Evaluating sorghums as green manure against root-knot nematodes

Caroline Djian-Caporalino, Thierry Mateille, Marc Bailly-Bechet, Nathalie Marteu, Ariane Fazari, Pierre Bautheac, Alizée Raptopoulo, van Luan Duong, Johannes Tavoillot, Bernard Martiny, et al.

Evaluating sorghums as green manure against root-knot nematodes

Caroline Djian-Caporalino[1*], Thierry Mateille[2], Marc Bailly-Bechet[1], Nathalie Marteu[1], Ariane Fazari[1], Pierre Bautheac[1], Alizée Raptopoulo[1], Luan Van Duong[1], Johannes Tavoillot[2], Bernard Martiny[2], Claire Goillon[3], Philippe Castagnone-Sereno[1]

[1] INRA, Université Côte d'Azur, CNRS, Institut Sophia Agrobiotech, Sophia Antipolis, France.

[2] CBGP, IRD, CIRAD, INRA, Montpellier SupAgro, Univ. Montpellier, Montpellier, France.

[3] APREL (Association Provençale de Recherche et d'Expérimentation Légumière), France.

*** Corresponding author:** caroline.caporalino@inra.fr

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.

Keywords: Meloidogyne sp.; *Sorghum vulgare var. sudanense*; sudangrass hybrids; cover crop; trap crop; biofumigation.

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; Séguy 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 (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 to 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 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. chitwoodi*. Wang et al. (2004) and Guerena (2006) reported that the prior use of sudangrass hybrids as a cover crop decreases the density of populations of *M. incognita* on *Secale cereal*e '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 (Orfonedes, 1995; Widner 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 futher 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 socio-economic 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 x 8 m x 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 & Bergé,

120 1978). During the experimental period, soil temperature at a depth of 15 cm varied from 5 to 15°C
121 from November to February, and from 15 to 30°C from April to October.
122 The plot was divided into two subplots, one for planting sudangrass 'Piper', the standard cover
123 crop commonly used by commercial farmers in France with low dhurrin content, the other for planting
124 sudangrass hybrid '270911' with high dhurrin content known for biofumigation purposes. '270911' is a
125 three-way hybrid generated from [*Sorghum bicolor* spp. *bicolor* x sudangrass] cross developed by UPL
126 France SAS™. The 4-year-field experiment lasted two seasons, the sorghum cover crop being grown
127 in the first and third years in order to duplicate the experiment. The sorghum seeds were sown in July
128 or August on rototilled soil with seedbed preparation before sowing, at a density of 50 kg seeds/ha for
129 'Piper' to reach 25 to 30 tons/ha of aboveground fresh weight. '270911' was sown at a density of 85
130 kg/ha in 2012 due to poor germination but at 30 kg seeds/ha in 2014 as recommended by UPL France
131 to reach the same amendment rate (Table 1). One month after sowing, the leaves and shoots were
132 mowed and soil incorporated with rotavator. The soil was rolled and left uncovered for one month to
133 allow biofumigation. To prevent phytotoxicity, the next cash crops were planted three weeks after
134 termination of biofumigation.
135 RKN-susceptible plant species were grown subsequent to these cover crops to assess the
136 suppression of RKN. Two types of RKN-susceptible plants were used: 1) a very susceptible crop, i.e.,
137 *Cucumis melo* (melon) in spring, and 2) susceptible crops in winter when the RKN cycle is slower,
138 such as *Lactuca* spp. (lettuce) or *Beta vulgaris* subsp. *vulgaris* (Swiss chard). Weak forms of R-crops
139 were cropped during the summer in the second and fourth years, to determine whether the use of a
140 green manure could decrease the RKN soil infestation potential enough to secure crop rotation with
141 them. RKN-resistant tomatoes (R-tomato) whose *Mi-1.2* resistance gene is inactivated at high
142 temperature were used. Sweet pepper varieties grafted onto resistant pepper rootstock (R-pepper)
143 that carries the major R-gene *Me3* weakened in the highly susceptible genetic background Doux-
144 Long-des-Landes, were also used and mixed with the R-tomatoes.
145 Sorghum biomass was estimated at the end of the cover crop by weighing the aerial part of plants
146 sampled from a 1 m^2 microplot before and after drying at 60°C for 48 h (Table 1). Rhizosphere soil
147 from eight 250 ml samples per subplot were sampled at a depth of 15 cm, before the experiment and
148 after each susceptible crop was terminated. Throughout the experiment, samples were systematically
149 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 dm^3 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.

Table 1.

Cropping schedule for cover crop in the commercial farm located in Lambesc 43.65N, 5.21E (80 m x 8 m x 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

167 *2.2. Effects of soil types, time of planting and time of biofumigation on nematocidal effects of*
168 *sorghums (greenhouse pot experiment)*

169 A greenhouse pot experiment was conducted to compare, in terms of nematode suppression, two
170 times of planting and of biofumigation for both sorghum varieties, 'Piper' and the hybrid '270911' in
171 three soil types: 1) sandy (82% sand, 9% loam, 4% clay, 1.6% OM), 2) clayey (21% sand, 24% loam,
172 33% clay, 3.9% OM), and 3) sandy-loamy (53% sand, 22% loam, 12% clay, 3.3% OM). *M. incognita,*
173 obtained from the collection maintained at INRA Sophia Antipolis, France, was inoculated at 1000
174 J2s.kg^{-1} soil in 12-liter pots. Experiments were conducted in a 2×3×2 (sorghum variety × soils ×
175 termination time) factorial designed experiment with 4 replications and repeated twice. Nine seeds of
176 '270911' and 15 seeds of 'Piper' were sown per pot, in accordance with UPL France SAS™
177 recommendations of 30 kg seeds ha^{-1} to reach the same amendment rate (1% after two months, 0.3%
178 after one month in g of fresh sorghum material per 100 g of soil). Sorghums were grown in sandy,
179 clayey and sandy-loamy soils for one or two months. The plants were chopped into pieces then soil
180 incorporated. The pots were irrigated to establish anaerobic soil conditions and tarped with a virtually
181 impermeable film (VIF) usually used as solarization mulch. The effect of biofumigation on RKN was
182 examined at 10 days after sorghum residues were incorporated into the soil. During this time, soil
183 temperature varied from 20 to 40°C. To evaluate the effect of time of biofumigation, sorghums were
184 grown in sandy-clayey soil for one month, incorporated into the soil, irrigated and tarped with VIF and
185 examined at 10 or 30 days. The experiment was conducted twice with 4 replicates each time. Soil
186 infestation potential (SIP) was evaluated by subsampling 1 kg of soil from each pot at a depth of 15
187 cm before sowing the sorghum seeds, after the growth period (one or two months), and after
188 biofumigation (10 or 30 days). Two-month-old tomato plants (RKN-susceptible cv Saint-Pierre,
189 provided by Vilmorin™ France) were transplanted into each pot filled with these soil samples and
190 maintained in the greenhouse. After six weeks, SIP was evaluated in each pot by immersing tomato
191 roots in cold aqueous eosin yellow solution as described by Roberts et al. (1990), observing under a
192 magnifying glass and determining the number of egg masses (EMs) present on them. The
193 multiplication rates Pf/Pi were compared between each modality.
194
195 *2.3. Variety screening for susceptibility (growth chamber conditions)*

196 'Piper' (Sg 'Piper') and '270911' (SbSg '270911') were compared for susceptibility to *M.*
197 *incognita*. Nine other sorghum varieties including *Sorghum bicolor* (Sb 'Nutrigrain'), four [*Sorghum*
198 *bicolor* x sudangrass] hybrids (SbSg1,2,3,4), three [*Sorghum bicolor x Sorghum bicolor*] hybrids
199 (SbSb1,2,3) and one [*S. bicolor* ssp. *saccharatum x S. bicolor* ssp. *saccharatum*] hybrid (SsSs)
200 provided by UPL France SAS™ were also analyzed for susceptibility to *M. incognita*. The susceptible
201 tomato cv Saint-Pierre was used as a control. Seeds of each variety were grown in 400 mL pots
202 containing steam-sterilized sandy soil covered by a 1 cm layer of loam and maintained in growth
203 chambers (16 h light/8 h dark cycle, mean temperature of 24±2°C, relative humidity of 60-70%). Each
204 variety was replicated in 8 pots and the experiment was conducted twice. At 4 weeks after seeding,
205 400 *M. incognita* J2s, obtained from the collection maintained at INRA Sophia Antipolis, France, were
206 inoculated per pot. At 6 weeks after nematode inoculation, the number of EMs per root system for
207 each plant was estimated as described above. Plants were considered to be susceptible if EMs > 100,
208 non-host if EMs =0, and poor host if 1 < EMs ≤ 100. After observation, the same roots were stained to
209 visualize RKN infection into the plant tissues by acid fuchsin method as described by Byrd et al.
210 (1983).
211
212 *2.4. Statistical analysis*
213 For the field trial, two-way ANOVA was performed to compare nematode concentration in soil in
214 function of time and sorghum type. Time points were not compared altogether but three comparisons
215 were done: T0 vs T3, T24 vs T27 (to measure the short-term impact of sorghum culture) and T0 vs
216 T43 (to measure the long-term effect of using sorghum culture). Interactions were included. Another
217 set of two-way ANOVA were performed to compare galls index on similar cultures at different time
218 points for both sorghum types. Cultures tested were melon, winter cultures (chard and lettuce
219 together, or lettuce only), and resistant cultures together (pepper and tomato). Interactions were
220 included.
221 For the greenhouse experiment, multifactorial ANOVA with all interactions was done on the mean
222 SIP data (over replicates) of differences between EM at the time considered and start of the
223 experiment. Two analyses were done, separately before and after biofumigation (with factors time x
224 soil x sorghum type, i.e. 2x3x2).

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/dm^3) 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_time<0.02, p_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_time<0.01, p_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_time<0.01, p_sorghum>0.5, no interaction) (final rate = 2,850±1,365 individuals/dm^3 in 'Piper' plot and 4,725±600 individuals/dm^3 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_time=0.46, p_sorghum=0.32, no interaction). For resistant cultures, very few galls were observed and 40 to 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_crop<10^{-3}, p_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_time<10^{-16}, p_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 to 45% of plants with no gall when sorghums were used before winter crops.

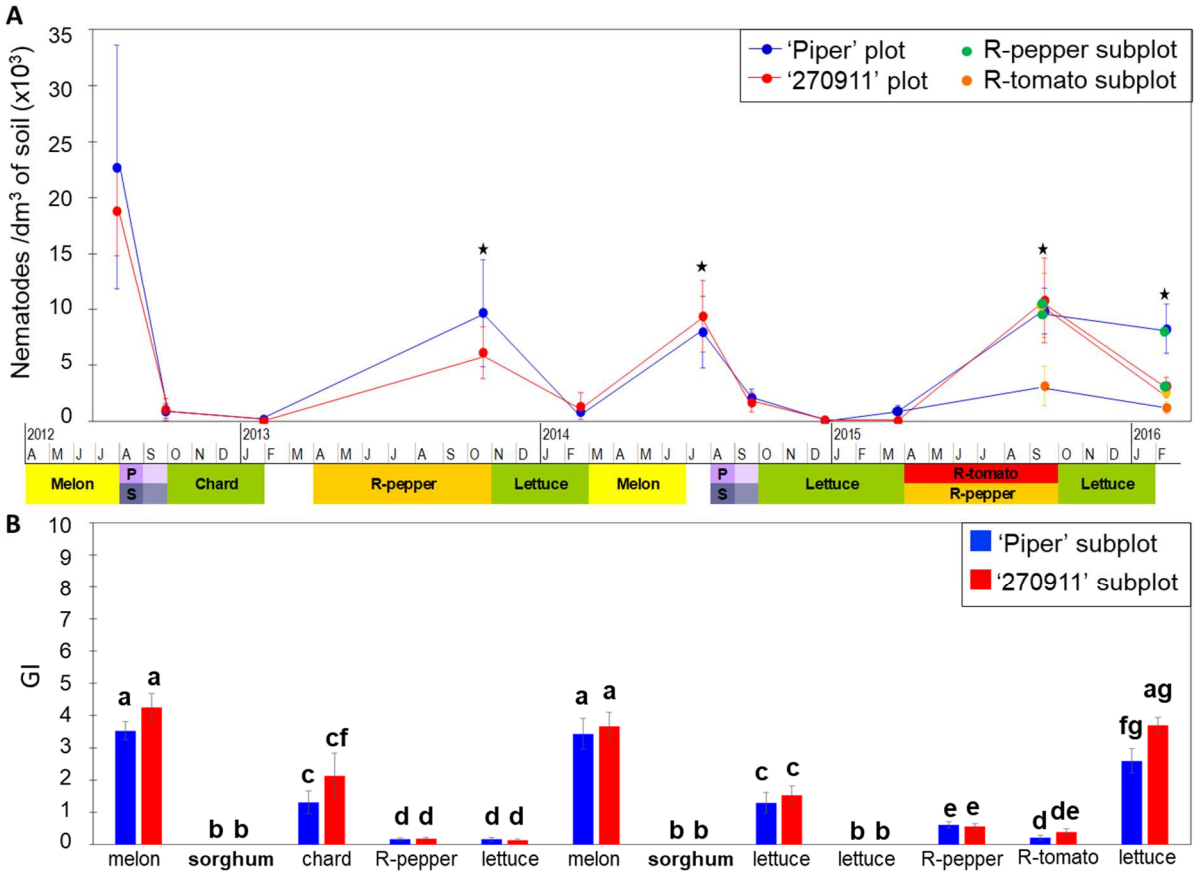

Fig. 1. (A) Kinetics of RKN populations in the soil over the 4-year field experiments in Lambesc: means (n=8) ± standard deviation followed by stars indicate significant differences (p < 0.05) between both sorghum plots at the sampling date; **and (B) Gall index (GI) on plants:** mean (n=36) ± standard 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.

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 x 3 x 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. 2x3x2, 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 < Pf/Pi after two months < 2.1 ; 0.1 < Pf/Pi < 0.4 after one month), and another strong soil effect ($p<10^{-15}$) showing a strong increase in clayey soils (Pf/Pi > 2). No effect relative to the sorghum type was 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.

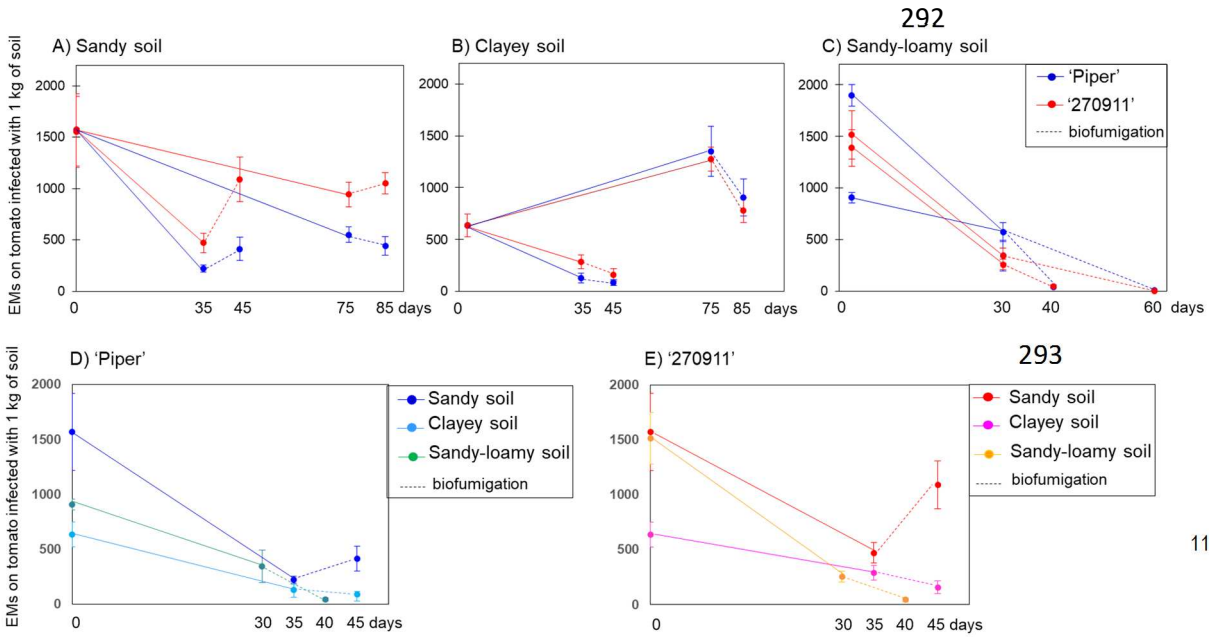

Fig. 2. Changes over time in RKN soil infestation potential (SIP) with 'Piper' and '270911' in greenhouse experiments, as expressed by the number of egg masses (EMs) on susceptible tomato plants maintained for six weeks in pots filled with 1 kg rhizosphere soil sampled from each pot: means (n=8) ± standard deviation. A multifactorial ANOVA was done on the mean variation in EM numbers along the experiment before and after biofumigation (time x soil x sorghum type, i.e. 2x3x2). 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.

	Before biofumigation			After biofumigation		
Variable	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, forth 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-val<0.1. Data are identical to those of the ANOVA table 2.

		Before biofumigation			After biofumigation		
Variable:ref	Modality	Effect	Std. Dev	p-val	Effect	Std. Dev	p-val
Soil:clayley	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'	S'270911'	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

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.

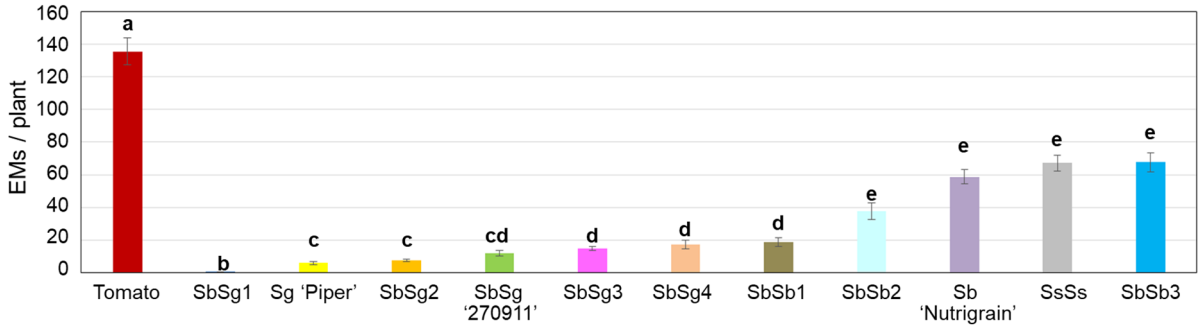

Fig. 3. Mean number of EMs per plant counted on several sorghum varieties and on tomato (as a control) maintained in a controlled-climate growth chamber after inoculation with 400 juveniles of *M. incognita:* means (n=16) ± standard deviation followed by different letters indicate significant differences ($p < 0.05$). Sg 'Piper' = Sudan grass (*Sorghum bicolor* ssp. *sudanense*) variety 'Piper'; SbSg '270911' = [*Sorghum bicolor* spp. *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.

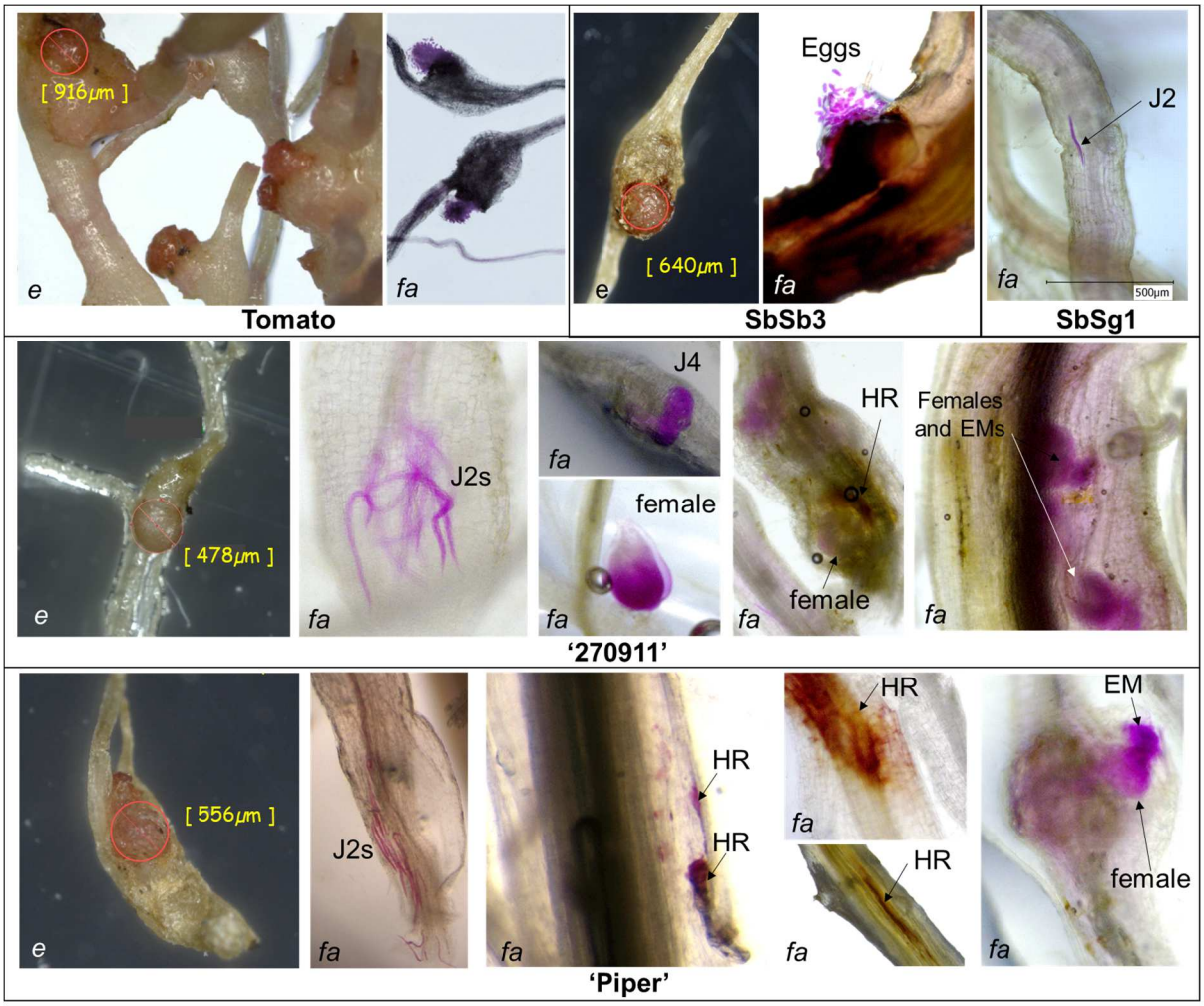

Fig. 4. Observation of the root system of the very poor hosts Sg 'Piper' and SbSg '270911', the non-host SbSg1, and the poor host 'SbSb3' under a stereomicroscope after inoculation with 600 juveniles of *M. incognita*. Tomato is used as a control (HR = hypersensitive-like reaction; EM = egg-mass; *e*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

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.

364

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

367 The results of the four-year trial and greenhouse pot experiment clearly demonstrated that both
368 sorghums, cultivated for one month or less to avoid RKN multiplication and then buried for one month,
369 were able to control RKN in vegetable production systems with the same efficiency: they significantly
370 reduced RKN populations in the soil by up to 70% compared to the rate before planting, thereby
371 protecting crop rotations including forthcoming susceptible hosts. The farm had sandy-loamy soil and
372 the greenhouse experiment confirmed the high efficiency of both sorghums in this type of soil as long
373 as they are used appropriately.

374 In the same way, both types of sorghum appeared to minimize damage to subsequent
375 susceptible winter crops in the rotation: Swiss chard and lettuce exhibited a very low mean GI after
376 both types of sorghum, whereas GI was higher if sorghum was not grown. Nevertheless, GI for these
377 plants also varied according to the date of plantation ($1.5 < GI < 4$ for October plantation and $0 < GI <$
378 0.2 for November and January plantations). Date of plantation is strongly related tothe soil
379 temperature; indeed, *M. incognita* and *M. arenaria* are not able to infect plants, develop and reproduce
380 at temperatures below 15°C (Evans and Perry, 2009; Thomason and Lear, 1961; Vrain et al., 1978). In
381 October, the mean soil temperature in Lambesc plot was around 15-20°C and in November, around 7-
382 15°C. Thus, we cannot conclude that the GI reduction observed on these winter crops was only due to
383 the cultivation of both sorghums.

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

393

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.

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 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 (Viane 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,

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

427 All these required conditions may explain why some authors found sorghums efficient to control
428 RKN populations in vegetable cropping systems (Everts, 2006; Kratochvil et al., 2004; Mojtahedi et al.,
429 1993; Viaene and Abawi, 1998; Widmer and Abawi, 2000), whereas other obtained disappointing
430 results (Collange et al., 2011; Crow et al., 2001; Kokalis-Burelle et al., 2013; Védie, 2010). Now, the
431 sustainable management of RKN populations should not only be considered in terms of managing
432 these nematodes but also in terms of managing the pathogenicity and the biodiversity of the whole
433 plant-parasitic and non-parasitic nematode communities (i.e., ecological sustainability) (Mateille et al.,
434 2008), because competitive interactions between nematodes may increase the sustainabilty of the
435 management strategy (Mateille et al., submitted).

436

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

438 Only one variety, SbSg1, was a non-host for *M. incognita* (no EM) and was as dhurrin-rich as
439 '270911' (7.5 ± 0.2 mg/g of dry matter after one month according to Gard et al., 2014): it could
440 therefore be grown for more than four weeks before burial without the risk of RKN multiplication. The
441 SbSg1 hybrid was obtained from a [*Sorghum bicolor* x sudangrass] cross. It is not yet developed,
442 registered or commercialized. Its dhurrin content reached 7.5 ± 0.2 mg/g of dry matter after one month
443 (Gard et al., 2014), a level slightly lower than that of SbSg '270911' after one month (8.7 ± 0.2 mg/g).
444 All the other varieties, SsSs hybrid, Sb 'Nutrigrain' and SbSb hybrids were more susceptible than Sg
445 'Piper' and SbSg hybrids, including SbSg '270911'. Birchfield (1983), Davis and Anderson (2012),
446 Fortnum and Currin (1988) and Mojtahedi et al. (1993) also reported a varietal effect on susceptibility
447 of grain and sweet sorghum Sb (*S. bicolor*), Sg (sudangrass), SbSg (Sorghum-sudangrass hybrids)
448 genotypes tested against various RKN species. This differential level of resistance cannot be
449 accounted for by dhurrin content, because Sg 'Piper' (low level of dhurrin) and SbSg '270911' (high
450 level of dhurrin) were both very poor hosts, with no significant difference in EM numbers on their roots.
451 In 2015, Harris-Shultz et al. mapped a major quantitative trait locus (QTL) to sorghum chromosome 3,
452 accounting for the resistance of one of Sb varieties to *M. incognita* race 3. Other inherited resistance
453 factors may be present in the sudangrass genome, accounting for the lack of RKN reproduction on Sg

454 'Piper' and the hybrids. Sg 'Piper' roots contained only a few RKN and showed many hypersensitive-
455 like reaction (HR) sites, indicating a response to infection similar to that in *Mi-1.2* resistant tomato
456 plants (Paulson and Webster, 1972); SbSg '270911' plants contained larger numbers of juveniles of all
457 stages, concealed within the roots, with little sign of a HR. Both sorghums acted as trap plants for
458 RKN because only few EMs were produced. In the roots of the non-host sorghum SbSg1, only a few
459 scattered J2s, with no further development, and a total absence of HR were observed. Thus, SbSg1
460 roots may repel juveniles, due to the toxic root exudates, as reported for several other non-host plants
461 (summarized in Djian-Caporalino et al., 2005), including two SbSg (*Sorghum bicolor x S. sudanense*
462 'SX-15' and 'SX-17') (Czarnota et al., 2003). Sorgoleone, the phenolic compound identified as a
463 predominant constituent in exudates, could be potentially responsible for the suppressive effect of
464 SbSg1. This hypothesis should be tested in future studies. This sorghum could be a more usable
465 candidate for farmers as it may be cultivated more than one month before burying for biofumigation
466 because it is non-host for RKN.
467

468 ## 5. Conclusion

469

470 The use of sorghums, sudangrass or hybrids, as a green manure was found to be an effective
471 strategy for decreasing the RKN infestation potential of soils, thereby protecting crop rotations
472 including both susceptible and partially resistant hosts. But the efficacy of sorghums clearly depends
473 on the management strategy to be set up in the field. In particular, incorporating sorghums into the soil
474 before the end of the RKN cycle plays a key role in the efficient and sustainable control of these
475 parasites. No effect relative to the sorghum type (with low or high dhurrin content) was detectable as
476 long as they were used correctly, i.e., cultivated during one month or less and left for one month for
477 biofumigation. This study thus provides information potentially useful to breeders and farmers for the
478 sustainable management of RKN in protected vegetable systems.
479

480 ## Acknowledgments

481 The authors would like to thank O. Arnaud for supplying the field site and performing all the
482 cropping practices. This work was part of the GEDUNEM project (Varietal and technical innovations
483 for the sustainable and integrated management of root-knot nematodes in protected vegetable

cropping systems) supported by the INRA metaprogram SMaCH (Sustainable Management of Crop Health, www.smach.inra.fr) and the GIS PicLeg (Scientific interest group on integrated vegetable production, www.picleg.fr).

References

Abawi, G.S., and Widmer, T.L. 2000. Impact of soil management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. Appl Soil Ecol 15:37-47.

Abd-Elgawad, M.M.M. 2014. Yield losses by phytonematodes: challenges and opportunities with special reference to Egypt. Egypt J Agronematol 13:75-94.

Adewusi, S.R.A. 1990. Turnover of dhurrin in green sorghum seedlings. Plant Physiol 94:1219-1224.

Asmus, G.L., Inomoto, M.M., and Cargnin, R.A. 2008. Cover crops for reniform nematode suppression in cotton: greenhouse and field evaluations. Trop Plant Pathol 23:85-89.

Benjamini, Y., and Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B Met 57 (1):289-300.

Birchfield, W. (1983). Wheat and grain sorghum varietal reaction to *Meloidogyne incognita* and *Rotylenchulus reniformis*. Plant Dis 67:41-42.

Byrd, DW, Kirkpatrick, T, Barker, K.R. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. J Nematol 15:142-143.

Chambliss, C.G. 2002. *Producing millets and sorghum*. Publication SS-AGR-89. Agronomy Department, Cooperative Extension Service, University of Florida, Gainesville, FL. http://edis.ifas.ufl.edu/AG157.

Chitwood, D.J. 2002. Phytochemical based strategies for nematode control. Annu Rev Phytopathol 40:221-49.

Colbran, R.C. 1979. Problems in tree replacement. III. The effects of cultural practices and soil fumigation on root lesion nematodes and on the growth of apple replants. Aust J Agr Res 30:113-123.

Collange, B., Navarrete, M., Peyre, G., Mateille, T., and Tchamitchian, M. 2011. Root-knot nematode (*Meloidogyne*) management in vegetable crop production: The challenge of an agronomic system analysis. Crop Prot 30:1251-1262.

513 Consortium REX 2012. Heterogeneity of selection and the evolution of resistance. Trends Ecol Evol
514 28:110-118.
515 Crow, W.T., Weingartner D.P., Dickson D.W., and McSorley R. 2001. Effect of sorghum Sudangrass
516 and velvetbean cover crops on plant-parasitic nematodes associated with potato production in
517 Florida. J Nematol 33 (4S):285-288.
518 Curto, G., Dallavalle, E., De Nicol, G.R., and Lazzeri, L. 2012. Evaluation of the activity of dhurrin
519 and sorghum towards *Meloidogyne incognita*. Nematology 14:759-769.
520 Curto, G., Dallavalle, E., Santi ,R., Casadei, N., D'Avino, L., and Lazzeri, L. 2015. The potential of
521 *Crotalaria juncea* L. as a summer green manure crop in comparison to Brassicaceae catch crops
522 for management of *Meloidogyne incognita* in the Mediterranean area. Eur J Plant Pathol
523 142:829–841
524 Curto, G., DallaValle, E., Matteo, R., and Lazzeri, L. 2016. Biofumigant effect of new defatted seed
525 meals against the southern root-knot nematode, *Meloidogyne incognita*. Annu Appl Biol 169:17-
526 26.
527 Czarnota, M.A., Rimando, A.M., and Weston, L.A. 2003. Evaluation of seven sorghum (*Sorghum* sp.)
528 accessions. J Chem Ecol 29:2073-2083.
529 Dalmasso, A., and Bergé, J. B., 1978. Molecular polymorphism and phylogenetic relationships in
530 some *Meloidogyne* spp. : application to the taxonomy of *Meloidogyne.* J Nematol 10:324-332.
531 Davis, R.F., and Anderson, W.F. 2012. Identification of widely varying levels of resistance to
532 *Meloidogyne incognita* in sweet sorghum. J Nematol 44:475.
533 De Nicola, G.R., Leoni, O., Malaguti, L., Bernardi, R., and Lazzeri, L. 2011. A simple analytical method
534 for dhurrin content evaluation in cyanogenic plants for their utilization in fodder and biofumigation.
535 J Agr and Food Chem 59:8065-8069.
536 Delamarre, C. 2011. Désinfection des sols sous abri froid. Fiche Chambre d'Agriculture du Lot-et-
537 Garonne/Invenio. http://lot-et-
538 garonne.chambagri.fr/fileadmin/telechargement/Productions_vegetales
539 /fiches_techniques_maraichage/desinfection-sols-abri-froid-aout-2011.pdf
540 Djian-Caporalino, C. 2012. Root-knot nematodes (*Meloidogyne* spp.), a growing problem in French
541 vegetable crops. EPPO Bulletin 42:127-137.

542 Djian-Caporalino, C., Bourdy, G., and Cayrol, J.C. 2005. Nematicidal and nematode-resistant plants.
543 Pp. 173-224 in Regnault-Roger, C., Philogène, B.Jr,. Vincent, C., ed. Biopesticides of plant origin.
544 UK, Intercept Ltd, and USA: Lavoisier Publishing Inc.
545 Djian-Caporalino, C., Navarrete, M., Palloix, A., Mateille, T., Lefevre, A., Barbary, A., Fazari, A.,
546 Marteu, N., Chapuis, M., Tchamitchian, M., Dufils, A., Sage-Palloix, A.M., Tavoillot, J., Pares, L.,
547 Vedie, H., Goillon, C., Taussig, C., Risso, S., Lanza, R., and Castagnone-Sereno, P. 2014.
548 Varietal and technical innovations for the sustainable and integrated managements of root knot
549 nematodes. J Nematol 46:154-156.
550 Dover, K., Wang, K.-H., and McSorley, R. 2012. *Nematode Management Using Sorghum and Its*
551 *Relative*s. Series of the Entomology and Nematology Department, UF/IFAS Extension, ENY716:5
552 pages.
553 EC Directive 2009 of the European Parliament and of the Council of 21 October 2009 concerning the
554 placing of plant protection products on the market and repealing Council. Directives 79/117/EEC
555 and 91/414/EEC. Official Journal of the European Union, 24.11.2009, L 309/1-50 (in French,
556 abstract available in English at http://eur-
557 lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF).
558 EIP-AGRI Focus Group on Soil-borne diseases: Final report 2015,
559 https://ec.europa.eu/eip/agriculture/en/publications/eip-agri-focus-group-soil-borne-diseases-final
560 Evans, A.A.F. and Perry, R.N. 2009. *Survival mechanisms*. Chapter: 9. Pp. 201-222 *in* Perry, R. N.
561 Moens, M. Starr J. L., ed. Root-knot nematodes: CABI.
562 Everts, K.L. 2006. Root-knot and root-lesion nematode suppression by cover crops, poultry litter, and
563 poultry litter compost. Plant Dis 90:487-492.
564 Fay, P., and Duke, W.B. 1977. An assessment of allelopathic potential in *Avena* germplasm. Weed Sci
565 25:224-228.
566 Ferraz, S., and de Freitas, L.G. 2004. *Use of antagonistic plants and natural products.* Pp. 931-977 *in*
567 Chen, Z.X., Chen, S.Y., and Dickson, D.W., ed. Nematology, Advances and perspectives, vol II.
568 Nematode management and utilization. Beijing, China and Wallingford UK. Tsinghua University
569 Press: CABI.
570 Fortnum, B.A., and Currin R.E. 1988. Host suitability of grain sorghum cultivars to *Meloidogyne* spp. J
571 Nematol 20 (Annals 2):61-4.

572 Fortuna, A., Harwood, R., Kizilkaya, K., and Paul, E.A. 2003. Optimizing nutrient availability and
573 potential carbon sequestration. Soil Biol Biochem 35:1005-1013.
574 Gamliel, A. and Stapleton, J.J. 1993. Characterization of antifungal volatile compounds evolved from
575 solarized soil amended with cabbage residues. Phytopathology 83:899-905
576 Gard, B., Goillon, C., Trottin-Caudal, Y., and Védie, H. 2014. Le sorgho fourrager comme engrais vert
577 à effet assainissant: évaluation agronomique et potentiel pour la biofumigation. Infos CTIFL
578 303:48-54.
579 Gill, H.K., and McSorley, R. 1994. Cover Crops for Managing Root-Knot Nematodes. ENY063, one of
580 a series of the Entomology and Nematology Department, UF/IFAS Extension.
581 http://edis.ifas.ufl.edu.
582 Goillon, C., Mateille, T., Tavoillot, J., Marteu, N., Fazari, A., and Djian-Caporalino, C. 2016. Utiliser le
583 sorgho pour lutter contre les nématodes à galles. Phytoma La défense des végétaux 698:39-44.
584 Gomes Carneiro, R. 1982. Seleçâo preliminar de plantas para rotaçâo de culturas em âreas
585 infestadas por *Meloidogyne incognita* nos anos de 1979 e 1980. Nematol Bras 6:141-148.
586 Guerena, M. 2006. Nematodes: Alternative controls. National sustainable agriculture information
587 service. ATTRA publication 28:1-20.
588 Hagan, A., Gazaway, W., and Sikora, E. 1998. Nematode suppressive crops. Alabama Cooperative
589 Extension System, ANR 856:5 pages.
590 Harris-Shultz, K.R. 2015. Inheritance and identification of a major quantitative trait locus (QTL) that
591 confers resistance to *Meloidogyne incognita* and a novel QTL for plant height in sweet sorghum.
592 Phytopathology 105:1522-1528.
593 Jones, J.T, Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K, Kikuchi, T.,
594 Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L., and Perry, R.N. 2013. Top 10
595 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946–961.
596 Kokalis-Burelle, N., Butler, D.M., and Rosskopf, E.N. 2013. Evaluation of cover crops with potential for
597 use in anaerobic soil disinfestation (ASD) for susceptibility to three species of *Meloidogyne*. J
598 Nematol 45:272-278.
599 Kratochvil, R.J., Sardanelli, S., Everts, K., and Gallagher, E. 2004. Evaluation of crop rotation and
600 other cultural practices for management of root-knot and lesion nematodes. Agron J 96:1419-
601 1428.

602 Kruger, D.H.M., Fourie, J.C., and Malan, A.P. 2013. Cover crops with biofumigation properties for the
603 suppression of plant-parasitic nematodes: a review. S Afr J Enol Vitic 34:287-295.
604 LaMondia, J.A., Elmer, W.H., Mervosh, T.L., and Cowles, R.S. 2002. Integrated management of
605 strawberry pests by rotation and intercropping. Crop Prot 21:837-846.
606 Mateille, T., Cadet, P., and Fargette, M. 2008. Control and management of plant-parasitic nematode
607 communities in a soil conservation approach. Pp. 79-97 *in* Ciancio, A., Mukerji, K.G., ed.
608 Integrated Management of Plant Pests and Diseases 2: Springer, Dordrecht.
609 Mateille, T., Tavoillot J., Goillon, C., Parès, L., Lefèvre, A., Védie, H., Navarrete, M., Palloix, A., Sage-
610 Palloix, A.M., Castagnone-Sereno, P., Fazari, A., Marteu, N., and Djian-Caporalino, C.
611 Interspecific competition in plant-parasitic nematode communities may question the sustainability
612 of control strategies in complex cropping systems. Submitted.
613 MBTOC 2006. *Report of the Methyl Bromide Technical Options Committee. Non-chemical Alternatives*
614 *Adopted as Replacements to Methyl Bromide on a Large Scale*. Pp. 39-73 *in* United Nation
615 Environmental Program, UNON Publishing Section Services, Nairobi (KE).
616 McSorley, R. 2001. Multiple cropping systems for nematode management: a review. Soil and Crop
617 Science Society of Florida Proceedings 60:132-142.
618 McSorley, R., and Dickson, D.W. 1995. Effect of tropical rotation crops on *Meloidogyne incognita* and
619 other plant-parasitic nematodes. Suppl J Nematol 27:535-544.
620 McSorley, R., and Gallaher, R.N. 1991. Nematode population changes and forage yields of six corn
621 and sorghum cultivars. Suppl J Nematol 23:673-677.
622 McSorley, R., Dickson, D.W., and deBrito, J.A. 1994. Host status of selected tropical rotation crops to
623 four populations of root-knot nematodes. Nematropica 24:45-53.
624 McGuidwin, A.E., and Layne, T.L. 1995. Response of nematode communities to sudangrass and
625 sorghum-sudangrass hybrids grown as green manure crops. Suppl J Nematol 27:609-616.
626 Merny, G., and Luc, M. 1969. *Les techniques d'échantillonnage des peuplements de nématodes dans*
627 *le sol.* Pp. 257-292 *in* Lamotte M., Boulière F., ed. *Problèmes d'écologie, l'échantillonnage des*
628 *peuplements animaux dans les milieux terrestres*: Masson, Paris.
629 Mojtahedi, H., Santo, G.S., and Ingham, R.E. 1993. Suppression of *Meloidogyne chitwoodi* with
630 Sudangrass cultivars as green manure. J Nematol 25:303-311.

631 Orfanedes, M. 1995. Sudangrass trials - What have we learned to date? Lake Plains Vegetable
632 Program, Cornell Cooperative Extension Program. Ithaca, NY.
633 Paulson, R.E., and Webster, J.M. 1972. Ultrastructure of the hypersensitive reaction in roots of
634 tomato, *Lycopersicon esculentum* L. to infection by the root-knot nematode, *Meloidogyne*
635 *incognita*. Physiol Plant Pathol 2:227-234.
636 Prasad P., Kumar J., and Pandey S. 2015. Biofumigation: success and prospects in soilborne plant
637 disease management. Int J Appl Pure Sci Agric 1:47-59.
638 Quaranta, B. 2009. Effet des plantes de service sur les bio-agresseurs des cultures.- Etude
639 bibliographique sur les plantes utilisées dans les systèmes de culture sur couverture végétale
640 (SCV) à Madagascar. *CIRAD,* http://agroecologie.cirad.fr
641 Ratnadass, A., Fernandes, P., Avelino, J., and Habib, R. 2012. Plant species diversity for sustainable
642 management of crop pests and diseases in agroecosystems: a review. Agron Sustain Dev
643 32:273-303.
644 Rhoades, H.L. 1983. Effects of cover crops and fallowing on populations of *Belonolaimus*
645 *longicaudatus* and Meloidogyne incognita and subsequent crop yields. Nematropica 13:9-16.
646 Ritzinger, C.H.S.P. and McSorley, R. 1998. Review: effect of fresh and dry organic amendments on
647 *Meloidogyne arenaria* in greenhouse experiments. Nematropica 28:173-185.
648 Roberts, P.A., Dalmasso, A., Cap, G.B., Castagnone-Sereno, P. 1990. Resistance in *Lycopersicon*
649 *peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. J Nematol 22:585-589.
650 Séguy, L., Husson, O., Charpentier, H., Bouzinac, S., Michellon, R., Chabanne, A., Boulakia, S., Tivet,
651 F., Naudin, K., Enjalric, F., Ramaroson, I., Rakotondramanana I. 2009. Principes et
652 fonctionnement des écosystèmes cultivés en semis direct sur couverture végétale. Pp. 32 *in*
653 Manuel du semis direct à Madagascar, vol.1, chap.1, GSDM and CIRAD, eds : Antananarivo.
654 Seinhorst, J.W. 1962. Extraction Methods for Nematodes Inhabiting Soil. Pp. 243-256 *in* Murphy,
655 P.W., Ed., Progress in Soil Zoology: Butterworths, London.
656 Sipes, B.S., and Arakaki A.S. 1997. Root-knot nematode management in dryland taro with tropical
657 cover crops. Suppl J Nematol 29:721-724.
658 Talavera, M., Sayadi, S., Chirosa-Rios, M., Salmeron, T., Flor-Peregrin, E., and Verdejo-Lucas, S.
659 2012. Perception of the impact of root-knot nematode-induced diseases in horticultural protected
660 crops of south-eastern Spain. Nematology 14:517-527.

661 Thoden, T.C., Korthals, G.W., and Termorshuizen, A.J. 2011. Organic amendments and their
662 influences on plant-parasitic and free-living nematodes: a promising method for nematode
663 management? Nematology 13:133-153.
664 Thomason, I. J., and Lear B. 1961. Rate of reproduction of *Meloidogyne* spp. as influenced by soil
665 temperature. Phytopathology 51:520-524.
666 Timper, P. and Wilson, J.P. 2006. Root-knot nematode resistance in pearl millet From West and East
667 Africa. Plant Dis 90:339-344.
668 Védie, H. 2010. Protection des nématodes à galles: intérêt de méthodes alternatives sur l'interculture
669 d'été. Fiche Maraîchage 2009:3 pages.
670 Védie, H., Geffroy, T., and Lambion, J. 2006. *Lutte contre les nématodes a galles : test de différents*
671 *engrais verts nématicides: compte-rendu d'essai 2005*. Groupe de Recherche en Agriculture
672 Biologique. Maraichage 2006, Fiches action L05 and L06 LR/02.
673 Viaene, N.M and Abawi, G.S. 1998. Management of *Meloidogyne hapla* on lettuce in organic soil with
674 Sudangrass as a cover crop. Plant Dis 82:945-952.
675 Vrain T.C., Barker, K.R., and Holtzman G.I. 1978. Influence of low temperature on rate of development
676 of *Meloidogyne incognita* and *M. hapla* larvae. J Nematol 10:166-71.
677 Wang, K.H., McSorley, R., and Gallaher, R.N. 2004. Winter cover crops and nematode densities. J
678 Nematol 36:517–523.
679 Wang, K.-H., McSorley, R., and Kokalis-Burelle, N. 2006. Effects of cover cropping, solarization, and
680 soil fumigation on nematode communities. Plant Soil 286:229-243.
681 Weingartner, D.P., McSorely, R., and Goth, R.W. 1993. Management strategies in potato for
682 nematodes and soil-borne diseases in subtropical Florida. Nematropica 23:233-245.
683 Widmer, T.L., and Abawi G.S. 2000. Mechanism of suppression of *Meloidogyne hapla* and its damage
684 by a green manure of Sudangrass. Plant Dis 84:562-568.
685 Widmer, T.L., and Abawi G.S. 2002. Relationship between levels of cyanide in Sudangrass hybrids
686 incorporated into soil and suppression of *Meloidogyne hapla*. J Nematol 34:16-22.
687 Zeck, W.M. 1971. A rating scheme for field evaluation of root-knot nematode infestations.
688 Pflanzenschutz-Nachrichten 24:141-144.
689
690

691 **“Declarations of interest: None”**

692

693 **Color should be used for any figure. Article online only.**

Melon roots infested by *Meloidogyne arenaria* and *M. incognita*

30-days of sorghum culture as nematocidal cover crop

Melon roots after sorghum