



Evaluating sorghums as green manure against root-knot nematodes

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ABSTRACT

Current restrictions on the use of chemical nematicides have led to an increase in root-knot nematode (RKN) damages in horticultural crops. The effects of two sorghums as summer cover crops, *Sorghum sudanense* sudangrass cv. 'Piper' or sudangrass hybrid [*S. bicolor* x *S. sudanense*] '270911', respectively with low and high dhurrin contents, were compared in their ability to suppress RKN in a vegetable production system. The use of both sorghums 'Piper' and '270911' as a green manure was found to be an effective strategy for decreasing RKN infestation in the soil, thereby protecting the subsequent planting of RKN susceptible crops (chard, lettuce or melon). Analytical experiments were further conducted in growth chamber and greenhouse pot experiments to investigate and compare the susceptibility of the sorghums and the factors affecting their efficacy for RKN management, in order to better explain the results obtained in the field trial. The two sorghums were poor hosts of RKN, acted as trap crops and as a biofumigant releasing hydrogen cyanide. Time of planting, time of biofumigation, and type of soil affected their efficacy for RKN management. For best RKN suppression, the sorghum cover crops need to be cultivated during one month or less and biofumigated for one month prior to crop planting. The trapping effect of both sorghums in clayey soil was less efficient than in sandy or sandy-loamy soils. Combining less than 30-days of sorghum culture and 10-days soil incorporation with solarization mulch was particularly efficient in suppressing nematodes. No effect relative to the sorghum type was detectable as long as they were used appropriately.

1. Introduction

Intensive monoculture of specialized horticultural crops is becoming vulnerable to damage from plant-parasitic nematodes (PPN), especially root-knot nematodes (RKN, *Meloidogyne* spp.) worldwide (Jones et al., 2013). In Mediterranean regions, RKN are most destructive to vegetable farms (Abd-Elgawad, 2014; Djian-Caporalino, 2012; Talavera et al., 2012). Accentuated by the current restrictions on the use of chemical nematicides (MBTOC, 2006; EC Directive, 2009), there is an urgent need to develop innovative, low-input, ecologically sound and efficient solutions for managing these pests.

Use of cover crops in rotation to vegetable crop production has been recommended to farmers as a mean to improve fertility, physical and chemical properties of soil (Fortuna et al., 2003; Séguéy et al., 2009). Cover crops improve the productivity of subsequent crops by reducing

pressure of pests and pathogens (Ratnadass et al., 2012), serving as non or poor hosts for RKN (Djian-Caporalino et al., 2005), and thus contribute to nematode management (McSorley, 2001). Cover crops documented to be capable of managing RKN include *Phacelia* spp., *Avena sativa* (Gomes Carneiro, 1982), brassicas, such as *Sinapis alba*, *Eruca sativa*, and *Raphanus sativus* (Curto et al., 2015; Kruger et al., 2013), and grasses, such as *Pennisetum glaucum*, *Sorghum bicolor* 'forage sorghum' or *Sorghum sudanense* 'sudangrass', formerly classified as *S. vulgare* var. *sudanense*, and *S. bicolor* x *S. sudanense* 'sudangrass hybrids' (McSorley et al., 1994; Mojtahedi et al., 1993; Sipes and Arakaki, 1997; Timper and Wilson, 2006). They have been tested as winter or summer cover crops for limiting RKN populations. These cover crops, which are used as green manure, can be used as trap crops if the nematodes penetrate the roots but cannot complete their cycle. They can also be used as biofumigant crops that release volatile compounds when soil incorporated

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(Djian-Caporalino et al., 2005). Interest in biofumigation for soilborne disease management has recently increased due to its compatibility with environmental friendly management (Kruger et al., 2013; Prasad et al., 2015). One group of cover crops with effective biofumigation effect against RKN is forage sorghum or sudangrass. Sorghum has been well known to release hydrogen cyanide (HCN) following the hydrolysis of dhurrin, a cyanogenic glycoside typically present in sorghum (Chitwood, 2002; Curto et al., 2012; De Nicola et al., 2011).

Sudangrass hybrids produce larger amounts of biomass than non-hybrid sudangrass. Both sudangrass and its hybrids have a long root system penetrating deep into the soil, and they are used as a green manure to improve soil porosity, and attract soil nutrients present at depth up towards the top soil layer (Abawi and Widmer, 2000; Kratochvil et al., 2004). Sudangrass require little management, beside seed sowing and sprinkler irrigation after soil tillage. The cover crop grows for 1.5–2 months, and its biomass is then mowed and soil incorporated (Delamarre, 2011). A number of studies, summarized in the review by Quaranta (2009), have reported activity against several pests and diseases. In the case of PPN, forage sorghum or sudangrass have been reported to be effective at reducing the numbers of *Helicotylenchus dihystera* (Wang et al., 2004), *Rotylenchus reniformis* (Asmus et al., 2008) and *Pratylenchus penetrans* (LaMondia et al., 2002), but were found to facilitate the multiplication of *Belonolaimus longicaudatus* (Rhoades, 1983; Weingartner et al., 1993), *Mesocriconema* sp. (Crow et al., 2001), *Paratrichodorus minor* (McSorley and Gallaher, 1991; McSorley et al., 1994; McSorley and Dickson, 1995) and *Tylenchorhynchus* sp. (Crow et al., 2001). McGuidwin and Layne (1995) reported the maintenance or an increase in populations of *Pratylenchus*, *Longidorus*, *Xiphinema* and *Paratrichodorus* after the incorporation of some varieties of sudangrass and sudangrass hybrids. Conversely, these plants were considered to be non-hosts or poor hosts for RKN (Colbran, 1979; Fay and Duke, 1977; Gomes Carneiro, 1982; Hagan et al., 1998; Ritzinger and McSorley, 1998; Védie et al., 2006), such as *Meloidogyne incognita* and *M. hapla* in particular (Chitwood, 2002; Ferraz and de Freitas, 2004; McSorley and Dickson, 1995; Viaene and Abawi, 1998; Wang et al., 2004). Mojtahedi et al. (1993) and Widmer and Abawi (2000) also reported that they released HCN, with biofumigation effects against *M. hapla* or *M. chitwoodii*. Wang et al. (2004) and Guereña (2006) reported that the prior use of sudangrass hybrids as a cover crop decreases the density of populations of *M. incognita* on *Secale cereale* 'rye', *Lupinus angustifolius* 'lupin' or *Glycine max* 'soybean'. However, poor results were obtained when the crops were combined and sudangrass or sudangrass hybrids residues were incorporated into the soil while still green (Orfanedes, 1995; Widmer and Abawi, 2002). The best results for RKN suppression in Florida were obtained with the use of forage sorghum or sudangrass hybrids as a rotation crop (Dover et al., 2012; Gill and McSorley, 1994), but the benefits of crop residues for nematode suppression were not assessed in this study. In France, sorghum residues have been found to be only partly effective in the field, often yielding variable results (Abawi and Widmer, 2000; Collange et al., 2011; McGuidwin and Layne, 1995; Thoden et al., 2011). However, interest in the use of biofumigation in vegetable crop rotations has recently increased (Curto et al., 2016; Goillon et al., 2016; Prasad et al., 2015), and deserves further work.

Sorghum is the most widely used green manure in vegetable cropping systems in the South of France. In this context, both analytical experiments under controlled conditions and a field experiment evaluating sorghums as green manure against RKN were performed. An innovative co-design process using a participatory approach involving scientists (geneticists, plant pathologists, agronomists), technical advisers and farmers, was used to take into account technical and socioeconomic constraints (Djian-Caporalino et al., 2014). This 'system approach', recommended by the 'EIP-AGRI Focus Group IPM practices for soil-borne diseases' (2015), allows the farmer to change some crops of the rotation and treatments according to market constraints and decision rules established during the co-design process. But the field

experiment has to last at least two seasons.

'Piper' is the most commonly used sudangrass variety in France as green manure but it has low dhurrin content (Mojtahedi et al., 1993), containing only 2.7 mg/g of dry matter after three weeks of growth (Gard et al., 2014). Low dhurrin content in sudangrass is selected for low animal toxicity when used as feed (Chambliss, 2002). On the other hand, high level of dhurrin has been found in sudangrass hybrid '270911' which was developed for biofumigation, containing 12.7 mg/g dhurrin in dry matter after three weeks of growth (Gard et al., 2014).

The objectives of this study were 1) to compare the effects as summer cover crops of the two sorghum varieties 'Piper' and '270911', respectively with low and high dhurrin content, in suppressing RKN in vegetable production systems; 2) to investigate in greenhouse conditions some factors affecting their efficiency in terms of nematode suppression; and 3) to screen both varieties and additional sorghum genotypes, either sudangrass or hybrids, for susceptibility to *M. incognita*.

2. Materials and methods

2.1. Nematode suppressive effects of sorghums in agroecosystem (field trial)

A four-year field trial was performed on a commercial organic farm near Lambesc (43.65N, 5.21E) located at Provence in southern France, with Mediterranean climate, from 2012 to 2016. The trial was carried out under a 80 m × 8 m × 3.5 m plastic cover plot. The soil was sandy-loamy (37.5% sand, 22.3% loam, 10.7% clay, 3.5% soil organic matter (OM) and with pH of 8.4) heavily infested with both *M. arenaria* and *M. incognita* as determined by their isoesterase phenotype (Dalmasso and Bergé, 1978). During the experimental period, soil temperature at a depth of 15 cm varied from 5 to 15 °C from November to February, and from 15 to 30 °C from April to October.

The plot was divided into two subplots, one for planting sudangrass 'Piper', the standard cover crop commonly used by commercial farmers in France with low dhurrin content, the other for planting sudangrass hybrid '270911' with high dhurrin content known for biofumigation purposes. '270911' is a three-way hybrid generated from [*Sorghum bicolor* spp. *bicolor* × sudangrass] cross developed by UPL France SASTM. The 4-year-field experiment lasted two seasons, the sorghum cover crop being grown in the first and third years in order to duplicate the experiment. The sorghum seeds were sown in July or August on rototilled soil with seedbed preparation before sowing, at a density of 50 kg seeds/ha for 'Piper' to reach 25–30 tons/ha of aboveground fresh weight. '270911' was sown at a density of 85 kg/ha in 2012 due to poor germination but at 30 kg seeds/ha in 2014 as recommended by UPL France to reach the same amendment rate (Table 1). One month after

Table 1

Cropping schedule for cover crop in the commercial farm located in Lambesc 43.65N, 5.21E (80 m × 8 m × 3.5 m plastic cover plot).

Year	2012	2014
Sorghum '270911' plot		
Sowing date ^a	23/07	16/08
Sowing density (kg/ha) (% germination)	85 (50%)	30 (100%)
Aboveground fresh weight (tonnes/ha)	29.2	29
Burial date ^b	23/08	12/09
Winter crop planting date ^a	03/10 (chard)	03/10 (lettuce)
Control 'Piper' plot		
Sowing date ^a	23/07	16/08
Sowing density (kg/ha) (% germination)	50 (100%)	50 (100%)
Aboveground fresh weight (tonnes/ha)	24.6	29
Burial date ^b	23/08	12/09
Winter crop planting date ^a	03/10 (chard)	03/10 (lettuce)

^a After planting, the crop was irrigated every three days initially and less frequently thereafter.

^b After incorporation with rotavator, the soil was rolled and left uncovered for one month to allow biofumigation.

sowing, the leaves and shoots were mowed and soil incorporated with rotavator. The soil was rolled and left uncovered for one month to allow biofumigation. To prevent phytotoxicity, the next cash crops were planted three weeks after termination of biofumigation.

RKN-susceptible plant species were grown subsequent to these cover crops to assess the suppression of RKN. Two types of RKN-susceptible plants were used: 1) a very susceptible crop, i.e., *Cucumis melo* (melon) in spring, and 2) susceptible crops in winter when the RKN cycle is slower, such as *Lactuca* spp. (lettuce) or *Beta vulgaris* subsp. *vulgaris* (Swiss chard). Weak forms of R-crops were cropped during the summer in the second and fourth years, to determine whether the use of a green manure could decrease the RKN soil infestation potential enough to secure crop rotation with them. RKN-resistant tomatoes (R-tomato) whose *Mi-1.2* resistance gene is inactivated at high temperature were used. Sweet pepper varieties grafted onto resistant pepper rootstock (R-pepper) that carries the major R-gene *Me3* weakened in the highly susceptible genetic background Doux-Long-des-Landes, were also used and mixed with the R-tomatoes.

Sorghum biomass was estimated at the end of the cover crop by weighing the aerial part of plants sampled from a 1 m² microplot before and after drying at 60 °C for 48 h (Table 1). Rhizosphere soil from eight 250 ml samples per subplot were sampled at a depth of 15 cm, before the experiment and after each susceptible crop was terminated. Throughout the experiment, samples were systematically taken from the same core site, to minimize the effects of heterogeneity in the distribution of nematodes over the plot. RKN were extracted by the Seinhorst (1962) elutriation procedure and counted under a stereomicroscope (x60 magnification) (Merny and Luc, 1969). Nematode population densities were determined and expressed per dm³ of fresh soil (mean of 8 replicates). To analyse the effect of the four-year-cropping systems on RKN soil populations, a multiplication rate Pf/Pi (final population Pf = nematode density in the soil at the end of the crop; initial population Pi = nematode density in the soil at planting or sowing) was compared between the plots. To determine the impact of the system on susceptible and resistant crops, RKN damage was estimated by determining the gall index (GI) on a scale of 0–10 (Zeck, 1971) for the root system of a representative subsample (36 plants per subplot) at the end of each crop. The number of RKN-infected plants was also recorded.

2.2. Effects of soil types, time of planting and time of biofumigation on nematocidal effects of sorghums (greenhouse pot experiment)

A greenhouse pot experiment was conducted to compare, in terms of nematode suppression, two times of planting and of biofumigation for both sorghum varieties, ‘Piper’ and the hybrid ‘270911’ in three soil types: 1) sandy (82% sand, 9% loam, 4% clay, 1.6% OM), 2) clayey (21% sand, 24% loam, 33% clay, 3.9% OM), and 3) sandy-loamy (53% sand, 22% loam, 12% clay, 3.3% OM). *M. incognita*, obtained from the collection maintained at INRA Sophia Antipolis, France, was inoculated at 1000 J2s.kg⁻¹ soil in 12-liter pots. Experiments were conducted in a 2 × 3 × 2 (sorghum variety × soils × termination time) factorial designed experiment with 4 replications and repeated twice. Nine seeds of ‘270911’ and 15 seeds of ‘Piper’ were sown per pot, in accordance with UPL France SASTM recommendations of 30 kg seeds ha⁻¹ to reach the same amendment rate (1% after two months, 0.3% after one month in g of fresh sorghum material per 100 g of soil). Sorghums were grown in sandy, clayey and sandy-loamy soils for one or two months. The plants were chopped into pieces then soil incorporated. The pots were irrigated to establish anaerobic soil conditions and tarped with a virtually impermeable film (VIF) usually used as solarization mulch. The effect of biofumigation on RKN was examined at 10 days after sorghum residues were incorporated into the soil. During this time, soil temperature varied from 20 to 40 °C. To evaluate the effect of time of biofumigation, sorghums were grown in sandy-clayey soil for one month, incorporated into the soil, irrigated and tarped with VIF and examined at 10 or 30 days. The experiment was conducted twice with 4 replicates each time. Soil

infestation potential (SIP) was evaluated by subsampling 1 kg of soil from each pot at a depth of 15 cm before sowing the sorghum seeds, after the growth period (one or two months), and after biofumigation (10 or 30 days). Two-month-old tomato plants (RKN-susceptible cv Saint-Pierre, provided by VilmorinTM France) were transplanted into each pot filled with these soil samples and maintained in the greenhouse. After six weeks, SIP was evaluated in each pot by immersing tomato roots in cold aqueous eosin yellow solution as described by Roberts et al. (1990), observing under a magnifying glass and determining the number of egg masses (EMs) present on them. The multiplication rates Pf/Pi were compared between each modality.

2.3. Variety screening for susceptibility (growth chamber conditions)

‘Piper’ (Sg ‘Piper’) and ‘270911’ (SbSg ‘270911’) were compared for susceptibility to *M. incognita*. Nine other sorghum varieties including *Sorghum bicolor* (Sb ‘Nutrigrain’), four [*Sorghum bicolor* × *Sorghum bicolor*] hybrids (SbSg1,2,3,4), three [*Sorghum bicolor* × *Sorghum bicolor*] hybrids (SbSb1,2,3) and one [*S. bicolor* ssp. *saccharatum* × *S. bicolor* ssp. *saccharatum*] hybrid (SsSs) provided by UPL France SASTM were also analyzed for susceptibility to *M. incognita*. The susceptible tomato cv Saint-Pierre was used as a control. Seeds of each variety were grown in 400 mL pots containing steam-sterilized sandy soil covered by a 1 cm layer of loam and maintained in growth chambers (16 h light/8 h dark cycle, mean temperature of 24 ± 2 °C, relative humidity of 60–70%). Each variety was replicated in 8 pots and the experiment was conducted twice. At 4 weeks after seeding, 400 *M. incognita* J2s, obtained from the collection maintained at INRA Sophia Antipolis, France, were inoculated per pot. At 6 weeks after nematode inoculation, the number of EMs per root system for each plant was estimated as described above. Plants were considered to be susceptible if EMs > 100, non-host if EMs = 0, and poor host if 1 < EMs ≤ 100. After observation, the same roots were stained to visualize RKN infection into the plant tissues by acid fuchsin method as described by Byrd et al. (1983).

2.4. Statistical analysis

For the field trial, two-way ANOVA was performed to compare nematode concentration in soil in function of time and sorghum type. Time points were not compared altogether but three comparisons were done: T0 vs T3, T24 vs T27 (to measure the short-term impact of sorghum culture) and T0 vs T43 (to measure the long-term effect of using sorghum culture). Interactions were included. Another set of two-way ANOVA were performed to compare galls index on similar cultures at different time points for both sorghum types. Cultures tested were melon, winter cultures (chard and lettuce together, or lettuce only), and resistant cultures together (pepper and tomato). Interactions were included.

For the greenhouse experiment, multifactorial ANOVA with all interactions was done on the mean SIP data (over replicates) of differences between EM at the time considered and start of the experiment. Two analyses were done, separately before and after biofumigation (with factors time × soil × sorghum type, i.e. 2 × 3 × 2).

Data from the host suitability assay in controlled conditions were assessed in a global Kruskal-Wallis test followed by post-hoc pairwise Wilcoxon tests with Benjamini-Hochberg correction for multiple testing, with a threshold of P = 0.05 (Benjamini and Hochberg, 1995).

3. Results

3.1. Impact of the sorghum cropping system on RKN in the field experiment

Mean RKN abundance was high over the entire plot in the trial at Lambesc (initial rate = 20,766 ± 5,620 individuals/dm³) after the first melon crop (Fig. 1A). We observed a strong and significant short term

diminution of RKN abundance in soil by using both sorghums. At the beginning of the study, the relative RKN concentration loss was of 94% for ‘270911’ and of 95% for ‘Piper’ (ANOVA, $p_{\text{time}} < 0.02$, $p_{\text{sorghum}} > 0.5$, no interaction). RKN populations subsequently remained low in the soil, below the initial rate, after the cultivation of highly susceptible chard or lettuce winter crops just after sorghum. Following chard or lettuce, the summer crops with low resistance to RKN (pepper in 2013, pepper and tomato in 2015) allowed an increase of RKN populations in the soil significantly higher for ‘270911’ plot than for ‘Piper’ plot. At mid-study, the relative concentration loss was of 81% for ‘270911’ and of 73% for ‘Piper’ (ANOVA, $p_{\text{time}} < 0.01$, $p_{\text{sorghum}} > 0.5$, no interaction). Throughout the four years of the experiment, the alternation of sorghum green manure and partially resistant crops maintained a decrease in the RKN population and the long-term trend was still beneficial, with a global diminution of 84% for ‘270911’ and 79% for ‘Piper’ (ANOVA, $p_{\text{time}} < 0.01$, $p_{\text{sorghum}} > 0.5$, no interaction) (final rate = $2,850 \pm 1,365$ individuals/dm³ in ‘Piper’ plot and $4,725 \pm 600$ individuals/dm³ in ‘270911’ plot).

After one month of cultivation in summer, no gall was present on the roots of ‘Piper’ or ‘270911’ (Fig. 1B). GI was similar and moderate (less than 4) on melon culture in 2012 and 2014 after both sorghum types ($p_{\text{time}} = 0.46$, $p_{\text{sorghum}} = 0.32$, no interaction). For resistant cultures, very few galls were observed and 40–70% of plants exhibited no gall during all the experiment, tomato showing an average GI lower than pepper, with no difference in both sorghum plots ($p_{\text{crop}} < 10^{-3}$, $p_{\text{sorghum}} = 0.73$, no interaction). The picture is more complex for winter cultures, with a significant difference between chard and lettuce, chard being more infected than lettuce (ANOVA, $p < 0.05$) and ‘Piper’ sorghum plot being less infected than ‘270911’ sorghum plot (ANOVA,

$p < 0.05$, no interaction with crop type). Moreover, results for lettuce only showed some variability, with early lettuce crops (harvested in October) being significantly less infected than late crops for both sorghum types (ANOVA, $p_{\text{time}} < 10^{-16}$, $p_{\text{sorghum}} > 0.05$). This late analysis also showed a weak interaction between time points and sorghum type (ANOVA, $p < 0.02$) essentially due to a diminution of GI on lettuce at the end of the experiment, only in plots cultivated with ‘Piper’, diminution which was not significant at other time points. GI reached almost 4 and only 3% of plants had no gall when no sorghum was used in the fourth year compared to GI < 2 and 30–45% of plants with no gall when sorghums were used before winter crops.

3.2. Effects of soil types, time of planting and time of biofumigation on nematocidal effects of sorghums in pot experiment

Before the sorghum was planted in the $2 \times 3 \times 2$ factorial experiment, the number of nematodes in the soil, as evaluated by determining SIP, was moderate in clayey and sandy-loamy soils, to heavy in sandy soils (Fig. 2). After one month of cultivation, the number of nematodes greatly decreased in the three types of soils, for both sorghum varieties: Pf/Pi was less than 0.4. The experiment being repeated twice with the same mean values, results of both experiments (thus for 8 pots) were combined and shown in Fig. 2. A multifactorial ANOVA on data before biofumigation (time x soil x sorghum type, i.e. $2 \times 3 \times 2$, Tables 2 and 3) showed a strong time effect ($p < 10^{-11}$) with an increase in EM numbers when comparing two months cultivation relative to one month ($0.4 < \text{Pf/Pi}$ after two months < 2.1 ; $0.1 < \text{Pf/Pi} < 0.4$ after one month), and another strong soil effect ($p < 10^{-15}$) showing a strong increase in clayey soils ($\text{Pf/Pi} > 2$). No effect relative to the sorghum type was

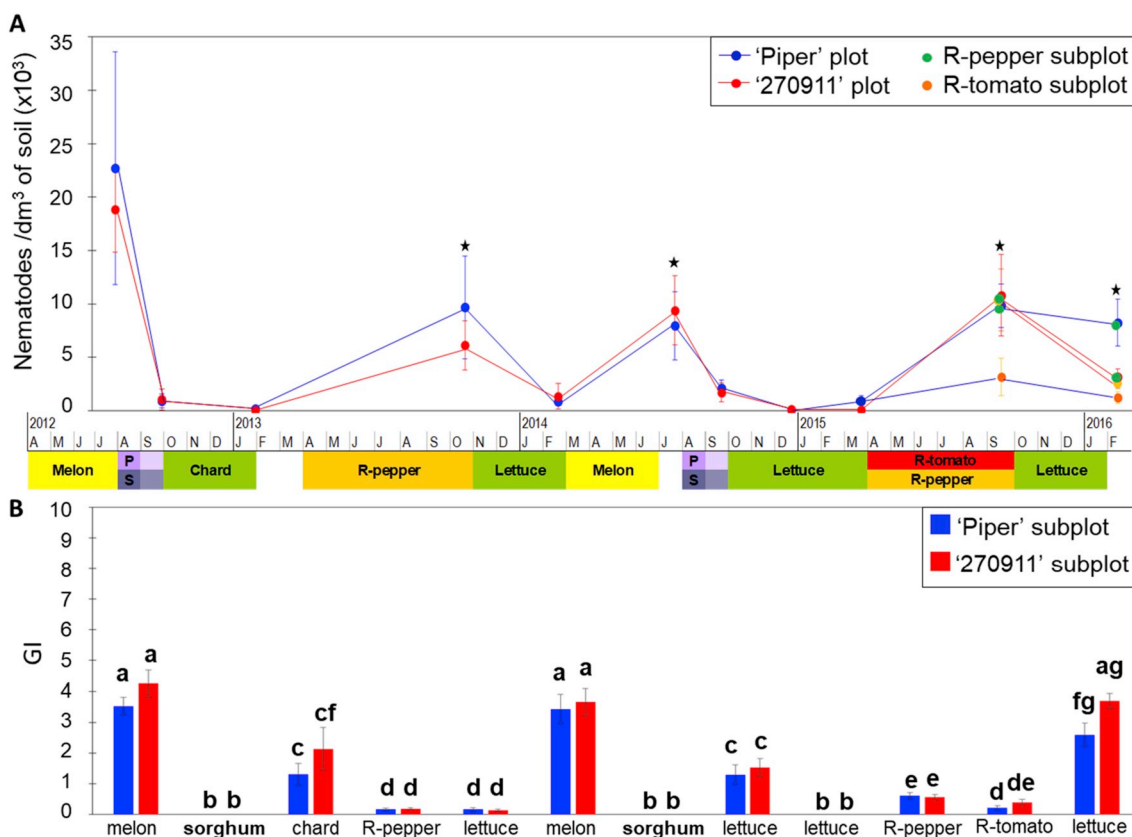


Fig. 1. (A) Kinetics of RKN populations in the soil over the 4-year field experiments in Lambesc: means ($n = 8$) \pm standard deviation followed by stars indicate significant differences ($p < 0.05$) between both sorghum plots at the sampling date; and (B) Gall index (GI) on plants: mean ($n = 36$) \pm standard deviation followed by different letters indicate significant differences. P = ‘Piper’, S = ‘270911’; chard = Swiss chard; lettuce = Batavia salad or oak leaf lettuce; R-pepper = several varieties of sweet peppers grafted onto a R-pepper rootstock carrying *Me3* R-gene in the susceptible genetic background Doux-Long-des-Landes; R-tomato = *Mi-1* R-tomato.

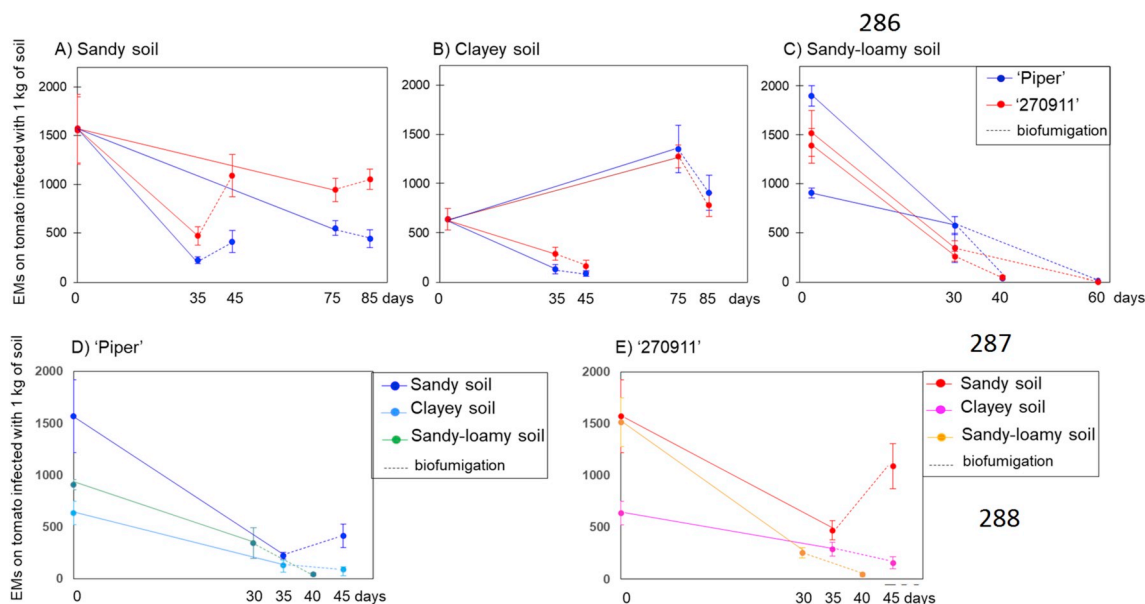


Fig. 2. Changes over time in RKN soil infestation potential (SIP) with ‘Piper’ and ‘270911’ in greenhouse experiments, as expressed by the number of egg masses (EMs) on susceptible tomato plants maintained for six weeks in pots filled with 1 kg rhizosphere soil sampled from each pot: means ($n = 8$) \pm standard deviation. A multifactorial ANOVA was done on the mean variation in EM numbers along the experiment before and after biofumigation (time \times soil \times sorghum type, i.e. $2 \times 3 \times 2$). In sandy soil (A) and clayey soil (B), both sorghums were grown for either one or two months, buried, irrigated, and tarped with VIF (a solarization mulch) during 10 days. In sandy-loamy soil (C), both sorghums were grown for one month, buried, irrigated, and tarped with VIF during 10 or 30 days. Soil effect was compared for ‘Piper’ (D) and ‘270911’ (E) grown for one month, buried, irrigated, and tarped with VIF during 10 days. Dotted lines indicate biofumigant effects. Note that panels D and E are based on the same experiments and data as panels A, B and C, and are presented under this format for easier comparison between sorghum types.

Table 2

ANOVA of soil infestation potential data before or after biofumigation and at the beginning of the experiment. df: degrees of freedom, Sum sq: sum of squares, p-val: p-value associated to the variable considered.

Variable	Before biofumigation			After biofumigation		
	df	Sum sq.	p-val	df	Sum sq.	p-val
Time	1	27572673	$<10^{-11}$	1	2220990	$<10^{-4}$
Soil	2	9309191	$<10^{-15}$	2	11691741	$<10^{-14}$
Sorghum type	1	40747	0.59	1	295017	0.09
Time*Soil	1	1867127	$<10^{-3}$	1	1968328	$<10^{-4}$
Time*Sorghum type	1	9350	0.79	1	67002	0.42
Soil*Sorghum type	2	1262920	0.014	2	4202318	$<10^{-6}$
Time*Soil*Sorghum type	1	138751	0.32	1	14354	0.70
Residuals	83	11648935		67	6847038	

Table 3

Linear model table of the soil infestation potential values before or after biofumigation. First column indicates variable and the corresponding reference level, second column the modality under study, third, fourth and fifth columns the estimate for the model, the std. deviation of the estimate and the associated p-value. Interactions are reported only for those showing a $p\text{-val} < 0.1$. Data are identical to those of the ANOVA Table 2.

Variable:ref	Modality	Before biofumigation			After biofumigation		
		Effect	Std. Dev	p-val	Effect	Std. Dev	p-val
Soil:clayey	Sandy	-844.32	132.45	$<10^{-4}$	-844.32	132.45	$<10^{-4}$
	Sandy-loamy	-384.18	166.04	0.023	-384.18	166.04	0.023
Time:Short	Long	1219.88	187.32	$<10^{-8}$	1219.88	187.32	$<10^{-8}$
	Sorghum type: ‘Piper’	156.63	187.32	0.40	156.63	187.32	0.40
Soil*time	Sandy*Long	-874.53	264.90	$<10^{-2}$	-874.53	264.90	$<10^{-2}$
Soil*Sorghum type	Sandy-loamy* S’270911’	-420.07	232.13	0.073	-420.07	232.13	0.073

detectable. Moreover, an advanced analysis including interaction terms showed that the increase in EM numbers due to two months-cultivation in clayey soils still exists, but in a much weaker form ($p < 10^{-3}$). Repeating the same analysis after 10 days of biofumigation (Tables 2 and 3) replicated the same results, with slightly different p-values: $p < 10^{-4}$ for the increase in EMs number with two months cultivation

($0.3 < Pf/Pi$ after two months < 1.4 ; $0.1 < Pf/Pi < 0.6$ after one month), $p < 10^{-14}$ for the soil effect with the same effect as previously, and still no sorghum type effect. This showed that 10 days of biofumigation did not remediate to the increase in EM numbers due to the two months-cultivation. In sandy-loamy soil after one month-cultivation, 30 days of biofumigation were slightly more efficient ($Pf/Pi < 0.003$) than 10

days of biofumigation ($Pf/Pi < 0.04$), resulting in the almost complete abolition of SIP in some pots. The temperature in the soil under the VIF varied from 22 to 47 °C over this 30-day period.

3.3. Tests of host suitability in controlled conditions and observations of RKN developmental stages in the roots

The experiment in growth chamber being repeated twice with the same mean values, results of both experiments (thus for 16 plants) were combined and shown in Fig. 3. Based on EM production, both the commonly used variety Sg ‘Piper’ and the recommended variety with high dhurrin SbSg ‘270911’ were equally resistant to *M. incognita*. SbSg2 exhibited the same EM numbers. Only SbSg1 was non-host. SbSg3, SbSg4, and SbSb1 could be compared to SbSg ‘270911’ and designated as poor hosts. SbSb2, SbSb3, Sb ‘Nutrigrain’ and SsSs were more susceptible to RKN compared to Sg ‘Piper’ and SbSg ‘270911’. The galls induced by *M. incognita* on the roots of all sorghum plants were much smaller than those observed on tomato roots. EMs were about 1 ± 0.05 mm in diameter on the roots of susceptible tomato plants, but only about 650 ± 60 µm in diameter on sorghums Sb, SbSb3 and SsSs, and about 500 ± 50 µm in diameter on the other types of sorghum, including Sg ‘Piper’ and SbSg ‘270911’. Moreover, EMs were often found inside sorghum roots, as seen with acid fuchsin staining, whereas those on tomato roots were always outside the gall (Fig. 4). Small numbers of second, third and fourth stage juveniles, females and EMs were observed in the roots of the poor hosts. Very few J2s penetrated the roots of the non-host SbSg1 and they did not develop into J3s after six weeks. Necrotic cells (hypersensitive-like reaction) were visible as a darkening of the orange staining in the epidermis and cortex of the roots at a higher frequency in the roots of Sg ‘Piper’ than in SbSg ‘270911’, where more juveniles of all stages were observed. No hypersensitive-like reaction was observed in the roots of SbSg1.

4. Discussion

In this study, the effects of two sorghums as summer cover crops, the hybrid ‘270911’ with high dhurrin content and the commercial ‘Piper’ with low dhurrin content, were compared in a vegetable production system by determining their impact on two main components of crop protection against RKN: the ability to decrease parasite levels in the soil and the potential to protect subsequent crops (susceptible or partially resistant) in the rotation. Analytical experiments in controlled conditions (growth chamber and greenhouse) were designed to provide explanatory elements to what was observed in the field.

Both sorghums with low and high dhurrin contents used as summer cover crop had the same potential to control RKN:

The results of the four-year trial and greenhouse pot experiment clearly demonstrated that both sorghums, cultivated for one month or

less to avoid RKN multiplication and then buried for one month, were able to control RKN in vegetable production systems with the same efficiency: they significantly reduced RKN populations in the soil by up to 70% compared to the rate before planting, thereby protecting crop rotations including forthcoming susceptible hosts. The farm had sandy-loamy soil and the greenhouse experiment confirmed the high efficiency of both sorghums in this type of soil as long as they are used appropriately.

In the same way, both types of sorghum appeared to minimize damage to subsequent susceptible winter crops in the rotation: Swiss chard and lettuce exhibited a very low mean GI after both types of sorghum, whereas GI was higher if sorghum was not grown. Nevertheless, GI for these plants also varied according to the date of plantation ($1.5 < GI < 4$ for October plantation and $0 < GI < 0.2$ for November and January plantations). Date of plantation is strongly related to the soil temperature; indeed, *M. incognita* and *M. arenaria* are not able to infect plants, develop and reproduce at temperatures below 15 °C (Evans and Perry, 2009; Thomason and Lear, 1961; Vrain et al., 1978). In October, the mean soil temperature in Lambesc plot was around 15–20 °C and in November, around 7–15 °C. Thus, we cannot conclude that the GI reduction observed on these winter crops was only due to the cultivation of both sorghums.

Both types of sorghum helped protect weak R-summer crops (tomato and pepper): very few galls were observed on the roots of these summer crops ($GI < 1$), and 40–70% of plants had no gall, even during the second crop rotation. Decreasing the number of parasites in the soil may increase the durability of R-genes, because the appearance and early increase in the frequency of virulence alleles in the pathogen population depend on the balance between mutation rates and population size (Consortium REX, 2012). However, longer experiments are required for firm conclusions about efficacy to be drawn, because these weak R-summer crops did not keep inoculum levels low in the soil. Nevertheless, the alternation of sorghum as summer cover crop and partially R-Solanaceae over a four-year period resulted in the sustainable control of RKN populations, with $0.05 < Pf/Pi < 0.36$.

Time of cultivation, time and quality of biofumigation, and type of soil affected sorghums efficacy in terms of nematode suppression:

The sorghums were cultivated only one month in summer before incorporation of residues into the soil of the field trial because they were suspected of favoring the long term multiplication of a few starting RKN. Experiments in controlled conditions confirmed this hypothesis, both sorghums being only poor hosts. Thus, duration of the growing period before burial of fresh material in the soil was of crucial importance: RKN significantly increased in the soil after 75 days cultivation (Pf/Pi up to 2), while they always significantly decreased with only 30 days cultivation ($0.1 < Pf/Pi < 0.4$), before completion of the RKN life cycle. This temporary trapping, reducing RKN infestation in the soil, masks the high risk of RKN multiplication if the sudangrass crop is destroyed too late.

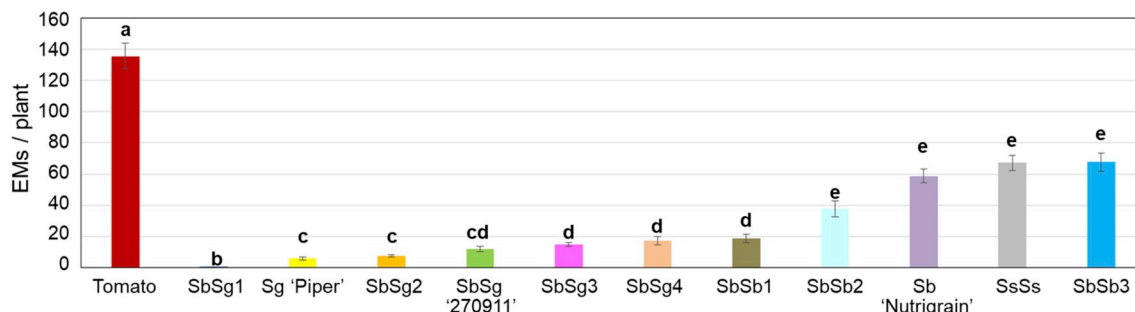


Fig. 3. Mean number of EMs per plant counted on several sorghum varieties and on tomato (as a control) maintained in a controlled-climate growth chamber after inoculation with 400 juveniles of *M. incognita*: means ($n = 16$) \pm standard deviation followed by different letters indicate significant differences ($p < 0.05$). Sg ‘Piper’ = Sudan grass (*Sorghum bicolor* ssp. *sudanense*) variety ‘Piper’; SbSg ‘270911’ = [*Sorghum bicolor* ssp. *bicolor* x Sudan grass] 3-way hybrid; Sb ‘Nutrigrain’ = *Sorghum bicolor*; SbSg1,2,3,4 = [*Sorghum bicolor* x Sudan grass] hybrids; SbSb1,2,3 = [*Sorghum bicolor* x *Sorghum bicolor*] hybrids; SsSs = [*S. bicolor* ssp. *saccharatum* x *S. bicolor* ssp. *saccharatum*] hybrid.

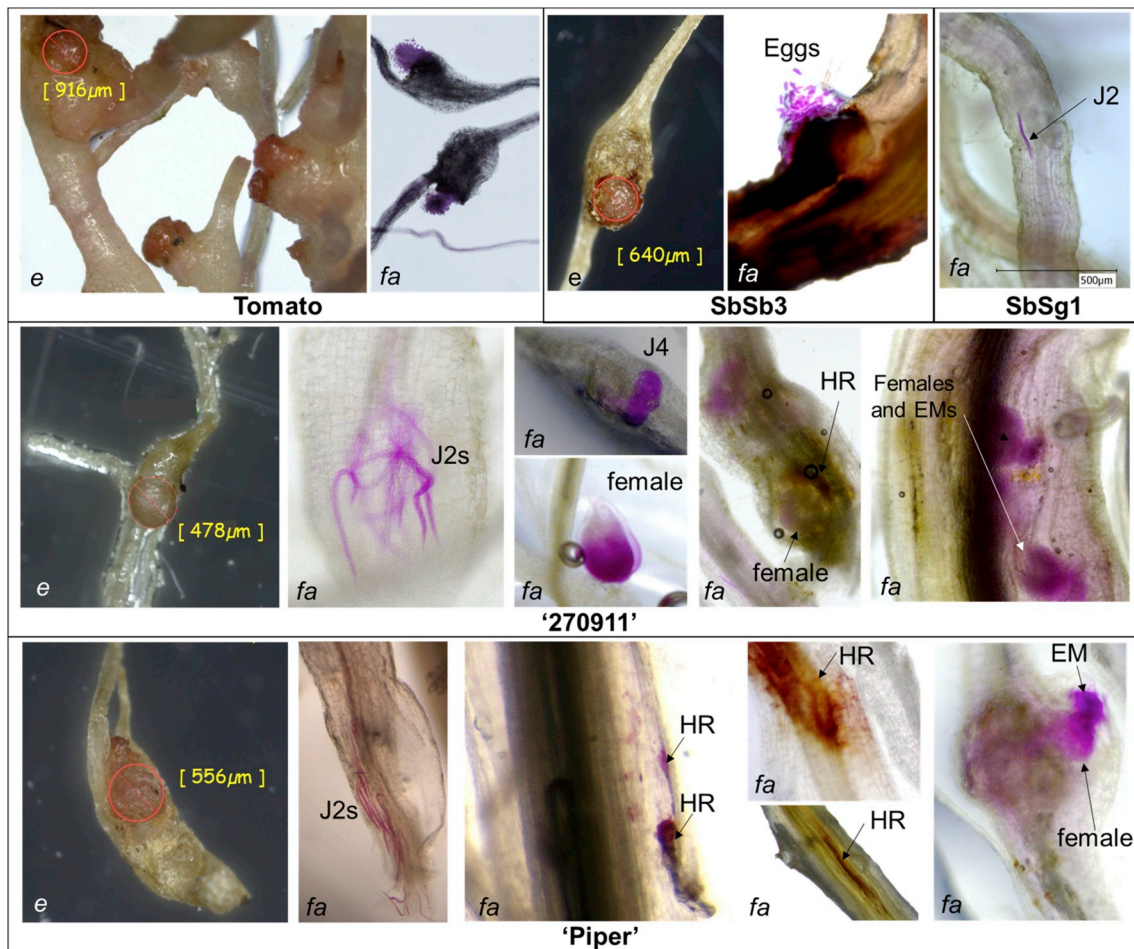


Fig. 4. Observation of the root system of the very poor hosts Sg 'Piper' and SbSg '270911', the non-host SbSg1, and the poor host 'SbSb3' under a stereomicroscope after inoculation with 600 juveniles of *M. incognita*. Tomato is used as a control (HR = hypersensitive-like reaction; EM = egg-mass; e = eosin staining; fa = fuchsin acid staining).

Moreover, young sorghums have a higher dhurrin content than old ones (Adewusi, 1990). It was confirmed by Gard et al. (2014): 12.7 and 2.68 mg/g of dry matter for 3-week-old '270911' and 'Piper', respectively, versus 8.7 and 2.3 mg/g of dry matter for 4-week-old '270911' and 'Piper', respectively, decreasing to 4.5 and 0.7 mg/g of dry matter for 6-week-old '270911' and 'Piper', respectively. Older tissues are also more bulky and may break down more slowly, thereby releasing smaller amounts of HCN into the soil (Viaene and Abawi, 1998).

The soil and temperature conditions may also affect the degradation of the fresh material buried in the soil and, thus, the release of HCN (Viaene and Abawi, 1998). It may explain why increasing the biofumigation time under VIF (that holds in the gaseous breakdown products; Gamliel and Stapleton, 1993) to 30 days in the greenhouse experiment, although a less important factor than the growing time, may nevertheless allow the almost complete elimination of RKN from soils when combined with the trapping effect. Wang et al. (2006) also found cover crop solarization to be an effective combined treatment for decreasing *Meloidogyne* spp. infestation, with an efficacy similar to that of methyl bromide fumigation at crop harvest, the most effective treatment used against soil-borne diseases and pests in conventional agriculture, before its prohibition.

The duration of burial, measured under controlled conditions, must be adjusted according to the growing conditions. Under cover in the South of France, this period may be estimated to be four weeks in spring, and three weeks in summer. In open-field conditions in northern France, this period could be extended to five weeks in spring and four weeks in summer. For farmers who consider green manure as essential and can

wait for two months before incorporating sorghum residues into the soil, an innovative double-sowing technique (two lots of sorghum grown one after another for three weeks each and then buried) could be proposed as it is currently tested efficiently in some farms following results obtained from the Lambesc farm experiment.

All these required conditions may explain why some authors found sorghums efficient to control RKN populations in vegetable cropping systems (Everts, 2006; Kratochvil et al., 2004; Mojtahedi et al., 1993; Viaene and Abawi, 1998; Widmer and Abawi, 2000), whereas other obtained disappointing results (Collange et al., 2011; Crow et al., 2001; Kokalis-Burelle et al., 2013; Védie, 2010). Now, the sustainable management of RKN populations should not only be considered in terms of managing these nematodes but also in terms of managing the pathogenicity and the biodiversity of the whole plant-parasitic and non-parasitic nematode communities (i.e., ecological sustainability) (Mateille et al., 2008), because competitive interactions between nematodes may increase the sustainability of the management strategy (Mateille et al., submitted).

The mode of action of sorghums to control RKN may depend on their genotypes:

Only one variety, SbSg1, was a non-host for *M. incognita* (no EM) and was as dhurrin-rich as '270911' (7.5 ± 0.2 mg/g of dry matter after one month according to Gard et al., 2014): it could therefore be grown for more than four weeks before burial without the risk of RKN multiplication. The SbSg1 hybrid was obtained from a [*Sorghum bicolor* x sudangrass] cross. It is not yet developed, registered or commercialized. Its dhurrin content reached 7.5 ± 0.2 mg/g of dry matter after one month

(Gard et al., 2014), a level slightly lower than that of SbSg '270911' after one month (8.7 ± 0.2 mg/g). All the other varieties, SsSs hybrid, Sb 'Nutrigrain' and SbSb hybrids were more susceptible than Sg 'Piper' and SbSg hybrids, including SbSg '270911'. Birchfield (1983), Davis and Anderson (2012), Fortnum and Currin (1988) and Mojtabehi et al. (1993) also reported a varietal effect on susceptibility of grain and sweet sorghum Sb (*S. bicolor*), Sg (sudangrass), SbSg (Sorghum-sudangrass hybrids) genotypes tested against various RKN species. This differential level of resistance cannot be accounted for by dhurrin content, because Sg 'Piper' (low level of dhurrin) and SbSg '270911' (high level of dhurrin) were both very poor hosts, with no significant difference in EM numbers on their roots. In 2015, Harris-Shultz et al. mapped a major quantitative trait locus (QTL) to sorghum chromosome 3, accounting for the resistance of one of Sb varieties to *M. incognita* race 3. Other inherited resistance factors may be present in the sudangrass genome, accounting for the lack of RKN reproduction on Sg 'Piper' and the hybrids. Sg 'Piper' roots contained only a few RKN and showed many hypersensitive-like reaction (HR) sites, indicating a response to infection similar to that in *Mi-1.2* resistant tomato plants (Paulson and Webster, 1972); SbSg '270911' plants contained larger numbers of juveniles of all stages, concealed within the roots, with little sign of a HR. Both sorghums acted as trap plants for RKN because only few EMs were produced. In the roots of the non-host sorghum SbSg1, only a few scattered J2s, with no further development, and a total absence of HR were observed. Thus, SbSg1 roots may repel juveniles, due to the toxic root exudates, as reported for several other non-host plants (summarized in Djian-Caporalino et al., 2005), including two SbSg (*Sorghum bicolor* x *S. sudanense* 'SX-15' and 'SX-17') (Czarnota et al., 2003). Sorgoleone, the phenolic compound identified as a predominant constituent in exudates, could be potentially responsible for the suppressive effect of SbSg1. This hypothesis should be tested in future studies. This sorghum could be a more usable candidate for farmers as it may be cultivated more than one month before burying for biofumigation because it is non-host for RKN.

5. Conclusion

The use of sorghums, sudangrass or hybrids, as a green manure was found to be an effective strategy for decreasing the RKN infestation potential of soils, thereby protecting crop rotations including both susceptible and partially resistant hosts. But the efficacy of sorghums clearly depends on the management strategy to be set up in the field. In particular, incorporating sorghums into the soil before the end of the RKN cycle plays a key role in the efficient and sustainable control of these parasites. No effect relative to the sorghum type (with low or high dhurrin content) was detectable as long as they were used correctly, i.e., cultivated during one month or less and left for one month for biofumigation. This study thus provides information potentially useful to breeders and farmers for the sustainable management of RKN in protected vegetable systems.

Declarations of interest

None.

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