that are not fully controlled (or not controlled at all) in commercial production may have either a direct effect on the BCA's (such as fluctuating temperatures and relative humidities) or an indirect affect by modifying the characteristics of the host plant (such as the metabolic state). Therefore, application of more than one BCA is suggested to reduce the variability and increase the reliability of biological control. However, the combined use of BCA's should not be recommended without clear understanding of their main biocontrol mechanisms and relative competitiveness (Xu et al. 2011). The same principles are also valid for the efficient biocontrol of soil-borne pathogens, including plant-parasitic nematodes (Meyer and Roberts 2002). A substantial amount of research has already been undertaken on the beneficial effects of dual colonisation of roots by AMF and rhizobia for plant growth promotion in a wide variety of plants such as common bean, Indian rosewood (*Dalbergia sissoo* Roxb.), lentil (*Lens culinaris* Medik.) and soybean (Nwoko and Sanginga 1999; Aryal et al. 2003; Zarei et al. 2006; Niranjan et al. 2007). However, few studies have focused on the suppressive effects of this type of dual colonisation on plant pathogens in general and plant-parasitic nematodes in particular. The majority of the recorded bio-protective effects of the dual colonisation of roots by AMF and rhizobia against plant-parasitic nematodes has focussed on root-knot nematodes (Jaizme-Vega et al. 2006; Akhtar and Siddiqui 2008; Reimann et al. 2008). To our knowledge, no information exists on the bio-protective effect of dual colonisation of roots by AMF and rhizobia on migratory endoparasitic nematodes, particularly *R. similis*. Therefore, the objective of our study was to examine the bio-protective effect of either single or dual colonisation of the roots of non-leguminous and leguminous banana intercrops differing in host response to *R. similis* on the reproduction of this important migratory endoparasitic nematode.

## Materials and methods

### Plant material

Based on the results of a previous study (Van der Veken et al. 2008) and their relevance in banana-based cropping systems, non-leguminous and leguminous intercrops combining an intermediate AMF compatibility with either a good, intermediate, poor or non-host response to *R. similis* infection were selected. The selected non-leguminous intercrops were: sorgho-Sudangrass (*Sorghum sudanense*), sweet potato cv. Inzovu and marigold (*Tagetes erecta* L.); the selected leguminous intercrops were: common bean, soybean and sunn hemp (*Crotalaria juncea* L.)(Table 1). Seeds of all intercrops, except sweet potato cv. Inzovu, were germinated in a plastic tray containing sterilised potting soil (autoclaved at 121 °C for 25 min. at 15 psi) for 5 days before transplanting. Sweet potato cv. Inzovu cuttings with one node were rooted in a plastic tray containing sterilised potting soil for 1 week before transplanting.

### AMF inoculum

A *Glomus mosseae* (= *Funneliformis mosseae*) isolate, originating from a banana field in the Canary Islands, was provided by M.C. Jaizme-Vega (ICIA, Tenerife), and established in a sterilised sand:potting soil mixture (2:1) using sorghum as a host (Jaizme-Vega et al. 1997). Mycorrhizal inoculum consisted of a 50 g mixture of soil and roots collected from a 6-month-old well-established AMF-sorghum pot culture.

### Rhizobial inoculum

*Rhizobium etli* CNPAF 512 and *Bradyrhizobium japonicum* USDA 110 were obtained from the Centre of Microbial and Plant Genetics (CMPG), University of Leuven, Belgium. *Rhizobium etli* CNPAF 512 was cultured for 2 to 3 days at 28 °C on a solid TY medium (5 g tryptone, 3 g yeast extract with 15 g plant agar/L distilled water; Bittinger and Handelsman 2000) followed by 1 day on a liquid TY medium at 28 °C and 200 rpm. After sterilising the media, 1% of

**Table 1** Non-leguminous and leguminous banana intercrops included in our study, and their host response to *Radopholus similis* infection as evaluated by Van der Veken et al. (2008)

| Scientific name | Common name | Accession | Source | Host response to *Radopholus similis*[*] |
|---|---|---|---|---|
| *Non-leguminous crops* | | | | |
| *Sorghum bicolor* x *S. sudanense* | Sorgho-Sudangrass | WH 15 | Lehle Seeds, USA | Good host |
| *Ipomoea batatas* | Sweet potato | cv. Inzovu | field site in Burundi | Intermediate |
| *Tagetes erecta* | Marigold | 2001-1253-56 | National Botanical Garden, Belgium | Non-host |
| *Leguminous crops* | | | | |
| *Phaseolus vulgaris* | Common bean | BAT 477 | CMPG, University of Leuven, Belgium | Intermediate |
| *Glycine max* | Soybean | NI 315 | National Botanical Garden, Belgium | Poor host |
| *Crotalaria juncea* | Sunn hemp | AusTRCF 71,013 | Tropical Crops & Forage Collection, Australia | Poor host |

[*]Host response *to Radopholus similis* infection was based on the reproduction ratio (Rr) which is the final nematode population density (Pf) divided by the initial (inoculated) nematode population density (Pi): good host ($Rr \geq 3$); intermediate host ($1 \leq Rr \leq 3$); poor host ($0.1 \leq Rr \leq 1$); non-host ($Rr \leq 0.1$) (Van der Veken et al. 2008)

a sterilised 10 mM $CaCl_2.H_2O$ solution was added to promote growth of the bacterial cell wall. *Bradyrhizobium japonicum* USDA 110 was cultured for 3 to 4 days at 28 °C on a solid YMA medium (0.2 g $K_2HPO_4$, 0.2 g $MgSO_4$, 10 g mannitol, 0.3 g yeast extract, 0.05 g NaCl and 20 g agar/L distilled water) followed by 2 days on a liquid YMA medium at 28 °C and 200 rpm. The optical density (O.D.) of the bacterial suspension was determined by measuring its absorbance at 600 nm wavelength using a spectrophotometer. The suspension was diluted in a sterile 10 mM $MgSO_4$ solution to obtain a bacterial inoculum containing about $10^6$ colony forming units (CFU) per ml. For common bean, *R. etli* CNPAF 512 and for soybean, *B. japonicum* USDA 110 were used as rhizobial inoculum. For sunn hemp, no compatible rhizobial strain was found (Van der Veken et al. 2008).

## Nematode inoculum

The *R. similis* population used in our study was isolated from a banana field in Uganda. Nematode specimens were identified under a dissecting microscope based on morphological characters associated with *R. similis* as described by Orton Williams and Siddiqi (1973). The nematode population was maintained on monoxenic carrot (*Daucus carota* L.) disc cultures (Speijer and De Waele 1997). Prior to inoculation, the nematodes were extracted using a maceration-sieving technique (Hooper et al. 2005).

## Experimental setup

All experiments were performed in a greenhouse at an ambient temperature of 20–27 °C with a 12 h photoperiod (170–190 PAR = Photo-synthetically Active Radiation) and a relative humidity of 50–70%. After germination, the selected non-leguminous and leguminous seedlings were transplanted in 1-L pots containing a sand:potting soil mixture (2:1) and a slow-release fertiliser (Osmocote®) applied. For AMF colonisation, the mycorrhizal inoculum was spread as a 5 cm layer beneath a 5 cm top layer of the soil mixture at transplanting. This allowed early mycorrhization of the emerging rootlets. For rhizobial colonisation, a 1 ml of the suspension containing the rhizobial inoculum was poured over the emerging rootlets of the common bean and soybean seedlings at transplanting allowing any excess to drip into the planting hole. For the other intercrops, there was no rhizobial treatment. Six weeks after transplanting, the seedlings were inoculated with 1,000 vermiform *R. similis life* stages, divided over three holes around the plant stem basis.

For the non-leguminous crops, a factorial design with two (AMF and *R. similis*) factors was set up. As such, four ($AMF^-/NEM^-$, $AMF^-/NEM^+$, $AMF^+/NEM^-$, $AMF^+/NEM^+$) treatments were obtained. For the leguminous crops, a factorial design with three (AMF, rhizobium and *R. similis*) factors was set up. As such, eight ($AMF^-/RHIZ^-/NEM^-$, $AMF^-/RHIZ^-/NEM^+$, $AMF^+/RHIZ^-/NEM^-$, $AMF^+/RHIZ^-/NEM^+$, $AMF^-/RHIZ^+/NEM^-$, $AMF^-/RHIZ^+/NEM^+$, $AMF^+/RHIZ^+/NEM^-$ and $AMF^+/RHIZ^+/NEM^+$) treatments were obtained.

## AMF and Rhizobial compatibility, and susceptibility to *Radopholus similis* infection

The plants were harvested at 8 weeks after nematode inoculation (WAI; *i.e.,* 14 weeks after transplanting). Fresh shoot weight (FSW) and fresh root weight (FRW), after gently washing off the substrate with tap water and gently drying the root systems, were recorded. Dry shoot weight (DSW) was determined by drying the leaves, stems and pods during

72 h in an oven at 70 °C. Visual quantification of the number of nodules and assessment of nodule quality (verifying the color for presence of leghemoglobin) were done after washing of the roots. 5 g root subsamples were taken for analysis of AMF colonisation and nematode reproduction.

For microscopic analysis of AMF colonisation, a 5 g root subsample was stained using the ink and vinegar technique (Vierheilig et al. 1998) and ten 1-cm root segments were mounted on a glass slide. Two slides per root subsample were scored for frequency (F%) and intensity (I%) of mycorrhizal colonisation. F% was calculated as the percentage of root segments colonised by either hyphae, arbuscules or vesicles; I% was calculated as the abundance of hyphae, arbuscules and vesicles in each mycorrhized root segment (Plenchette and Morel 1996). F% and I% values above 50% are considered high, 35% to 50% as moderate and below 35% as low. The relative mycorrhizal dependency (RMD) was determined by expressing the difference between the average DSW of the mycorrhized plants and non-mycorrhized plants as a percentage of the DSW of the mycorrhized plants (Plenchette et al. 1983).

*Radopholus similis* reproduction was assessed by extracting the nematodes from a 5 g root subsample using a maceration-sieving technique (Hooper et al. 2005). The number of juveniles, females and males were counted in two 2-ml sub-samples of a 50 ml suspension using a light microscope (Leitz Dialux, 120 x magnification). The average of two counts was used to determine the number of *R.similis* computed per root system. Reproduction factor (Rf) was computed as final nematode population/initial population (Windham and Williams 1987).

### Experimental design and statistical analysis

Each experiment was arranged in a randomized block design with eight replications per treatment. Mycorrhizal colonisation data (F% and I%) were arcsin (x/100) transformed and final nematode population densities/root system were log (x + 1) transformed prior to statistical analysis. ANOVA (one-way and two-way) and post-hoc Tukey HSD tests were performed using the Statistica 7.1 package after verifying the ANOVA assumptions (Anonymous 2007).

## Results

### Sorgho-Sudangrass (good *Radopholus similis* host)

In sorgho-Sudangrass, high F% (59–65) and low I% (11–15) values were observed (Table 2); the RMD value was 47%. FSW and FRW were significantly ($P \leq 0.01$ and 0.001, respectively) higher in the mycorrhized treatments compared with the non-mycorrhized treatments, both in the absence

**Table 2** Effect of single inoculation of *Glomus mosseae* on plant growth, mycorrhizal colonisation and reproduction of *Radopholus similis* on sorgho-Sudangrass, sweet potato cv. Inzovu, marigold and sunn hemp. Seedlings were inoculated with *G. mosseae* ($AMF^+$) at transplanting; with 1,000 vermiform *R. similis* ($NEM^+$) 6 weeks after transplanting. Plants were harvested 14 weeks after transplanting

| Treatment | Sorgho-Sudangrass | | | | | Sweet potato cv. Inzovu | | | | | Marigold | | | | | Sunn hemp | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | FSW (g) | FRW (g) | F% | I% | No. *R. similis*/ root system | FSW (g) | FRW (g) | F% | I% | No. *R. similis*/root system | FSW (g) | FRW (g) | F% | I% | No. *R. similis*/root system | FSW (g) | FRW (g) | F% | I% | No. *R. similis*/ root system |
| $AMF^-/NEM^-$ | 18.9 *B* A | 17.1 A | | | | 4.2 | 16.3 | | | | 7.0 | 4.3 | | | | 3.4 | 5.7 A | | | |
| $AMF^-/NEM^+$ | 14.6 *A* A | 16.4 A | | | 3,083 B | 4.6 | 14.0 | | | 52 B | 7.8 | 3.7 | | | 22 | 4.3 | 4.1 A | | | 88 |
| $AMF^+/NEM^-$ | 30.3 *B* B | 33.5 B | 59 | 11 | | 4.2 | 13.2 | 40 | 15 | | 7.0 | 5.3 | 99 | 38 | | 4.4 | 7.2 B | 74 *B* | 19 | |
| $AMF^+/NEM^+$ | 22.3 *A* B | 25.8 B | 65 | 15 | 1,574 A | 5.2 | 15.6 | 35 | 14 | 3 A | 6.8 | 4.0 | 99 | 41 | 16 | 4.9 | 7.4 B | 43 *A* | 17 | 16 |
| *P* (NEM*AMF) | | n.s | | | | n.s | n.s | | | | n.s | n.s | | | n.s | n.s | n.s | | | |
| *P* (NEM) | * | n.s | n.s | n.s | | n.s | n.s | n.s | n.s | | n.s | n.s | n.s | n.s | | n.s | n.s | ** | n.s | |
| *P* (AMF) | ** | *** | | | * | n.s | n.s | | | ** | n.s | n.s | | | n.s | n.s | ** | | | n.s |

FSW: fresh shoot weight; FRW: fresh root weight. F%: frequency of mycorrhizal colonisation; I%: intensity of mycorrhizal colonisation. In the same column, different italic capital letters indicate a significant main effect of the presence of nematodes and non-italic capital letters indicate a significant main effect of the presence of AMF according to Tukey's HSD test

*, ** and ***indicate *P* values ≤ 0.05, 0.01 and 0.001, respectively. n.s.: not significant

(60.3% and 58.5%, respectively) and presence (52.7% and 57.3%, respectively) of *R. similis*. Infection with *R. similis* significantly ($P \leq 0.05$) reduced FSW compared with the uninfected treatments, as well in the absence (22.8%) as in the presence (26.4%) of *G. mosseae*. Infection with *R. similis* did not significantly affect FRW compared with the uninfected treatments nor in the non-mycorrhized or the mycorrhized treatments. Nematode reproduction was relatively high (Rf = 3.08) at 8 WAI when on average 3,083 *R. similis*/root system were observed. The presence of *G. mosseae* significantly ($P \leq 0.05$) reduced the number of *R. similis*/root system with 48.9% compared with the non-mycorrhized treatments. No significant effect of *R. similis* on colonisation by *G. mosseae* (F%, I%) was observed.

### Sweet potato cv. Inzovu (intermediate *Radopholus similis* host)

In sweet potato cv. Inzovu, moderate F% (35–40) and low I% (14–15) values were observed (Table 2); the RMD value was -44%. Hyphae and arbuscules were visible in the majority of the root systems. Vesicles, however, were observed only in two root systems. Colonisation by *G. mosseae* and inoculation with *R. similis* had no significant effect on FSW and FRW compared with the non-colonised and uninoculated treatments. Nematode reproduction was low (Rf = 0.05) at 8 WAI when on average 52 *R.* similis/root system were observed. The presence of *G. mosseae* significantly ($P \leq 0.01$) reduced the number of *R. similis*/root system with 94.2% compared with the non-mycorrhized treatments. No significant effect of *R. similis* on colonisation by *G. mosseae* (F%, I%) was observed.

### Marigold (non-host of *Radopholus similis*)

In marigold, high F% (99) and moderate I% (38–41) values were observed (Table 2); the RMD value was 8%. Colonisation by *G. mosseae* and inoculation with *R. similis* had no significant effect on FSW and FRW compared with the non-colonised and uninoculated treatments. Nematode reproduction was low (Rf = 0.02) at 8 WAI when on average 22 *R. similis*/root system were observed. The presence of *G. mosseae* had no significant effect on the number of *R. similis*/root system compared with the non-mycorrhized treatments. No significant effect of *R. similis* on colonisation by *G. mosseae* (F%, I%) was observed.

### Sunn hemp (poor *Radopholus similis* host)

In sunn hemp, moderate to high F% (43–74) and low I% (17–19) values were observed (Table 2); the RMD values was 11%. FRW was significantly ($P \leq 0.01$) higher in the mycorrhized treatments compared with the non-mycorrhized treatments, both in the absence (26.3%) and presence (80.5%) of *R. similis*. Colonisation by *G. mosseae* had no significant effect on FSW and inoculation with *R. similis* had no significant effect on both FSW and FRW compared with the uninoculated treatments. Nematode reproduction was low (Rf = 0.09) at 8 WAI when on average 88 *R.* similis/root system were observed. Although the presence of *G. mosseae* reduced the number of *R. similis*/root system with 81.8% compared with the non-mycorrhized treatments, these reductions were not significant due to high variation of the data. Inoculation with *R. similis* significantly ($P \leq 0.01$) reduced the F% value with 41.9% compared with the uninoculated treatment but not the I% value.

### Common bean (intermediate *Radopholus similis* host)

In common bean, high F% (99) and low I% (21–24) values were observed in the treatment that was only colonised by *G. mossea* (Table 3). In this treatment, hyphae, arbuscules and spores were observed in all mycorrhized root systems while the RMD value was 65%. In the treatments that were not colonised by *R. etli* CNPAF 512, FSW and FRW were significantly ($P \leq 0.001$) higher in the mycorrhized treatments compared with the non-mycorrhized treatments, both in the absence (3.3 and 3.6 times, respectively) and presence (10 and 9.2 times, respectively) of *R. similis*. In the treatments that were not colonised by *G. mosseae*, FSW and FRW were significantly ($P \leq 0.001$) higher in the treatments colonised by *R. etli* CNPAF 512 compared with the non-colonised treatments, both in the absence (1.7 and 2.2 times, respectively) and presence (4.8 and 5.8 times, respectively) of *R. similis*. In the treatments that were not inoculated with *R. similis*, FSW and FRW were 2 and 1.6 times, respectively, higher ($P \leq 0.001$) in the treatments colonised by *G. mosseae* only compared with the treatments colonised by *R. etli* CNPAF 512 only. Dual colonisation by *G. mosseae* and *R. etli* CNPAF 512 did not result in an additive effect on FSW and FRW, neither in the absence nor presence of *R. similis*. In the absence of *G. mosseae* and *R. etli* CNPAF 512, infection with *R. similis* reduced FSW and FRW with 61.5% and 61.7%, respectively, compared with the uninfected treatments. Nematode reproduction was low at 8 WAI when on average 139 *R. similis*/root system were observed. In the absence of *R. etli* CNPAF 512, the presence of *G. mosseae* significantly ($P \leq 0.05$) reduced the number of *R. similis*/root system with 40% compared with the non-mycorrhized treatments. In the absence of *G. mosseae*, the presence of *R. etli* CNPAF 512 significantly ($P \leq 0.05$) reduced the number of *R. similis*/root system with 35.3% compared with the treatments not colonised by *R. etli* CNPAF 512.

Dual root colonisation by *G. mosseae* and *R. etli* CNPAF 512 reduced the number of *R. similis*/root system with about

**Table 3** Effect of single and dual inoculation of *Glomus mosseae* and either *Rhizobium etli* CNPAF 512 or *Bradyrhizobium japonicum* USDA 110 on plant growth, mycorrhizal colonisation and reproduction of *Radopholus similis* on common bean and soybean. Seedlings were inoculated with *G. mosseae* ($AMF^+$) and either *R. etli* CNPAF 512 (common bean; $RHIZ^+$) or *B. japonicum* USDA 110 (soybean; $RHIZ^+$) at transplanting; with 1,000 vermiform *R. similis* ($NEM^+$) 6 weeks after transplanting. Plants were harvested 14 weeks after transplanting

| Treatment | Common bean | | | | | Soybean | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | FSW (g) | FRW (g) | F% | I% | No. *R. similis*/root system | FSW (g) | FRW (g) | F% | I% | No. *R. similis*/root system |
| $AMF^-/RHIZ^-/NEM^-$ | 12.6 A | 3.4 A | | | | 13.7 ab | 13.5 | | | |
| $AMF^-/RHIZ^-/NEM^+$ | 4.9 A | 1.3 A | | | 139 B | 8.0 a | 6.5 | | | 131 AB |
| $AMF^+/RHIZ^-/NEM^-$ | 43.4 D | 12.4 D | 99 | 21 | | 10.1 a | 9.8 | 90 *B* | 32 *B* | |
| $AMF^+/RHIZ^-/NEM^+$ | 49.6 D | 12.0 C | 99 | 24 | 84 A | 13.6 ab | 7.4 | 54 *A* | 19 *A* | 162 AB |
| $AMF^-/RHIZ^+/NEM^-$ | 21.4 B | 7.6 B | | | | 6.4 a | 4.7 | | | |
| $AMF^-/RHIZ^+/NEM^+$ | 23.7 B | 7.5 B | | | 90 A | 14.8 ab | 12.1 | | | 556 B |
| $AMF^+/RHIZ^+/NEM^-$ | 42.3 C | 12.0 C | 100 | 27 | | 32.4 b | 25.3 | 77 *B* | 30 *B* | |
| $AMF^+/RHIZ^+/NEM^+$ | 43.0 C | 11.8 C | 99 | 25 | 24 A | 7.7 a | 6.4 | 49 *A* | 16 *A* | 116 A |
| *P* (NEM*Treatment) | n.s | n.s | | | | ** | n.s | | | |
| *P* (NEM) | n.s | n.s | n.s | n.s | | n.s | n.s | *** | *** | |
| *P* (Treatment) | *** | *** | | | * | n.s | n.s | | | ** |

FSW: fresh shoot weight; FRW: fresh root weight. F%: frequency of mycorrhizal colonisation; I%: intensity of mycorrhizal colonisation. In the same column, different italic capital letters indicate a significant main effect of the presence of nematodes and non-italic capital letters indicate a significant main effect of the treatment according to Tukey's HSD test; small letters indicate a significant interaction effect of both the presence of nematodes and treatment; *, ** and *** indicate *P* values ≤ 0.05, 0.01 and 0.001, respectively. n.s.: not significant

70% compared with single colonisation by either *G. mosseae* or *R. etli* CNPAF 512 but this difference was not significant. No significant effect of *R. etli* CNPAF 512 and *R. similis* on colonisation (F%, I%) by *G. mosseae* was observed. No significant effect of *G. mosseae* on root nodulation by *R. etli* CNPAF 512 was observed (data not shown; number of nodules ranged 30–83).

## Soybean (poor *Radopholus similis* host)

In soybean, high F% (54–90) and low I% (19–32) values were observed in the treatment that was only colonised by *G. mossea* (Table 3). In this treatment, hyphae, arbuscules and spores were observed in all mycorrhized root systems while the RMD value was 32%. In the treatments that were not colonised by *B. japonicum* USDA 110, FSW and FRW were not significantly higher in the mycorrhized treatments compared with the non-mycorrhized treatments, both in the absence and presence of *R. similis*. In the treatments that were not colonised by *G. mosseae*, this was also the case for the treatments that were colonised by *B. japonicum* USDA 110 compared with the non-colonised treatments. In the treatments that were not inoculated with *R. similis*, FSW and FRW were not significantly different in the treatments colonised by *G. mosseae* only compared with the treatments colonised by *B. japonicum* USDA 110 only. Dual colonisation by *G. mosseae* and *B. japonicum* USDA 110 in the treatments that were not infected with *R. similis* resulted in FSW which was 3.2 and 5.1 times higher ($P \leq 0.01$) compared with single colonisation by either *G. mosseae* or *B. japonicum* USDA 110, respectively. Nematode reproduction was low at 8 WAI when on average 131 *R. similis*/root system were observed. In the absence of *B. japonicum* USDA 110, the presence of *G. mosseae* did not reduce the number of *R. similis*/root system compared with the non-mycorrhized treatments. In the absence of *G. mosseae*, the presence of *B. japonicum* USDA 110 also did not reduce the number of *R. similis*/root system compared with the treatments not colonised by *B. japonicum* USDA 110.

Dual colonisation by *G. mosseae* and *B. japonicum* USDA 110 did not reduce the number of *R. similis*/root system compared with single colonisation by *G. mosseae* but significantly ($P \leq 0.01$) reduced the number of *R. similis*/root system with 79.1% compared with single colonisation by *B. japonicum* USDA 110 (Table 3). No significant effect of *B. japonicum* USDA 110 on colonisation (F%, I%) by *G. mosseae* and vice versa was observed. In the absence of *R. similis*, on average 7 nodules were observed in root systems colonised by *B. japonicum* USDA 110 only compared with on average 29 nodules in root systems also colonised by *G. mosseae* (data not shown). With *R.similis* the F% and I% values were about 40% lower ($P \leq 0.001$) in the absence of *B. japonicum* USDA 110 and 36.4% to 46.7%, respectively, lower ($P \leq 0.001$) in the presence of

*B. japonicum* USDA 110, compared with tretaments without *R. similis*.

## Discussion

In general, we observed a significant plant growth-promoting effect of single AMF and rhizobial colonisation in the good and intermediate *R. similis* hosts sorgho-Sudangrass (AMF) and common bean (AMF and rhizobium), respectively, whereas this plant growth-promoting effect was absent in the other intercrops included in our study with the exception of sunn hemp in which a significant plant growth-promoting effect of AMF colonisation on FRW was observed. No plant growth-promoting effect of AMF colonisation was observed on the moderate *R. similis* host sweet potato cv. Inzovu. In this intercrop, the RMD value (-44%) was negative. In contrast with our findings, Gai et al. (2006) observed RMD values varying from 5 to 20% in sweet potato. Relative mycorrhizal dependency is cultivar and AMF-dependent (Elsen et al. 2003b), and the difference in the sweet potato genotypes and *G. mosseae* isolates used in our study and Gai et al. (2006) may explain the observed differences in RMD value. Also, our observations were made at an early stage of AMF colonisation when F% and I% values may be low due to an initial draw back in root growth (lag-phase). An additive plant growth-promoting effect of dual AMF and rhizobial colonisation (on FSW) was only observed in the poor *R. similis* host soybean.

In addition to a plant growth-promoting effect, single AMF and rhizobial colonisation also resulted in a significant bio-protective effect against *R. similis* in sorgho-Sudangrass (AMF), sweet potato cv. Inzovu (AMF) and common bean (AMF and rhizobium): final population densities of *R. similis* were significantly reduced in these either good or intermediate *R. similis* hosts. In the poor and non- *R. similis* hosts sunn hemp and marigold, AMF colonisation did not suppress the already low *R. similis* population densities more. For poor or non- *R. similis* hosts, AMF colonisation has no additional bio-protective effect against *R. similis*. These findings are in agreement with previous reports of the suppressive effect of AMF on migratory endoparasitic nematodes in general (Hol and Cook 2005) and *R. similis* (on banana) in particular (Elsen et al. 2003a,b,c). No additive bio-protective effect of dual AMF and rhizobial colonisation was observed in common bean. In soybean, single rhizobial colonisation resulted in a final population density of *R. similis* which was significantly higher compared with dual AMF and rhizobial colonisation. This observation is in contrast with previous findings that root-nodulation by rhizobia usually suppress the reproduction of plant-parasitic nematodes (Huang 1987; Sharma and Tiagi 1990; Fazal et al. 1992; Khan et al. 2018). However, all these reports dealt with the bio-protective effect of root-nodulation on root-knot nematodes and one should be careful when extrapolating these findings to *R. similis*, a migratory endoparasite. Root-knot nematodes can suppress root-nodulation (Taha and Raski 1969; Mani and Sethi 1984; Huang 1987; Upadhyay and Dwivedi 1987; Nejad and Khan 1997; Vovlas et al. 1998; Khan et al. 2002, 2018; Desaeger et al. 2005; Wood et al. 2018) and Germani et al. (1984) reported that *Pratylenchus sefaensis* Fortuner, 1973, a migratory endoparasite, also suppressed root-nodulation on soybean. In contrast, Hussey and Barker (1976) found that *Pratylenchus penetrans* Cobb, 1917, another migratory endoparasite, stimulated root-nodulation in soybean. In our study, on average 7 nodules were formed on the root systems of soybean plants colonised by *Bradyrhizobium japonicum* USDA 110 but not infected with *R. similis vs* on average 18 nodules on the root systems of soybean plants infected with *R. similis*, thus confirming that migratory endoparasitic nematodes may stimulate root-nodulation. The observation that dual and rhizobial colonisation resulted in a final population density of *R. similis* which was significantly lower compared with single rhizobial colonisation suggests that rhizobial colonisation resulted in an increase in the nematode population but that this effect was mitigated by AMF colonisation. The nematode-suppressive effects of AMF have been attributed to, *inter alia*, competition between AMF and nematodes for resources and space (Schouteden et al. 2015). Elsen et al. (2008) also suggested that AMF were able to induce systemic resistance against *R. similis* and *P. coffeae*, another migratory endoparasitic nematode in banana roots.

The growth-promoting and bio-protective effects of AMF colonisation were clearly present in the good and intermediate *R. similis* hosts with high RMD values ranging from 47% (sorgho-Sudangrass) to 65% (common bean) but absent in the poor and non- *R. similis* hosts with RMD values ranging from -44% to 32%. Apparently, *G. mosseae* colonisation achieves these beneficial effects from a certain RMD value threshold onwards; below this threshold no beneficial effects can be achieved.

Overall, no effect of *R. similis* infection on AMF and rhizobial colonisation was observed except in soybean where AMF colonisation (F% and I%) was significantly reduced upon *R. similis* infection and in sunn hemp where F% was significantly reduced. In banana, Elsen et al. (2003b) observed a significant lower F% in plants infected with *R. similis* compared with uninfected plants. They suggested that migration of *R. similis* inside the roots results in the destruction of cortical parenchyma which in turn results in a decrease in root tissues that can be colonised by AMF.

Since the production of legumes is often limited by the low availability of P in the soil, legume cultivars with high RMD values and a good response to P application are suitable candidates for cultivation in P-deficient soils. The RMD

values of the leguminous intercrops included in our study varied from 11% for sunn hemp, over 32% for soybean to 65% for common bean. This suggests that the soybean and common bean genotypes used in our study classify as highly AMF-dependent plants (RMD value > 30%) and are thus highly dependent of AMF colonisation for maximum plant growth and development. As such, it makes them suitable candidates for cultivation in P-deficient soils (Nwoko and Sanginga 1999). Among the non-leguminous intercrops included in our study, sorgho-Sudangrass with an RMD value of 47% could be a suitable candidate. AMF colonisation of sunn hemp and marigold resulted in RMD values of 11% and 8%, respectively, and the crops were classified as intermediate (10% > RMD > 30%) and non (RMD < 10%) AMF-dependent (Nwoko and Sanginga 1999).

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethics approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for publication** Authors have seen and approved the manuscript and have taken a valid role through either study design, data generation, or manuscript preparation.

**Availability of data and material** All data and materials comply with the standards set by the journal.

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