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1 **Impact of native plant-parasitic nematode communities on the establishment of *Meloidogyne***
2 ***chitwoodi*.**

3

4 N. Garcia^{a, b, *}, E. Grenier^b, C. Sarniguet^a, A. Buisson^a, F. Ollivier^a, L. Folcher^a

5

6 ^a ANSES, Plant Health Laboratory – Nematology Unit, Domaine de la Motte au Vicomte - BP 35327 -
7 35653 Le Rheu Cédex - France

8 ^b IGEPP, Agrocampus-Ouest, INRA, Rennes 1 University, Domaine de la Motte au Vicomte - BP 35327
9 - 35653 Le Rheu Cédex - France

10 * Corresponding author: Nathan Garcia: natgarcia.pro@gmail.com

11

12 Running head: PPNs competition with *M. chitwoodi*

13

14 Abstract

15 Among plant-parasitic nematodes (PPNs), the species *Meloidogyne chitwoodi* constitutes an
16 important agricultural issue and is listed as a quarantine species in the EU. Here we investigate
17 whether PPN communities can affect the establishment and expansion of *M. chitwoodi* during a
18 simulated introduction. We developed an original experimental design on potato in glasshouse
19 involving four PPN communities derived from a single natural one that was initially grown on
20 different host plants. *M. chitwoodi* was inoculated in two different densities (50 or 1000 second-
21 stage juveniles (J2) per pot). After four months, *M. chitwoodi* was more abundant in the community
22 showing a low overall abundance of PPNs, decreased in the other PPN communities and failed to
23 establish in five pots out of 80. Results showed that establishment - even starting from a very low
24 inoculum - can occur in all communities even if indigenous PPNs in the community affect the
25 expansion through interspecific competition. The reverse interaction was also observed as PPN taxa
26 were generally less abundant in the 1000 *M. chitwoodi* J2 inoculation treatments. The proportion of

27 produced *M. chitwoodi* males was higher in the community showing low overall PPN abundance
28 suggesting a strong sensitivity of *M. chitwoodi* to intraspecific competition. The presented results
29 suggest that a low abundance of indigenous PPNs present a higher risk of expansion of *M. chitwoodi*
30 even if the introduced inoculum is low.

31

32 Keywords

33 Competition phenomenon, original experimental design, plant-parasitic nematode communities,
34 potato, quarantine species

35

36 **Introduction**

37

38 Plant-parasitic nematodes (PPNs) are widely distributed organisms that feed on plant tissues and
39 can cause severe damage to crops (Bridge & Starr, 2007). Among PPNs, root-knot nematodes
40 (*Meloidogyne* spp.) can lead to high economic losses. These endoparasitic organisms have an
41 extremely wide host range and can multiply profusely on numerous types of crop (Trudgill, 1997;
42 Trudgill & Blok, 2001), producing galls on roots and severely reducing yields (Bridge & Starr, 2007).
43 *Meloidogyne chitwoodi* is one of the most damaging species in Europe owing to its broad range of
44 host plants including many crops such as root crops, tomato, wheat or maize (O'Bannon *et al.*, 1982;
45 Karssen, 2002; EPPO bulletin, 2004). Furthermore, this species reproduces parthenogenetically
46 (Trudgill, 1997; Castagnone-Sereno, 2006), multiplying rapidly and massively through female
47 production only. Males are sometimes produced under adverse environmental conditions, such as
48 poor food quality or competition (Trudgill, 1972; Triantaphyllou, 1973). In contrast to the tropical
49 root-knot nematode species that reproduces by obligate mitotic parthenogenesis (Castagnone-
50 Sereno, 2006), *M. chitwoodi* reproduces by facultative meiotic parthenogenesis (Van der Beek &
51 Karssen, 1997); therefore, when males of *M. chitwoodi* are present in the populations, amphimixis
52 can occur (Castagnone-Sereno, 2006). This species has been classified as a quarantine species in the
53 European Union and because it is considered as invasive, it has to be immediately contained upon
54 detection in a cultivated field. In France, this species was detected in only a few foci between 1996
55 and 2014: the Nord-Pas de Calais, Picardy, Lower Normandy, Brittany, Ile-de-France and Aquitaine
56 administrative regions (Gamon & Lenne, 2012). Upon detection, French legislation requires that
57 farmers practice bare fallow for at least one year. Published studies carried out on the management
58 of *M. chitwoodi* mainly focus on plant-nematode interactions, including nematode virulence and
59 plant resistance (van der Beek *et al.*, 1998 ; Janssen *et al.*, 1995; Brinkman *et al.*, 1996; Jensen &
60 Griffin, 1997; Castagnone-Sereno, 2002) or plant diffusates (Wesemael *et al.*, 2006; Khokon *et al.*,
61 2009).

62 Other approaches, including preventive steps to assess and thus limit the risk of PPNs
63 establishment, are important to consider. A monitoring plan is carried out annually by French
64 authorities across the country in crop fields to detect *M. chitwoodi* introductions. However, these
65 fields are defined as “at-risk” only on the basis of the host crop grown, and not according to the
66 existing native PPN community. During an invasion, the introduced species comes into interaction
67 with the organisms already present in the community and possible antagonistic interactions, such as
68 competition, can occur and limit the establishment and/or multiplication of the introduced PPNs.
69 Three of the six categories of competition proposed by Schoener (1983), pre-emptive competition,
70 chemical competition and consumptive competition, are useful for summarising competitive
71 interactions among PPNs. Pre-emptive competition refers to ecological niche occupation by other
72 species, thereby preventing another species from entering that space. Chemical competition occurs
73 when a species produces a toxin or induces the production of allelochemicals that suppress another
74 species. Consumptive competition occurs when some essential resources, usually food, is consumed
75 by one species, reducing or depleting the quantity available to the competing species. Competition
76 (for example for access to resources or space) has already been reported between nematode species
77 (Freckman & Caswell, 1985; Umesh *et al.*, 1994). Although other PPN species have already been
78 shown to have a negative impact on the development, multiplication rate or hatching of root-knot
79 nematodes (Diez *et al.*, 2003; Brinkman *et al.*, 2008), few studies have explored *M. chitwoodi*
80 interactions with other PPNs. For instance, (Umesh *et al.*, 1994) observed that *Pratylenchus neglectus*
81 can limit *M. chitwoodi* growth or hatching in barley if it penetrates the roots first. Similar studies
82 have been conducted in more complex systems involving other organisms (Al-Rehiyani *et al.*, 1999)
83 and have highlighted three-way interactions between a bacteria (*Bacillus megaterium*), a PPN
84 (*Pratylenchus neglectus*) and green manure on the development of *M. chitwoodi*. Various types of
85 competition can be observed depending on the feeding habit; endoparasitic nematode species likely
86 compete mainly for space in the roots and ectoparasitic species for access to the resource (Umesh *et*
87 *al.*, 1994).

88 In this study, we focused on PPN community interactions with *M. chitwoodi* to assess the
89 potential of these communities for limiting *M. chitwoodi* multiplication or even its establishment
90 after introduction. We modified a natural field community by using different host plants to obtain
91 four contrasted PPN communities with the same taxonomic richness but varying in global and
92 relative abundance of PPNs or feeding type (*i.e.* ectoparasite vs endoparasite) and tested the impact
93 of these PPN communities on the multiplication rate of *M. chitwoodi*.

94

95 **Materials and Methods**

96 **Initial community and preliminary experiment**

97

98 To test and assess the effect of various types of PPN community but minimise the heterogeneity
99 of other variables between the communities (such as soil properties or history of cultural practices),
100 an initial PPN community was sampled in a field free of root-knot nematodes near the French
101 National Institute for Agricultural Research in Brittany (48°06'30"N 1°47'34.0"W). PPNs were
102 extracted from 400 g of soil previously homogenised according to EPPO bulletin PM 7/119 part 3.4
103 (2013) for morphological identification at the genus level when possible. The soil was mixed with 1/3
104 of PPN-free sand to give a texture allowing better plant growth and nematode movements and
105 extraction. Nineteen 22 L pots were filled with this soil and planted with either wheat (three pots),
106 maize (four pots), sugar beet (four pots), carrot (three pots) or potato (three pots). Two additional
107 pots were left free of any host plant. We let the initial PPN community develop in the different pots
108 for four months (25°C, L:D 12:8 and watered twice a week) to manipulate the abundance of PPN
109 taxa. After four months, PPNs were extracted and counted in 400 g of homogenised soil from each
110 pot according to PM 7/119 part 3.4 (2013) and endoparasitic nematodes (*i.e.* *Pratylenchus*) were
111 extracted from maize and carrot roots using a mist chamber. Those two plants were chosen as they
112 are known to be good host plants for *Pratylenchus* (Bridge & Starr, 2007).

113

114 **Experimental design**

115

116 From the preliminary experiment, we obtained contrasted communities (Table 1). Soil from wheat
117 and potato pots was not used in further experiments in order to keep only the most different
118 communities in terms of either total abundance or endoparasite vs ectoparasite abundance. The soil
119 from each pot was thoroughly mixed, homogenised and distributed to 106 new individual 2.8 kg pots
120 for the main experiment. Soil from maize, enriched with 150 *Pratylenchus* per pot, was used for
121 modality 1 (HT). Soil from uncultivated pots was used for modality 2 (LT). Soil from carrot, enriched
122 with 1500 *Pratylenchus* per pot, was used for modality 3 (HEN). Soil from sugar beet was used for
123 modality 4 (HEC). Control 3 (C3, Table 1) was obtained by mixing all the previous soils (without
124 *Pratylenchus* enrichment) to create the average community. PPN-free soil, from the same field but
125 stored several years in a container, also mixed with 1/3 of PPN-free sand was used for Controls 1 and
126 2 (C1 and C2, Table 1). Pots were planted with one potato tuber (var. Désirée) and distributed
127 randomly in the greenhouse. *M. chitwoodi* (population 13-765 from Lower-Normandy) was
128 inoculated in each pot 10 days after planting, in three spots around the potato tuber, near the roots.
129 This population was previously reared on tomato in the greenhouse and is part of the ANSES - Plant
130 Health Laboratory collection. To obtain second-stage juveniles (J2) for inoculation, roots harbouring
131 egg masses were placed in a mist chamber until hatching. For each community type and C2, 10 pots
132 were inoculated with 1000 *M. chitwoodi* J2 and 10 other pots with 50 J2. Three replicates of both C1
133 and C3 were also prepared for a total of 106 individual pots. We ran this experiment for one potato
134 growth cycle (about four months) at 25°C, 12 hours photoperiod and watered twice a week. At the
135 end of the experiment, above-ground plant parts were eliminated. PPNs were extracted again from
136 400 g of homogenised soil from each pot. Roots and tubers were separated, weighed and digested
137 overnight according to PM 7/119 part 2.7 (2013). PPNs were then recovered from the enzymatic
138 digestion by centrifugal flotation (PM 7/119 part 3.5, 2013). PPNs from the three extracts (soil,
139 tubers and roots) of each pot were identified based on morphological criteria using a

140 stereomicroscope. For standardisation, each taxon was counted, after a dilution step (depending on
141 the PPNs concentration) in 5 mL of the dilution. Only *M. chitwoodi* males and females (J4 and adults)
142 were counted separately in each extracts to obtain information on the proportion of inoculated J2
143 that reached the adult stage. *M. chitwoodi* J2 (J2 and J3) were not counted as we were not able to
144 distinguish those inoculated that have not reach the adult stage for any reason from those of the
145 following generations. For all the other taxa, all the developmental stages were counted.

146

147 **Statistical analyses**

148

149 The growth rate of each PPN taxon was calculated based on the initial density using the following
150 equation: $(P_f - P_i) / P_i$, where P_i is the initial population density of each taxon and P_f is the final
151 population density of each taxon at the end of the experiment. For the growth rate, no distinction
152 was made between *M. chitwoodi* females and males because undifferentiated J2 were used for the
153 inoculations.

154 All statistical analyses were conducted using R software (R Core Team, 2016). Comparisons
155 between taxon abundances and growth rate across the communities were performed using
156 generalised linear models (GLMs). We used Poisson models for abundance data and Gaussian model
157 for growth rate and percentage data. Multiple comparisons between community types were
158 performed using the estimated marginal means (EMMeans) method with the emmeans R package
159 (Lenth, 2018). Comparisons between C2 and all the other modalities for abundance and growth rate
160 of *M. chitwoodi* were performed first (results are presented in Figure S1 and Figure S2). It appeared
161 that C2 was always significantly different from the other modalities (except considering growth rate,
162 when C2 was compared to LT in pots inoculated with 1000 J2). Thus, comparisons between
163 modalities, without C2, were then performed in order to highlight more precisely the variation.

164

165 **Results**

166 **Preliminary experiment**

167

168 Five PPN taxa were identified in the initial community from the sampled field: *Pratylenchus*,
169 *Helicotylenchus*, Telotylenchidae, *Paratylenchus* and *Criconemoides*. Table 2 shows the abundance of
170 each PPN taxon before the preliminary experiment and after the development in the different pots
171 containing different host plants. Most importantly, *Paratylenchus* (ectoparasite) reproduced
172 extremely well on sugar beet (1960 ± 183 individuals per 400 g of soil) compared with the other host
173 plants and *Pratylenchus* (endoparasite) was recovered in high quantity (12139 ± 4086 individuals)
174 from the roots of maize and used to adjust the modalities HT and HEN. Soils from bare soil, maize,
175 sugar beet and carrot were used to design four modalities (HT to HEC, Table 1) as described in the
176 Material and Method section.

177

178 **Impact of PPN communities on *M. chitwoodi* populations**

179

180 After 4 months of potato culture using the modalities previously designed, abundance of *M.*
181 *chitwoodi* was determined in all pots. *M. chitwoodi* abundance was limited by the other PPNs
182 because its total abundance was significantly lower in all the modalities than in the control
183 containing *M. chitwoodi* alone on potato (Figure S1), regardless the inoculation treatment ($609.50 \pm$
184 73.88 in pots inoculated with 50 J2 and 1009.73 ± 127.73 in pots inoculated with 1000 J2). Figure 1
185 shows comparison of *M. chitwoodi* abundances between modalities without the control. *M.*
186 *chitwoodi* females and males followed similar abundance patterns, although males were always less
187 abundant than females (Figure 1). They showed their highest abundance in LT (164.00 ± 37.49 and
188 742.80 ± 86.93 females per pot, in the 50 and 1000 J2 inoculation treatment, respectively, and 83.28
189 ± 23.79 and 170.35 ± 17.58 males per pot, in the 50 and 1000 J2 inoculation treatment, respectively)
190 (Figure 1). None of the pots inoculated with 1000 *M. chitwoodi* J2 was found free of *M. chitwoodi* at

191 the end of the experiment. Only four pots out of the 10 for HT and 1 pot out of the 10 for HEN
192 inoculated with 50 J2 were found free of *M. chitwoodi* adults at the end of the experiment. Other
193 pots inoculated with 50 *M. chitwoodi* J2 showed mean final abundance of 12 ± 3.12 , 247 ± 54.44 , $8 \pm$
194 2.72 and 59 ± 23.64 for HT, LT, HEN and HEC respectively.

195
196 In order to understand the variations of abundance observed, we also compared the growth rate
197 of each taxon among the communities using Gaussian models. Results about *M. chitwoodi* are given
198 in Figure 2. *M. chitwoodi* multiplication was significantly more important in the control (mean C2 =
199 11.19 ± 1.48) than in the other modalities for pots inoculated with 50 J2 but did not vary from
200 multiplication in LT for pots inoculated with 1000 J2 (Figure S2). In HT, HEN and HEC, *M. chitwoodi*
201 populations showed a decrease (Figure 2a). Significant difference between inoculation treatments
202 among each modality appeared only for LT (3.95 ± 1.05 for pots inoculated with 50 J2 compare to -
203 0.09 ± 0.09) but not for the other modalities (Figure 2b). However, it should also be noticed that
204 while growth rate values differ drastically among the modalities in the 50 J2 inoculum, these values
205 appear to vary in a much narrower range in the 1000 J2 inoculum. This suggests that the strength of
206 the effect of the community on *M. chitwoodi* growth rate depends also of the invasive inoculum size.

207

208 **Effect of *M. chitwoodi* densities on other PPN**

209

210 Comparisons between final abundance of PPN in the two inoculation treatments among the
211 modalities are presented in Figure 3. Significantly higher abundance in 50 J2 inoculation treatment
212 than in 1000 J2 inoculation treatment was observed in HT, HEN and HEC for *Pratylenchus*, in LT and
213 HEC for *Helicotylenchus*, in HT, LT and HEN for *Paratylenchus*, in LT and HEC for Telotylenchidae and
214 in HT and LT for *Criconemoïdes*. On the contrary, significantly higher abundance in 1000 J2
215 inoculation treatment than in 50 J2 inoculation treatment was observed in a much more limited

216 number of situations: only in HT for *Helicotylenchus*, only in HEC for *Paratylenchus*, in HT and HEN for
217 Telotylenchidae and only in HEC for *Criconemoïdes*.

218

219 For each PPN taxon, growth rate was compared between modalities in pots inoculated with 50
220 and 1000 *M. chitwoodi* J2 (Figure 4a) and between inoculation treatments within each modality
221 (Figure 4b). It appears that for *Paratylenchus* and *Criconemoïdes*, a negative growth rate was
222 observed in all the modalities and no significant difference was observed between inoculation
223 treatments (Figure 4). There was globally no significant difference in PPN growth rate between the
224 *M. chitwoodi* inoculation treatments. In only two cases (*i.e.* *Paratylenchus* HEC and *Helicotylenchus* LT)
225 a significant difference appeared between the *M. chitwoodi* inoculation treatments and there was
226 always a more important multiplication in the 50 J2 inoculation treatment than in the 1000 J2
227 inoculation treatment (Figure 4b).

228

229 **Proportion of *M. chitwoodi* males**

230

231 To further investigate the observations about *M. chitwoodi* abundance, we studied the
232 proportions of males among communities and inoculation treatments using Gaussian models (Figure
233 5). Interestingly, males were more abundant in LT compare to the other modalities regardless the
234 inoculation treatment (Figure 5a). Furthermore, in LT, the percentage of males was higher in the 50
235 J2 inoculation treatment than in the 1000 J2 inoculation treatment ($35.82\% \pm 5.28\%$ vs. $19.70\% \pm$
236 3.82% , respectively) (Figure 5b).

237

238 **Discussion**

239

240 This study highlighted for the first time the impact of natural PPN communities on *M. chitwoodi*.
241 We choose to count only adults of *M. chitwoodi*, firstly because it was interesting to know the

242 abundance of J2 that have reached the adult stage and thus possibly multiplied, to study the
243 establishment of *M. chitwoodi*. Secondly, since at least two generations of *M. chitwoodi* occurred, it
244 was not possible to distinguish the J2 from the first generation (inoculated J2) which would not have
245 further developed into adults from the J2 of the second or even third generations. Considering this,
246 final abundance and growth rate based on adults bring a relevant information about the impact of
247 PPN communities on *M. chitwoodi* establishment but as J2 were present in the modalities, the
248 abundance figures presented in this study were slightly underestimated but much more comparable
249 among each other.

250 We created different community types from a single community to limit variation in variables
251 other than PPN community composition. Although abiotic factors were fully controlled during our
252 experiment, we cannot exclude the possibility that biotic factors other than PPNs (such as
253 microorganisms or non-phytoparasitic nematodes that were present in our soils) are also involved
254 and contribute to the observed decrease of *M. chitwoodi* (Akhtar & Malik, 2000). However, the
255 potential of the initial soil loading for microorganisms other than PPNs was the same because each
256 modality was established on the basis of the same soil and the experiment was conducted over a 4-
257 month period and only using potato. Therefore questions remain: would the observed decrease in *M.*
258 *chitwoodi* continue over a longer period and successive cropping or would *M. chitwoodi* ultimately
259 adapt to its competitors? Would similar results be observed using the same community types but
260 different host plants? Additional experiments will provide more information and should be now
261 considered to confirm these first results.

262

263 The abundance of *M. chitwoodi* greatly decreases during a potato cycle in three modalities (HT,
264 HEN and HEC) which corresponded to a high overall abundance of indigenous PPNs, irrespective of
265 their feeding habit (endoparasite vs. ectoparasite). Similar results were observed for *Meloidogyne*
266 *maritima* whose development is reduced by *Pratylenchus penetrans* and *Heterodera arenaria* in a
267 PPN community on dune grass (Brinkman *et al.*, 2005). Furthermore, dune grass biomass is lower

268 when *M. maritima* is added alone to the plant than when all three species are present. The two latter
269 PPN species may “protect” their host plant by delaying the development of *M. maritima*, which then
270 occurs later in the growing season when infection by *M. maritima* has less impact on the plant
271 (Brinkman *et al.*, 2005). However, it is unlikely that such effect occur in our study because the fresh
272 weight of roots and tubers did not significantly vary between the three controls: C1 (potato alone),
273 C2 (*M. chitwoodi* alone) and C3 (average PPN community) (data not shown). Instead, our results
274 appear to rather support interspecific competition between the native PPN communities and the
275 introduced species for access to roots, with the PPNs already present in soil colonising the roots first
276 and preventing *M. chitwoodi* from penetrating them and achieve its development. However, if the
277 PPNs abundance is low, the invasive species seems to have enough room to penetrate and multiply
278 among roots and tubers.

279 Overall abundance of PPN in HEN was however lower than in HT and HEC (in pots inoculated with
280 50 *M. chitwoodi* J2 for instance, HEN total PPN abundance was 267 ± 55 whereas HT and HEC total
281 abundance were respectively 1201 ± 138 and 946 ± 160). In this modality in particular, relative
282 abundance of *Pratylenchus* was higher than in the other, and could highlight an impact of this taxon
283 on *M. chitwoodi* as it has been observed in Umesh *et al.* (1994). Even if we cannot exclude the
284 competitor effect of species of *Pratylenchus* towards *M. chitwoodi*, our results highlight the impact of
285 the overall abundance of the PPN community as failure of establishment of *M. chitwoodi* was
286 observed predominantly in the HT community type and not the HEN community type. It should also
287 be emphasized that such failure of establishment was only observed for low invasive inoculum size
288 (*i.e.* 50 J2). In natural agrosystems, this number could for example be due to soil transfer through
289 agricultural machinery (highlighted for cyst nematodes in Plantard *et al.* (2008)) or contaminated
290 seedling tubers.

291 Observed *M. chitwoodi* growth rate variations between the modalities appear to confirm the
292 abundance results. We observed around a five-fold increase in *M. chitwoodi* in LT pots inoculated
293 with 50 J2 compared with the other modalities for which the *M. chitwoodi* population has decreased,

294 even until elimination in few pots. This confirms the existence of strong interspecific competition
295 that can be useful to prevent establishment when the invasive inoculum is low.

296

297 To simulate an indigenous PPN community which is already present and established in a field, *M.*
298 *chitwoodi* inoculations were performed 10 days after planting potato tubers to allow other PPNs to
299 colonise the roots first. Despite the observed decrease of the quarantine species, an inverse effect
300 was also noticed on the other PPN taxa. Except in LT, abundance of *Pratylenchus* for example was
301 lower in the 1000 J2 inoculation treatment than in the 50 J2 inoculation treatment despite the
302 delayed *M. chitwoodi* inoculation. Similar results were observed for the other PPN taxa present in
303 our study. This type of inverse effect has already been observed in previous studies (Villenave &
304 Cadet, 1998; Melakeberhan & Dey, 2003), suggesting reciprocal interspecific competition between
305 the indigenous PPNs and the invasive species. Furthermore, growth rates of these taxa were either
306 not significantly different or lower in the 1000 J2 inoculation treatment than in the 50 J2 inoculation
307 treatment. However, it should be noticed that *Paratylenchus* and *Criconemoïdes* populations have
308 decreased in all modalities, likely due to poor host (*i.e.* potato) suitability (Nickle, 1991; Bridge &
309 Starr, 2007) rather than to a direct effect of *M. chitwoodi* on these taxa. Accordingly, in the
310 preliminary experiment, *Paratylenchus* and *Criconemoïdes* have not well multiplied on potato.

311 Our results therefore support previous findings showing effects of endoparasitic nematodes (*i.e.*
312 *M. chitwoodi*) on the development of ectoparasitic nematodes (such as *Helicotylenchus* or
313 Telotylenchidae) (Brinkman *et al.*, 2004), as well as on other endoparasitic nematodes such as
314 *Pratylenchus* (Lasserre *et al.*, 1994; Melakeberhan & Dey, 2003; Brinkman *et al.*, 2008). We
315 hypothesise that the observed inverse effect is mainly due to interspecific competition for access to
316 roots and/or food, because previous results on *Pratylenchus* and *M. chitwoodi* have failed to show
317 any indirect competition effect by stimulating root defences (Umesh *et al.*, 1994; Brinkman *et al.*,
318 2008).

319 The decrease in the growth rate for the indigenous taxa appeared to be due to *M. chitwoodi*
320 density because at least one of the community types showed significant differences between the *M.*
321 *chitwoodi* inoculation treatments. However, we also sometime observed that the growth rates for
322 PPN taxa were higher in LT - lowest overall PPN abundance - suggesting that PPN communities may
323 regulate *M. chitwoodi* expansion and also partially regulate themselves through interspecific
324 competition.

325

326 Interestingly, we observed unexpected variation in the male proportions among the community
327 types and inoculations. In the agriculturally important root-knot nematode species, reproduction is
328 generally parthenogenetic (Trudgill, 1997; Castagnone-Sereno, 2006). In some of these species, such
329 as *M. chitwoodi*, parthenogenesis is facultative. Hence, males can participate in reproduction
330 (Castagnone-Sereno, 2006). Although the sexual determinism of these facultative parthenogenetic
331 species is not fully understood, it nonetheless appears to be a phenomenon mainly linked to
332 environmental conditions (Castagnone-Sereno, 2006). Juvenile transformation is not pre-determined
333 and males are produced when the environment is not suitable for female development, for example
334 due to poor host quality, small size of the giant cells or strong competition (Triantaphyllou, 1973;
335 Trudgill, 1997). Juvenile differentiation thus depends on the amount of food consumed (Trudgill,
336 1972). Unexpectedly, we observed that the proportion of males was higher in LT than in the other
337 PPN community types, and among this modality, even higher in the 50 J2 inoculation treatment than
338 in the 1000 J2 inoculation treatment. This male production seems in contradiction with our other
339 results that highlight a high growth rate of *M. chitwoodi* in LT where the overall PPNs abundance was
340 low (*i.e.* lower interspecific competition). In cyst nematodes, which are also sedentary endoparasites
341 like root-knot nematodes, the production of males is attributed to heavy root infestation and thus
342 high intra-host density (Mugnéry and Phillips, 2007). Keeping in mind that two to even three
343 generations of *M. chitwoodi* took place during the time of our experiment, we hypothesize that
344 intraspecific competition occurred in the roots heavily infested with *M. chitwoodi* in LT by the end of

345 our experiment after an important multiplication. In this case, interspecific competition was weak
346 and many *M. chitwoodi* J2 penetrated the roots to initiate feeding sites. It is therefore probable that
347 those J2 became females and in turn produced many J2, explaining the high population growth rate.
348 However, the second or third generation may have represented a threshold for the host plant which
349 then produced inferior quality feeding sites due to heavy intraspecific competition, inducing the
350 production of males.

351

352 This original experimental design allowed us to test the impact of different indigenous PPN
353 communities on the development and establishment success of *M. chitwoodi*. Clearly, soils showing a
354 poor PPN community in terms of abundance present a higher risk of successful establishment of *M.*
355 *chitwoodi*. It could be therefore interesting to consider this parameter in risk assessments and
356 monitoring plans in regard to this quarantine species. As discussed, the results presented in this
357 study need to be confirmed with other *M. chitwoodi* populations, but also other host plants and
358 longer experiments in order to propose a relevant risk assessment tool. Furthermore, studying the
359 impact of cultural practices on PPN communities in various agrosystems may allow identify the
360 practices that can help to maintain native and non-damaging PPN communities. This opens new
361 research areas for the management of nematode diversity in soils as a mean to regulate some soil-
362 borne invasive species.

363

364 **Acknowledgements**

365

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451

452 **Figures captions**

453

454 Fig. 1: Mean final abundance (number of individuals per pot) of *M. chitwoodi* total adults, only
455 females or only males, among the different modalities. Light grey indicate pots where *M. chitwoodi*
456 J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2.
457 Error bars indicate standard error. Different letters indicate significant differences between
458 modalities according to multiple comparison tests using the estimated marginal means method.
459 Scale varies between graphs.

460

461 Fig. 2: (a) Growth rate of *M. chitwoodi* among the different modalities. (b) Comparison of *M.*
462 *chitwoodi* growth rate between the inoculation treatments among each modality. Light grey indicate
463 pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2
464 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant
465 differences between modalities (a) or between inoculation treatments among the modalities (b)
466 according to multiple comparison tests using the estimated marginal means method. Scale varies
467 between graphs.

468

469 Fig. 3: Comparison of mean final abundance (number of individuals per pot) of each taxon between
470 the inoculation treatments among the modalities. Light grey indicate pots where *M. chitwoodi* J2
471 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error

472 bars indicate standard error. Different letters indicate significant differences between inoculation
473 treatments among the modalities according to multiple comparison tests using the estimated
474 marginal means method. Dotted line indicates the mean abundance value of each PPN taxon in the
475 control pots (C3, see Table 1). Scale varies between graphs.

476

477 Fig. 4: (a) Growth rate of PPN taxa among the different modalities. (b) Growth rate comparison of
478 each PPN taxon between the inoculation treatments among each modality. Light grey indicate pots
479 where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2
480 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant
481 differences between modalities (a) or between inoculation treatments among the modalities (b)
482 according to multiple comparison tests using the estimated marginal means method. Dotted line
483 indicates the mean abundance value of each PPN taxon in the control pots (C3, see Table 1). Scale
484 varies between graphs.

485

486 Fig. 5: (a) Percentage of *M. chitwoodi* males for each modality. (b) Comparison of *M. chitwoodi* males
487 percentage between the inoculation treatments among each modality. Light grey indicate pots
488 where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey indicate pots where *M. chitwoodi* J2
489 inoculum was 1000 J2. Error bars indicate standard error. Different letters indicate significant
490 differences between modalities (a) or between inoculation treatments among the modalities (b)
491 according to multiple comparison tests using the estimated marginal means method. Scale varies
492 between graphs.

493

494 Fig. S1: Mean final abundance (number of individuals per pot) of *M. chitwoodi* total adults, only
495 females or only males, among the different modalities, including control pots where *M. chitwoodi* J2
496 were inoculated alone. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark
497 grey indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error bars indicate standard error.

498 Different letters indicate significant differences between modalities according to multiple
499 comparison tests using the estimated marginal means method. Scale varies between graphs.

500

501 Fig. S2: (a) Growth rate of *M. chitwoodi* among the different modalities, including control pots where
502 *M. chitwoodi* J2 were inoculated alone. (b) Comparison of *M. chitwoodi* growth rate between the
503 inoculation treatments among each modality, including control pots where *M. chitwoodi* J2 were
504 inoculated alone. Light grey indicate pots where *M. chitwoodi* J2 inoculum was 50 J2 and dark grey
505 indicate pots where *M. chitwoodi* J2 inoculum was 1000 J2. Error bars indicate standard error.
506 Different letters indicate significant differences between modalities (a) or between inoculation
507 treatments among the modalities (b) according to multiple comparison tests using the estimated
508 marginal means method. Scale varies between graphs.

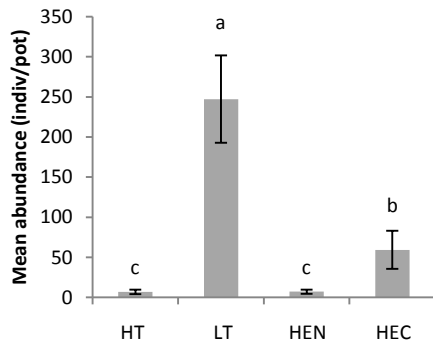
Table 1 Characteristics of the community types used in the experiment

Treatment	Abbreviation	Characteristics
Control 1	C1	No PPNs
Control 2	C2	<i>M. chitwoodi</i> alone
Control 3	C3	Average PPN community composed of M1, M2, M3, M4 soil mixed together (prior to <i>Pratylenchus</i> inoculation), without <i>M. chitwoodi</i>
Modality 1	HT	High total abundance of PPNs (around 600 individuals per 400 g of soil, proportion of endo- and ectoparasites relatively similar)
Modality 2	LT	Low total abundance of PPNs (around 50 individuals per 400 g of soil, proportion of endo- and ectoparasites relatively similar)
Modality 3	HEN	High relative abundance of endoparasitic PPNs (around 65%, global abundance around 350 individuals per 400 g of soil)
Modality 4	HEC	High relative abundance of ectoparasites PPNs (around 98%, global abundance around 650 individuals per 400 g)

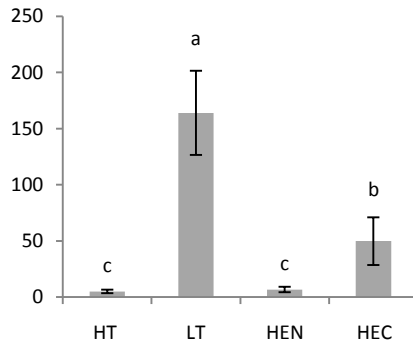
Table 2 Initial community and mean abundance \pm standard error of each PPN taxon at the end of the preliminary experiment

Host plant	Soil (individuals per 400 g of soil)					Roots (total individuals)
	<i>Pratylenchus</i>	<i>Helicotylenchus</i>	Telotylenchidae	<i>Paratylenchus</i>	<i>Criconemoïdes</i>	<i>Pratylenchus</i>
Initial community	384	128	240	224	4	-
Bare soil	8 \pm 3.00	7 \pm 4.00	48 \pm 5.50	29 \pm 11.00	3 \pm 0.50	-
Maize	62 \pm 15.10	174 \pm 17.09	97 \pm 27.34	505 \pm 95.39	10 \pm 2.00	12,139 \pm 4085.72
Sugar beet	44 \pm 5.16	60 \pm 18.62	86 \pm 18.29	1960 \pm 182.87	6 \pm 2.00	-
Wheat	73 \pm 24.52	121 \pm 24.01	516 \pm 205.30	474 \pm 222.43	11 \pm 5.33	-
Carrot	12 \pm 4.62	11 \pm 3.48	51 \pm 7.17	117 \pm 81.79	6 \pm 2.03	107 \pm 47.19
Potato	68 \pm 29.41	28 \pm 6.39	191 \pm 62.78	73 \pm 18.03	6 \pm 2.85	-

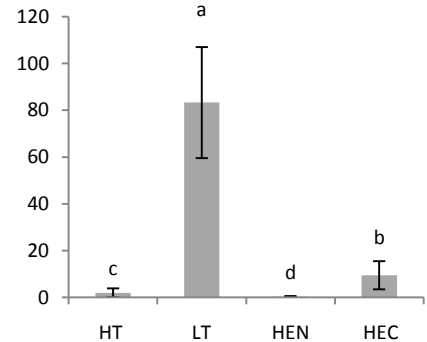
***M. chitwoodi* total
50 J2 inoculation**



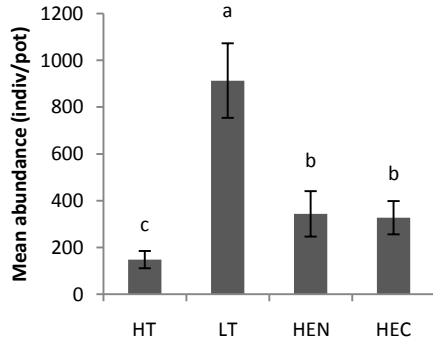
***M. chitwoodi* females
50 J2 inoculation**



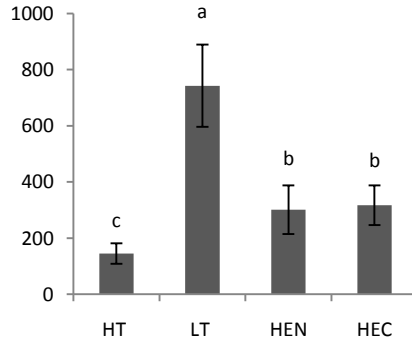
***M. chitwoodi* males
50 J2 inoculation**



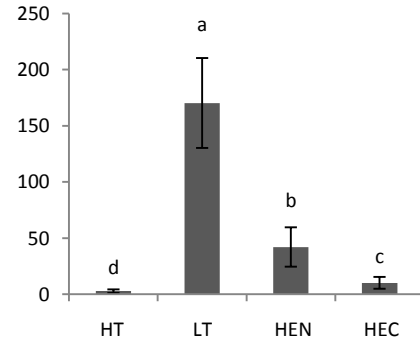
***M. chitwoodi* total
1000 J2 inoculation**



***M. chitwoodi* females
1000 J2 inoculation**

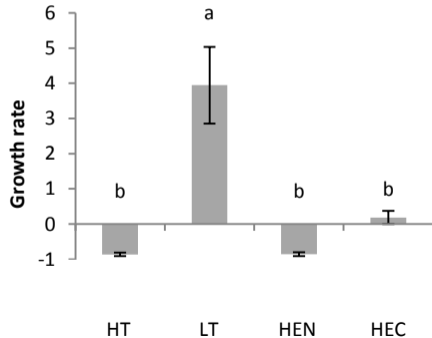


***M. chitwoodi* males
1000 J2 inoculation**

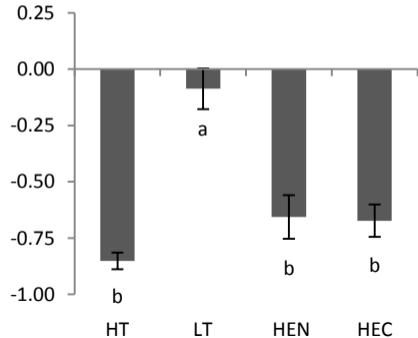


(a)

M. chitwoodi total
50 J2 inoculation

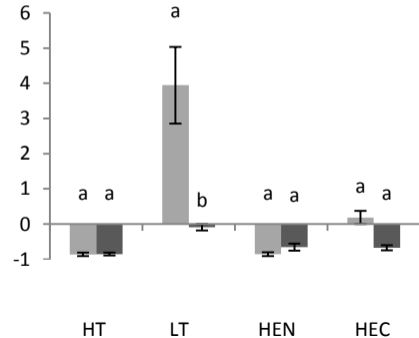


M. chitwoodi total
1000 J2 inoculation

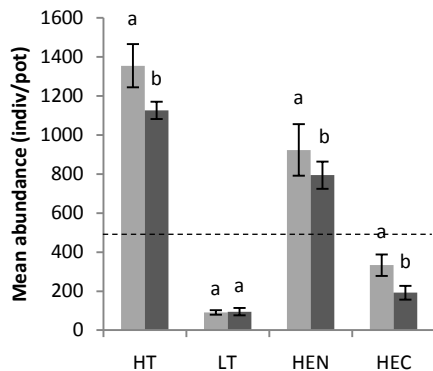


(b)

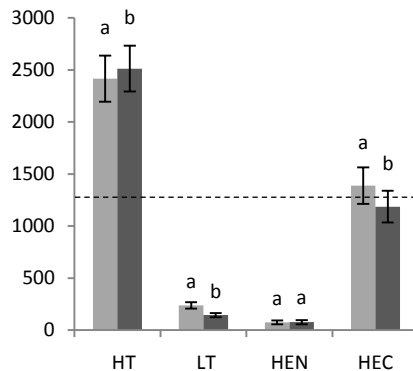
M. chitwoodi total
comparison between inoculum



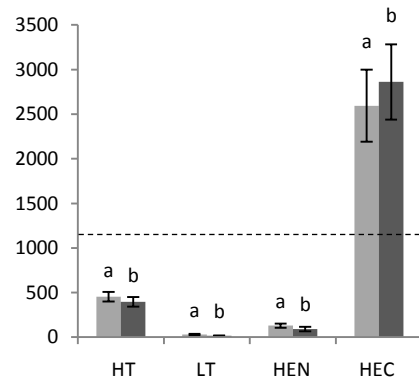
Pratylenchus



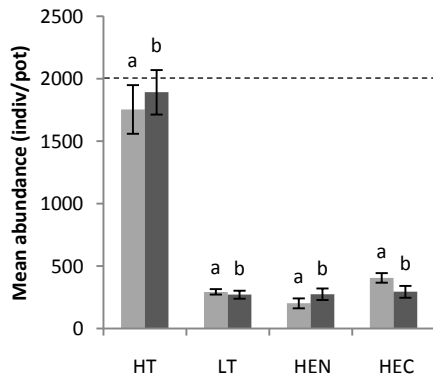
Helicotylenchus



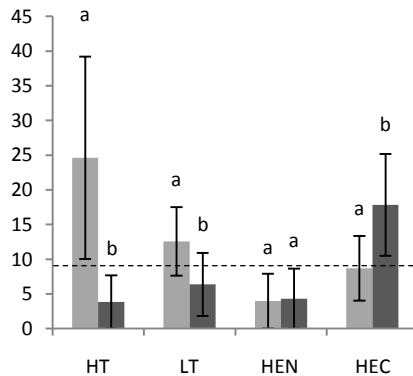
Paratylenchus



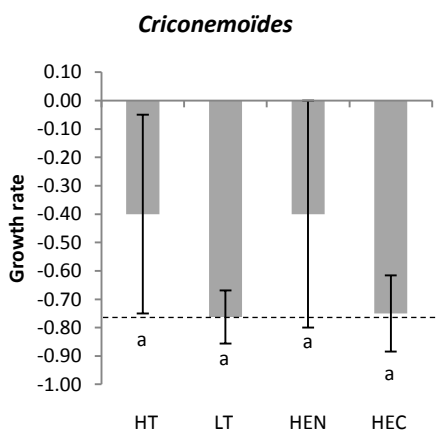
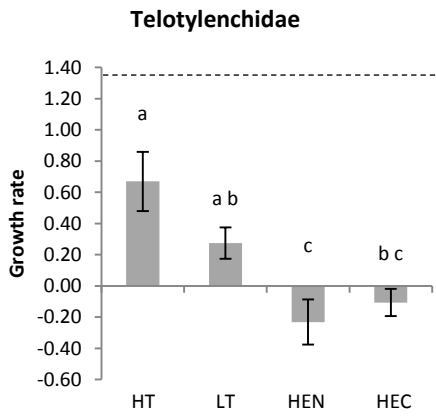
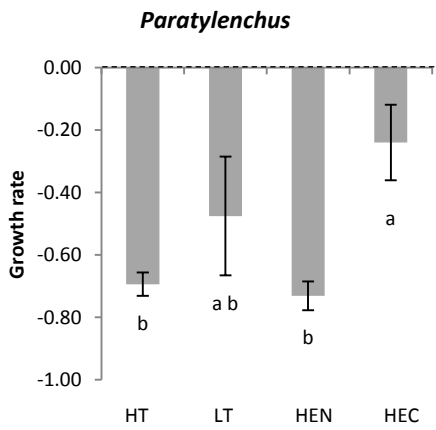
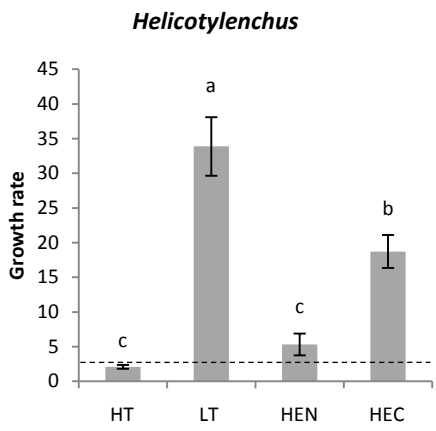
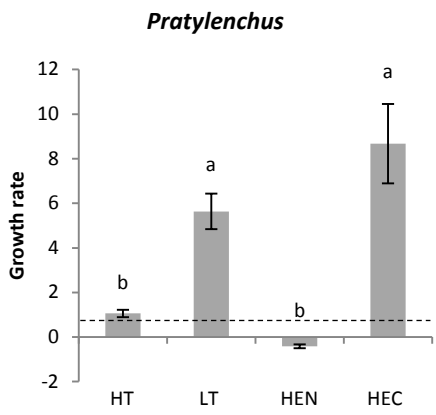
Telotylenchidae



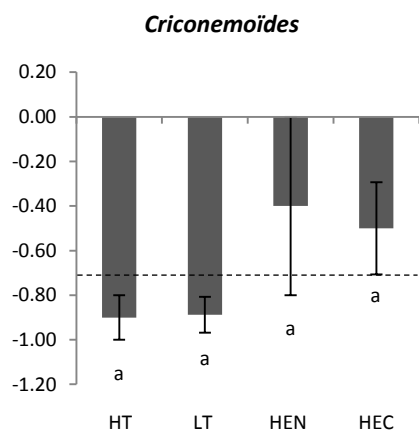
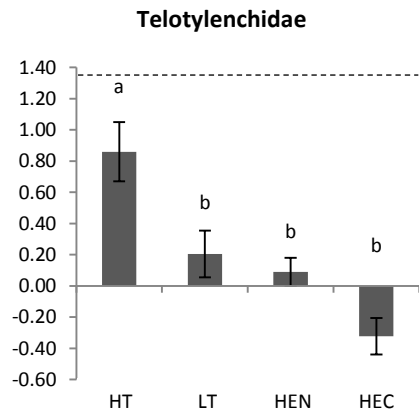
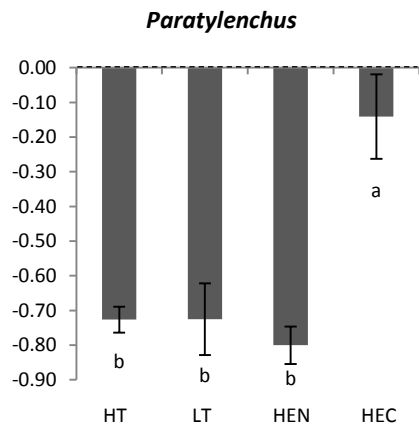
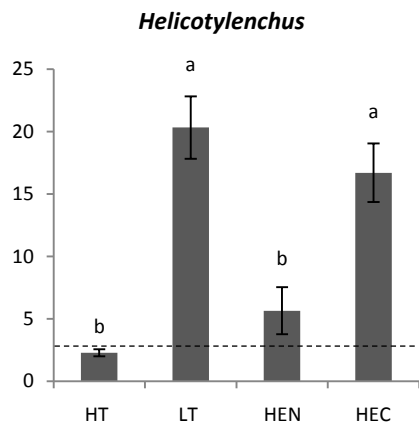
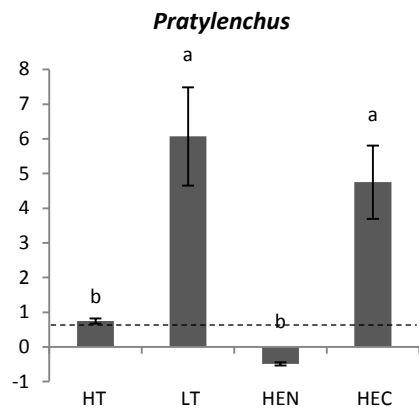
Criconemoides



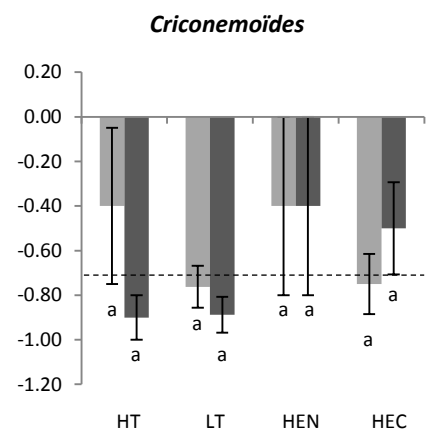
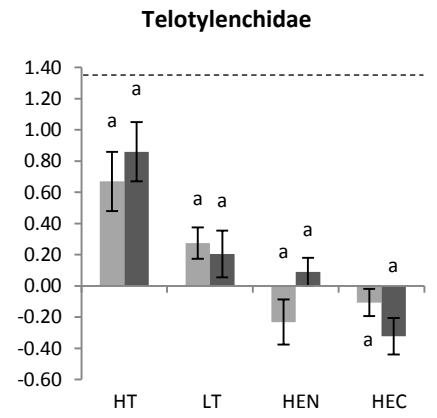
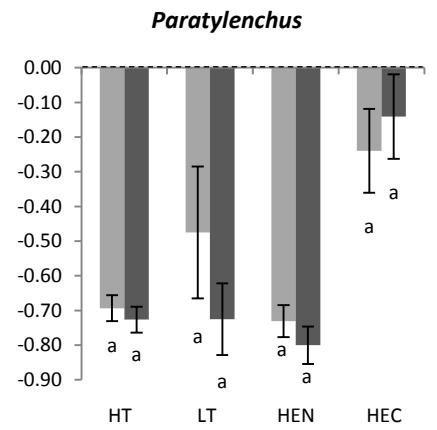
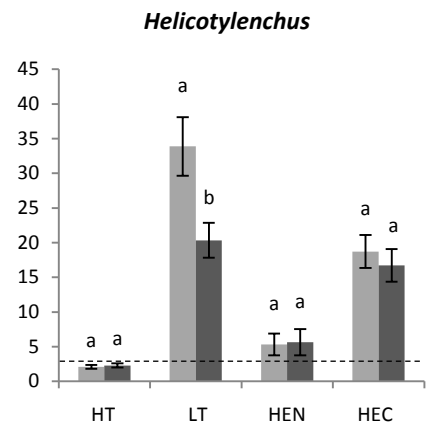
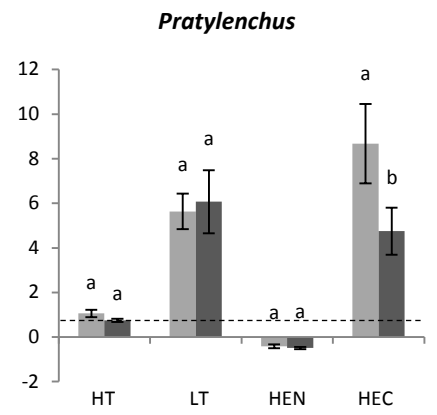
(a) 50 *M. chitwoodi* J2 inoculation



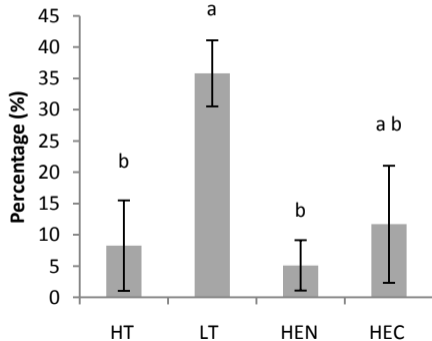
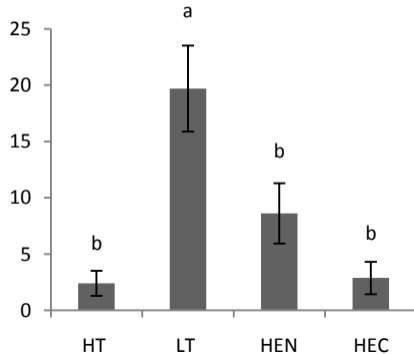
1000 *M. chitwoodi* J2 inoculation



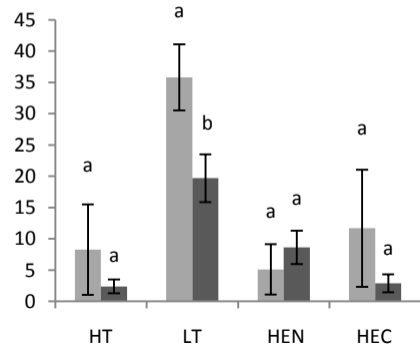
(b) Comparison between *M. chitwoodi* J2 inoculum



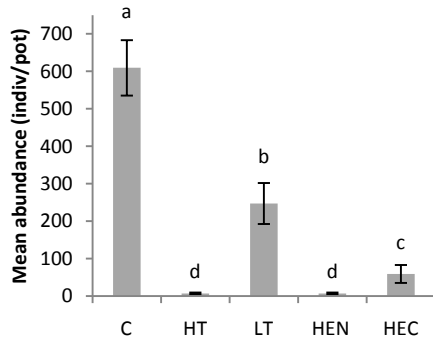
(a)

***M. chitwoodi* males
50 J2 inoculation*****M. chitwoodi* males
1000 J2 inoculation**

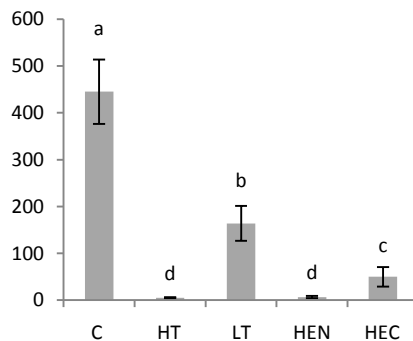
(b)

***M. chitwoodi* males
comparison between inoculum**

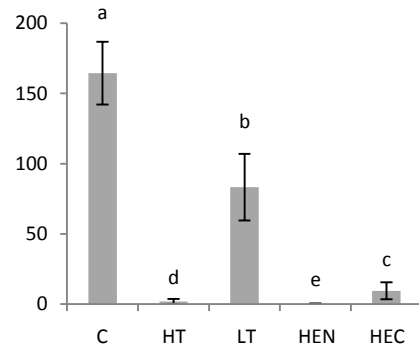
***M. chitwoodi* total
50 J2 inoculation**



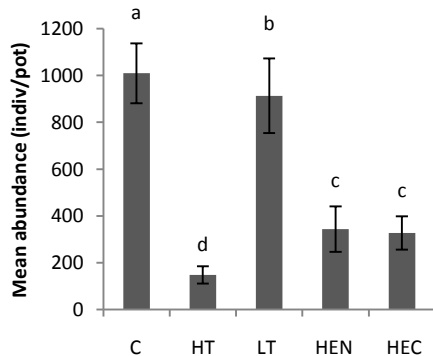
***M. chitwoodi* females
50 J2 inoculation**



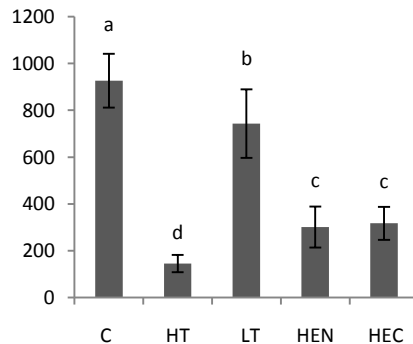
***M. chitwoodi* males
50 J2 inoculation**



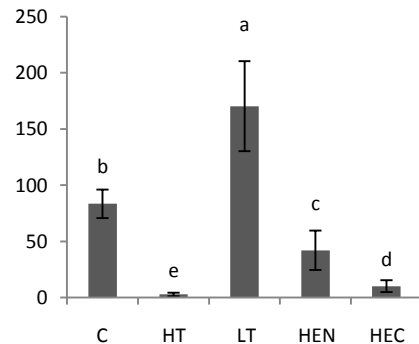
***M. chitwoodi* total
1000 J2 inoculation**



***M. chitwoodi* females
1000 J2 inoculation**

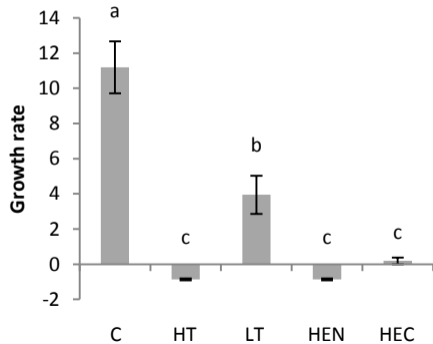


***M. chitwoodi* males
1000 J2 inoculation**

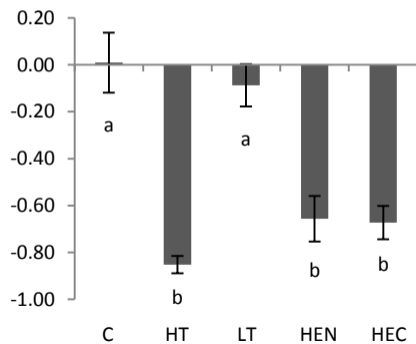


(a)

M. chitwoodi total
50 J2 inoculation



M. chitwoodi total
1000 J2 inoculation



(b)

M. chitwoodi total
Comparison between inoculum

