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## 1 **Evaluating sorghums as green manure against root-knot nematodes**

2

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6

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11

12 **Abstract:** Current restrictions on the use of chemical nematicides have led to an increase in root-  
13 knot nematode (RKN) damages in horticultural crops. The effects of two sorghums as summer cover  
14 crops, *Sorghum sudanense* sudangrass cv. 'Piper' or sudangrass hybrid [*S. bicolor* x *S. sudanense*]  
15 '270911', respectively with low and high dhurrin contents, were compared in their ability to suppress  
16 RKN in a vegetable production system. The use of both sorghums 'Piper' and '270911' as a green  
17 manure was found to be an effective strategy for decreasing RKN infestation in the soil, thereby  
18 protecting the subsequent planting of RKN susceptible crops (chard, lettuce or melon). Analytical  
19 experiments were further conducted in growth chamber and greenhouse pot experiments to  
20 investigate and compare the susceptibility of the sorghums and the factors affecting their efficacy for  
21 RKN management, in order to better explain the results obtained in the field trial. The two sorghums  
22 were poor hosts of RKN, acted as trap crops and as a biofumigant releasing hydrogen cyanide. Time  
23 of planting, time of biofumigation, and type of soil affected their efficacy for RKN management. For  
24 best RKN suppression, the sorghum cover crops need to be cultivated during one month or less and  
25 biofumigated for one month prior to crop planting. The trapping effect of both sorghums in clayey soil  
26 was less efficient than in sandy or sandy-loamy soils. Combining less than 30-days of sorghum culture  
27 and 10-days soil incorporation with solarization mulch was particularly efficient in suppressing  
28 nematodes. No effect relative to the sorghum type was detectable as long as they were used  
29 appropriately.

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

32

33

## 34 **1. Introduction**

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

41 Use of cover crops in rotation to vegetable crop production has been recommended to farmers as  
42 a mean to improve fertility, physical and chemical properties of soil (Fortuna et al., 2003; Séguy et al.,  
43 2009). Cover crops improve the productivity of subsequent crops by reducing pressure of pests and  
44 pathogens (Ratnadass et al., 2012), serving as non or poor hosts for RKN (Djian-Caporalino et al.,  
45 2005), and thus contribute to nematode management (McSorley, 2001). Cover crops documented to  
46 be capable of managing RKN include *Phacelia* spp., *Avena sativa* (Gomes Carneiro, 1982), brassicas,  
47 such as *Sinapis alba*, *Eruca sativa*, and *Raphanus sativus* (Curto et al., 2015; Kruger et al., 2013), and  
48 grasses, such as *Pennisetum glaucum*, *Sorghum bicolor* 'forage sorghum' or *Sorghum sudanense*  
49 'sudangrass', formerly classified as *S. vulgare var. sudanense*, and *S. bicolor* x *S. sudanense*  
50 'sudangrass hybrids' (McSorley et al., 1994; Mojtahedi et al., 1993; Sipes and Arakaki, 1997; Timper  
51 and Wilson, 2006). They have been tested as winter or summer cover crops for limiting RKN  
52 populations. These cover crops, which are used as green manure, can be used as trap crops if the  
53 nematodes penetrate the roots but cannot complete their cycle. They can also be used as biofumigant  
54 crops that release volatile compounds when soil incorporated (Djian-Caporalino et al., 2005). Interest  
55 in biofumigation for soilborne disease management has recently increased due to its compatibility with  
56 environmental friendly management (Kruger et al., 2013; Prasad et al., 2015). One group of cover  
57 crops with effective biofumigation effect against RKN is forage sorghum or sudangrass. Sorghum has  
58 been well known to release hydrogen cyanide (HCN) following the hydrolysis of dhurrin, a cyanogenic  
59 glycoside typically present in sorghum (Chitwood, 2002; Curto et al., 2012; De Nicola et al., 2011).

60 Sudangrass hybrids produce larger amounts of biomass than non-hybrid sudangrass. Both  
61 sudangrass and its hybrids have a long root system penetrating deep into the soil, and they are used  
62 as a green manure to improve soil porosity, and attract soil nutrients present at depth up towards the  
63 top soil layer (Abawi and Widmer, 2000; Kratochvil et al., 2004). Sudangrass require little  
64 management, beside seed sowing and sprinkler irrigation after soil tillage. The cover crop grows for  
65 1.5 to 2 months, and its biomass is then mowed and soil incorporated (Delamarre, 2011). A number of  
66 studies, summarized in the review by Quaranta (2009), have reported activity against several pests  
67 and diseases. In the case of PPN, forage sorghum or sudangrass have been reported to be effective  
68 at reducing the numbers of *Helicotylenchus dihystera* (Wang et al., 2004), *Rotylenchus reniformis*  
69 (Asmus et al., 2008) and *Pratylenchus penetrans* (LaMondia et al., 2002), but were found to facilitate  
70 the multiplication of *Belonolaimus longicaudatus* (Rhoades, 1983; Weingartner et al., 1993),  
71 *Mesocriconema* sp. (Crow et al., 2001), *Paratrichodorus minor* (McSorley and Gallaher, 1991;  
72 McSorley et al., 1994; McSorley and Dickson, 1995) and *Tylenchorhynchus* sp. (Crow et al., 2001).  
73 McGuidwin and Layne (1995) reported the maintenance or an increase in populations of *Pratylenchus*,  
74 *Longidorus*, *Xiphinema* and *Paratrichodorus* after the incorporation of some varieties of sudangrass  
75 and sudangrass hybrids. Conversely, these plants were considered to be non-hosts or poor hosts for  
76 RKN (Colbran, 1979; Fay and Duke, 1977; Gomes Carneiro, 1982; Hagan et al., 1998; Ritzinger and  
77 McSorley, 1998; Védie et al., 2006), such as *Meloidogyne incognita* and *M. hapla* in particular  
78 (Chitwood, 2002; Ferraz and Freitas, 2004; McSorley and Dickson, 1995; Viaene and Abawi, 1998;  
79 Wang et al., 2004). Mojtahedi et al. (1993) and Widmer and Abawi (2000) also reported that they  
80 released HCN, with biofumigation effects against *M. hapla* or *M. chitwoodi*. Wang et al. (2004) and  
81 Guerena (2006) reported that the prior use of sudangrass hybrids as a cover crop decreases the  
82 density of populations of *M. incognita* on *Secale cereale* 'rye', *Lupinus angustifolius* 'lupin' or *Glycine*  
83 *max* 'soybean'. However, poor results were obtained when the crops were combined and sudangrass  
84 or sudangrass hybrids residues were incorporated into the soil while still green (Orfonedes, 1995;  
85 Widner and Abawi, 2002). The best results for RKN suppression in Florida were obtained with the use  
86 of forage sorghum or sudangrass hybrids as a rotation crop (Dover et al., 2012; Gill and McSorley,  
87 1994), but the benefits of crop residues for nematode suppression were not assessed in this study. In  
88 France, sorghum residues have been found to be only partly effective in the field, often yielding  
89 variable results (Abawi and Widmer 2000; Collange et al., 2011; McGuidwin and Layne 1995; Thoden

90 [et al. 2011](#)). However, interest in the use of biofumigation in vegetable crop rotations has recently  
91 increased ([Curto et al. 2016](#); [Goillon et al. 2016](#); [Prasad et al. 2015](#)), and deserves further work.

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

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

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

112

## 113 **2. Materials and methods**

### 114 *2.1. Nematode suppressive effects of sorghums in agroecosystem (field trial)*

115 A four-year field trial was performed on a commercial organic farm near Lambesc (43.65N,  
116 5.21E) located at Provence in southern France, with Mediterranean climate, from 2012 to 2016. The  
117 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%  
118 sand, 22.3% loam, 10.7% clay, 3.5% soil organic matter (OM) and with pH of 8.4) heavily infested with  
119 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<sup>2</sup> 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

150 over the plot. RKN were extracted by the [Seinhorst \(1962\)](#) elutriation procedure and counted under a  
 151 stereomicroscope (x60 magnification) ([Merny and Luc, 1969](#)). Nematode population densities were  
 152 determined and expressed per dm<sup>3</sup> of fresh soil (mean of 8 replicates). To analyse the effect of the  
 153 four-year-cropping systems on RKN soil populations, a multiplication rate Pf/Pi (final population Pf =  
 154 nematode density in the soil at the end of the crop; initial population Pi = nematode density in the soil  
 155 at planting or sowing) was compared between the plots. To determine the impact of the system on  
 156 susceptible and resistant crops, RKN damage was estimated by determining the gall index (GI) on a  
 157 scale of 0-10 ([Zeck, 1971](#)) for the root system of a representative subsample (36 plants per subplot) at  
 158 the end of each crop. The number of RKN-infected plants was also recorded.

159

160 **Table 1.**

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

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

163 <sup>a</sup> After planting, the crop was irrigated every three days initially and less frequently thereafter; <sup>b</sup> after  
 164 incorporation with rotavator, the soil was rolled and left uncovered for one month to allow  
 165 biofumigation

166

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<sup>-1</sup> 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<sup>-1</sup> 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).

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

228

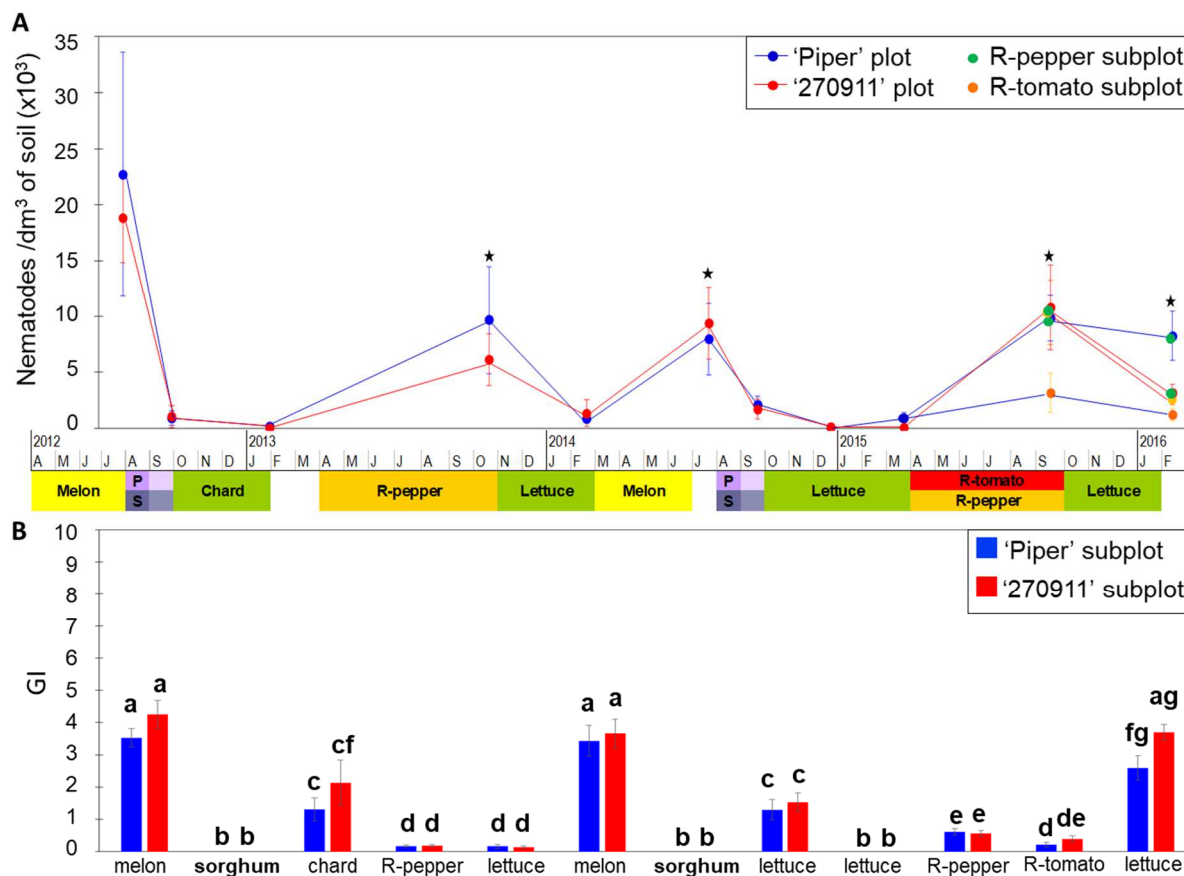
### 229 **3. Results**

#### 230 *3.1. Impact of the sorghum cropping system on RKN in the field experiment*

231 Mean RKN abundance was high over the entire plot in the trial at Lambesc (initial rate = 20,766  
232  $\pm 5,620$  individuals/dm<sup>3</sup>) after the first melon crop (Fig. 1A). We observed a strong and significant short  
233 term diminution of RKN abundance in soil by using both sorghums. At the beginning of the study, the  
234 relative RKN concentration loss was of 94% for '270911' and of 95% for 'Piper' (ANOVA,  $p_{\text{time}} < 0.02$ ,  
235  $p_{\text{sorghum}} > 0.5$ , no interaction). RKN populations subsequently remained low in the soil, below the  
236 initial rate, after the cultivation of highly susceptible chard or lettuce winter crops just after sorghum.  
237 Following chard or lettuce, the summer crops with low resistance to RKN (pepper in 2013, pepper and  
238 tomato in 2015) allowed an increase of RKN populations in the soil significantly higher for '270911'  
239 plot than for 'Piper' plot. At mid-study, the relative concentration loss was of 81% for '270911' and of  
240 73% for 'Piper' (ANOVA,  $p_{\text{time}} < 0.01$ ,  $p_{\text{sorghum}} > 0.5$ , no interaction). Throughout the four years of  
241 the experiment, the alternation of sorghum green manure and partially resistant crops maintained a  
242 decrease in the RKN population and the long-term trend was still beneficial, with a global diminution of  
243 84% for '270911' and 79% for 'Piper' (ANOVA,  $p_{\text{time}} < 0.01$ ,  $p_{\text{sorghum}} > 0.5$ , no interaction) (final rate  
244 =  $2,850 \pm 1,365$  individuals/dm<sup>3</sup> in 'Piper' plot and  $4,725 \pm 600$  individuals/dm<sup>3</sup> in '270911' plot).

245 After one month of cultivation in summer, no gall was present on the roots of 'Piper' or '270911'  
246 (Fig. 1B). GI was similar and moderate (less than 4) on melon culture in 2012 and 2014 after both  
247 sorghum types ( $p_{\text{time}} = 0.46$ ,  $p_{\text{sorghum}} = 0.32$ , no interaction). For resistant cultures, very few galls  
248 were observed and 40 to 70% of plants exhibited no gall during all the experiment, tomato showing an  
249 average GI lower than pepper, with no difference in both sorghum plots ( $p_{\text{crop}} < 10^{-3}$ ,  
250  $p_{\text{sorghum}} = 0.73$ , no interaction). The picture is more complex for winter cultures, with a significant  
251 difference between chard and lettuce, chard being more infected than lettuce (ANOVA,  $p < 0.05$ ) and  
252 'Piper' sorghum plot being less infected than '270911' sorghum plot (ANOVA,  $p < 0.05$ , no interaction  
253 with crop type). Moreover, results for lettuce only showed some variability, with early lettuce crops  
254 (harvested in October) being significantly less infected than late crops for both sorghum types

255 (ANOVA,  $p_{\text{time}} < 10^{-16}$ ,  $p_{\text{sorghum}} > 0.05$ ). This late analysis also showed a weak interaction between  
 256 time points and sorghum type (ANOVA,  $p < 0.02$ ) essentially due to a diminution of GI on lettuce at the  
 257 end of the experiment, only in plots cultivated with 'Piper', diminution which was not significant at other  
 258 time points. GI reached almost 4 and only 3% of plants had no gall when no sorghum was used in the  
 259 fourth year compared to  $GI < 2$  and 30 to 45% of plants with no gall when sorghums were used before  
 260 winter crops.

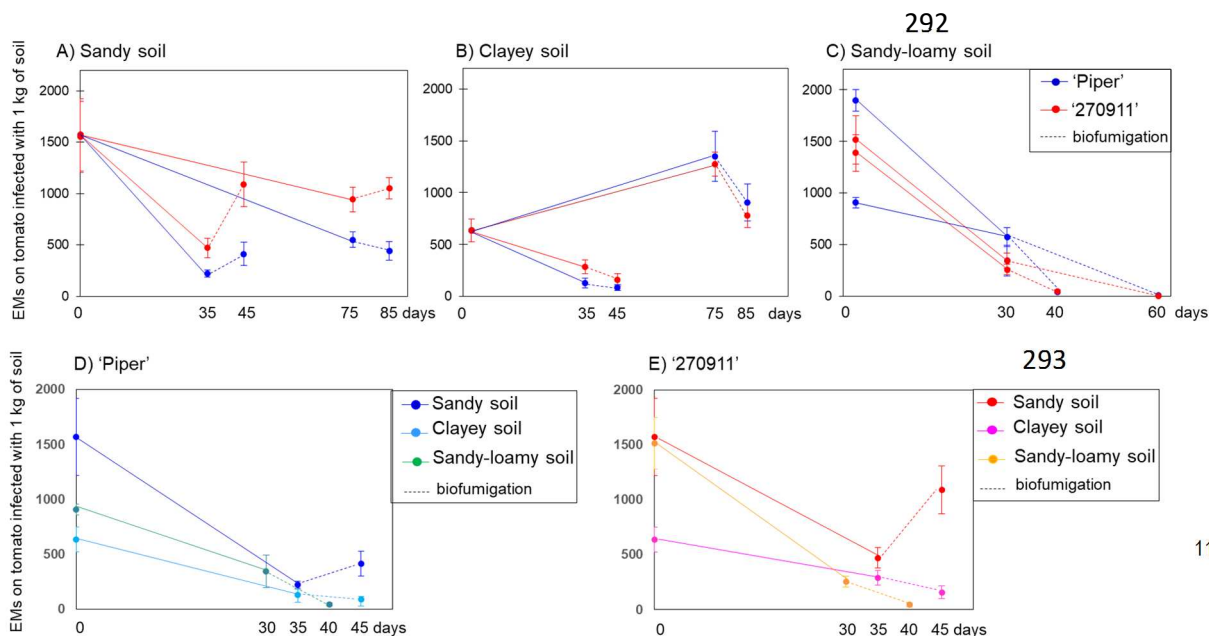


261  
 262 **Fig. 1. (A) Kinetics of RKN populations in the soil over the 4-year field experiments in Lambesc:**  
 263 means ( $n=8$ )  $\pm$  standard deviation followed by stars indicate significant differences ( $p < 0.05$ ) between  
 264 both sorghum plots at the sampling date; **and (B) Gall index (GI) on plants:** mean ( $n=36$ )  $\pm$  standard  
 265 deviation followed by different letters indicate significant differences. P = 'Piper', S = '270911'; chard =  
 266 Swiss chard; lettuce = Batavia salad or oak leaf lettuce; R-pepper = several varieties of sweet peppers  
 267 grafted onto a R-pepper rootstock carrying *Me3* R-gene in the susceptible genetic background Doux-  
 268 Long-des-Landes; R-tomato = *Mi-1* R-tomato.

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270 3.2. Effects of soil types, time of planting and time of biofumigation on nematocidal effects of  
 271 sorghums in pot experiment

272 Before the sorghum was planted in the 2 x 3 x 2 factorial experiment, the number of nematodes  
 273 in the soil, as evaluated by determining SIP, was moderate in clayey and sandy-loamy soils, to heavy  
 274 in sandy soils (Fig. 2). After one month of cultivation, the number of nematodes greatly decreased in  
 275 the three types of soils, for both sorghum varieties: Pf/Pi was less than 0.4. The experiment being  
 276 repeated twice with the same mean values, results of both experiments (thus for 8 pots) were  
 277 combined and shown in Fig. 2. A multifactorial ANOVA on data before biofumigation (time x soil x  
 278 sorghum type, i.e. 2x3x2, Tables 2 and 3) showed a strong time effect ( $p < 10^{-11}$ ) with an increase in  
 279 EM numbers when comparing two months cultivation relative to one month ( $0.4 < Pf/Pi$  after two  
 280 months  $< 2.1$  ;  $0.1 < Pf/Pi < 0.4$  after one month), and another strong soil effect ( $p < 10^{-15}$ ) showing a  
 281 strong increase in clayey soils ( $Pf/Pi > 2$ ). No effect relative to the sorghum type was detectable.  
 282 Moreover, an advanced analysis including interaction terms showed that the increase in EM numbers  
 283 due to two months-cultivation in clayey soils still exists, but in a much weaker form ( $p < 10^{-3}$ ). Repeating  
 284 the same analysis after 10 days of biofumigation (Tables 2 and 3) replicated the same results, with  
 285 slightly different p-values:  $p < 10^{-4}$  for the increase in EMs number with two months cultivation ( $0.3 <$   
 286  $Pf/Pi$  after two months  $< 1.4$ ;  $0.1 < Pf/Pi < 0.6$  after one month),  $p < 10^{-14}$  for the soil effect with the  
 287 same effect as previously, and still no sorghum type effect. This showed that 10 days of biofumigation  
 288 did not remediate to the increase in EM numbers due to the two months-cultivation. In sandy-loamy  
 289 soil after one month-cultivation, 30 days of biofumigation were slightly more efficient ( $Pf/Pi < 0.003$ )  
 290 than 10 days of biofumigation ( $Pf/Pi < 0.04$ ), resulting in the almost complete abolition of SIP in some  
 291 pots. The temperature in the soil under the VIF varied from 22 to 47°C over this 30-day period.



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**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.

310 **Table 2.** ANOVA of soil infestation potential data before or after biofumigation and at the beginning of  
 311 the experiment. df: degrees of freedom, Sum sq: sum of squares, p-val: p-value associated to the  
 312 variable considered.

Variable	Before biofumigation			After biofumigation		
	df	Sum sq.	p-val	df	Sum sq.	p-val
Time	1	27572673	< 10 <sup>-11</sup>	1	2220990	< 10 <sup>-4</sup>
Soil	2	9309191	< 10 <sup>-15</sup>	2	11691741	< 10 <sup>-14</sup>
Sorghum type	1	40747	0.59	1	295017	0.09
Time*Soil	1	1867127	<10 <sup>-3</sup>	1	1968328	<10 <sup>-4</sup>
Time*Sorghum type	1	9350	0.79	1	67002	0.42
Soil*Sorghum type	2	1262920	0.014	2	4202318	<10 <sup>-6</sup>
Time*Soil*Sorghum type	1	138751	0.32	1	14354	0.70

Residuals	83	11648935	67	6847038
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314 **Table 3.** Linear model table of the soil infestation potential values before or after biofumigation. First  
 315 column indicates variable and the corresponding reference level, second column the modality under  
 316 study, third, fourth and fifth columns the estimate for the model, the std. deviation of the estimate and  
 317 the associated p-value. Interactions are reported only for those showing a p-val<0.1. Data are identical  
 318 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:clayley	Sandy	-844.32	132.45	<10 <sup>-4</sup>	-844.32	132.45	<10 <sup>-4</sup>
	Sandy-loamy	-384.18	166.04	0.023	-384.18	166.04	0.023
Time:Short	Long	1219.88	187.32	<10 <sup>-8</sup>	1219.88	187.32	<10 <sup>-8</sup>
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 <sup>-2</sup>	-874.53	264.90	<10 <sup>-2</sup>
Soil*Sorghum type	Sandy-loamy* S'270911'	-420.07	232.13	0.073	-420.07	232.13	0.073

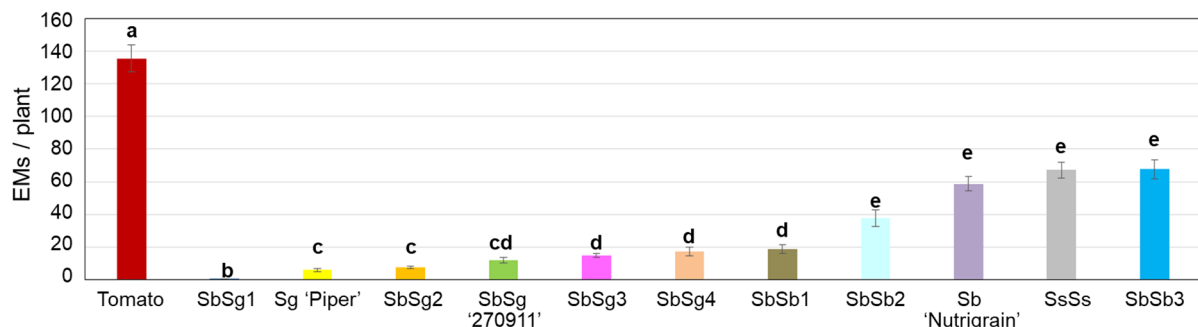
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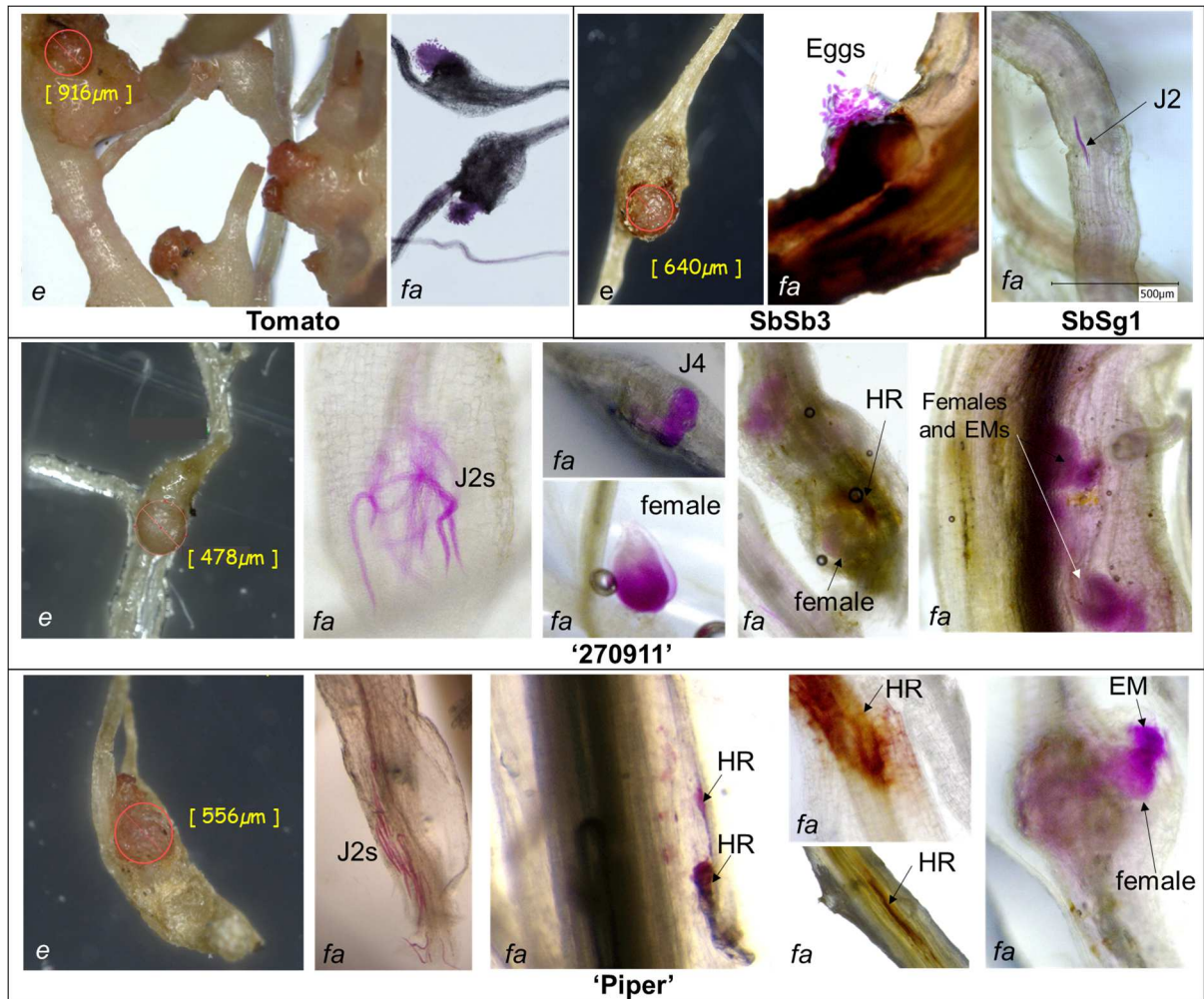
### 321 3.3. Tests of host suitability in controlled conditions and observations of RKN developmental stages in 322 the roots

323 The experiment in growth chamber being repeated twice with the same mean values, results of  
 324 both experiments (thus for 16 plants) were combined and shown in Fig. 3. Based on EM production,  
 325 both the commonly used variety Sg 'Piper' and the recommended variety with high dhurrin SbSg  
 326 '270911' were equally resistant to *M. incognita*. SbSg2 exhibited the same EM numbers. Only SbSg1  
 327 was non-host. SbSg3, SbSg4, and SbSb1 could be compared to SbSg '270911' and designated as  
 328 poor hosts. SbSb2, SbSb3, Sb 'Nutrigrain' and SsSs were more susceptible to RKN compared to Sg  
 329 'Piper' and SbSg '270911'. The galls induced by *M. incognita* on the roots of all sorghum plants were  
 330 much smaller than those observed on tomato roots. EMs were about 1 ± 0.05 mm in diameter on the

331 roots of susceptible tomato plants, but only about  $650 \pm 60 \mu\text{m}$  in diameter on sorghums Sb, SbSb3  
 332 and SsSs, and about  $500 \pm 50 \mu\text{m}$  in diameter on the other types of sorghum, including Sg 'Piper' and  
 333 SbSg '270911'. Moreover, EMs were often found inside sorghum roots, as seen with acid fuchsin  
 334 staining, whereas those on tomato roots were always outside the gall (Fig. 4). Small numbers of  
 335 second, third and fourth stage juveniles, females and EMs were observed in the roots of the poor  
 336 hosts. Very few J2s penetrated the roots of the non-host SbSg1 and they did not develop into J3s after  
 337 six weeks. Necrotic cells (hypersensitive-like reaction) were visible as a darkening of the orange  
 338 staining in the epidermis and cortex of the roots at a higher frequency in the roots of Sg 'Piper' than in  
 339 SbSg '270911', where more juveniles of all stages were observed. No hypersensitive-like reaction was  
 340 observed in the roots of SbSg1.



341 **Fig. 3. Mean number of EMs per plant counted on several sorghum varieties and on tomato (as**  
 342 **a control) maintained in a controlled-climate growth chamber after inoculation with 400**  
 343 **juveniles of *M. incognita*: means (n=16)  $\pm$  standard deviation followed by different letters indicate**  
 344 **significant differences ( $p < 0.05$ ). Sg 'Piper' = Sudan grass (*Sorghum bicolor* ssp. *sudanense*) variety**  
 345 **'Piper'; SbSg '270911' = [*Sorghum bicolor* spp. *bicolor* x Sudan grass] 3-way hybrid; Sb 'Nutrigrain' =**  
 346 ***Sorghum bicolor*; SbSg1,2,3,4 = [*Sorghum bicolor* x Sudan grass] hybrids; SbSb1,2,3 = [*Sorghum***  
 347 ***bicolor* x *Sorghum bicolor*] hybrids; SsSs = [*S. bicolor* ssp. *saccharatum* x *S. bicolor* ssp. *saccharatum*]**  
 348 **hybrid.**  
 349



350

351 **Fig. 4. Observation of the root system of the very poor hosts Sg 'Piper' and SbSg '270911', the**  
 352 **non-host SbSg1, and the poor host 'SbSb3' under a stereomicroscope after inoculation with**  
 353 **600 juveniles of *M. incognita*. Tomato is used as a control (HR = hypersensitive-like reaction; EM =**  
 354 **egg-mass; e = eosin staining; fa= fuchsin acid staining)**

355

#### 356 4. Discussion

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

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

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

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

410 The soil and temperature conditions may also affect the degradation of the fresh material buried  
411 in the soil and, thus, the release of HCN ([Viane and Abawi, 1998](#)). It may explain why increasing the  
412 biofumigation time under VIF (that holds in the gaseous breakdown products; [Gamliel and Stapleton,](#)  
413 [1993](#)) to 30 days in the greenhouse experiment, although a less important factor than the growing  
414 time, may nevertheless allow the almost complete elimination of RKN from soils when combined with  
415 the trapping effect. [Wang et al. \(2006\)](#) also found cover crop solarization to be an effective combined  
416 treatment for decreasing *Meloidogyne* spp. infestation, with an efficacy similar to that of methyl  
417 bromide fumigation at crop harvest, the most effective treatment used against soil-borne diseases and  
418 pests in conventional agriculture, before its prohibition.

419 The duration of burial, measured under controlled conditions, must be adjusted according to the  
420 growing conditions. Under cover in the South of France, this period may be estimated to be four  
421 weeks in spring, and three weeks in summer. In open-field conditions in northern France, this period  
422 could be extended to five weeks in spring and four weeks in summer. For farmers who consider green  
423 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 sustainability 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' ( $7.5 \pm 0.2$  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  $7.5 \pm 0.2$  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 ( $8.7 \pm 0.2$  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

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485 Health, [www.smach.inra.fr](http://www.smach.inra.fr)) and the GIS PicLeg (Scientific interest group on integrated vegetable  
486 production, [www.picleg.fr](http://www.picleg.fr)).

487

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

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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