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1 **Impact of the genetic diversity of three cyst nematodes on the effectiveness**
2 **of root exudates to induce hatching**
3

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14 **SUMMARY**

15 Cyst nematodes are sedentary endoparasites of plants which cause important economic losses
16 worldwide. New nematode control measures are needed since the removal of effective chemical
17 nematicides from the market due to their negative impact on environment and human health.
18 Induced hatching of second-stage juveniles in the absence of host plant using root exudates,
19 also named “suicide hatching”, could be a sustainable alternative method to reduce nematode
20 population densities in infested soils. Unfortunately, biocontrol methods are often less effective
21 in agricultural fields than in laboratory or greenhouse and this could be due to, among other
22 reasons, a different susceptibility of nematode populations to root exudate stimulation. Testing
23 this intra-specific variability would help to anticipate any drop in the efficiency of this new
24 biocontrol strategy. A selection of root exudates from different plant species, maximizing the
25 hatching level highlighted in a previous study, was tested on the hatching of representative
26 populations of the genetic diversity for *Globodera pallida*, *Heterodera schachtii* and
27 *Heterodera carotae*. Results showed significant differences between populations for given
28 nematode species but not correlated with the genetic structure. Overall, root exudates tested
29 provide a high level of hatching of these three nematode species. Surprisingly, the root exudate
30 from broccoli induces hatching of European populations of the potato cyst nematode *G. pallida*.

31

32

33 *Keywords:* Biocontrol - *Globodera pallida* – *Heterodera schachtii* – *Heterodera carotae* –
34 Suicide hatching

35 1. INTRODUCTION

36 The most important and damaging plant-parasitic nematodes of cultivated crops, causing severe
37 economic losses in the agriculture worldwide, are root-knot (*Meloidogyne*) and cyst
38 (*Heterodera* and *Globodera*) nematodes (Molinari, 2011). Cyst nematodes are characterised by
39 the presence of a survival stage in the soil: the cyst, which is the dead body of the female
40 containing hundreds of eggs. In some *Heterodera* species, eggs are contained in the cyst but
41 also in a gelatinous eggsac. Basically, the second-stage juveniles (J2) hatch from the cyst under
42 suitable climate conditions and less or more (both depending on the species) after the perception
43 of chemical cues released from roots of the host plant. The J2 then migrates to the host,
44 penetrates into the root and induces a feeding site, the syncytium, for its nutrition. Nematodes
45 moult through the third and fourth stages before becoming a male or a female adult. Adult males
46 leave the root in order to mate the females. Eggs form within the females which later die and
47 form cysts. The cyst constitutes an effective structure to spread and survive in the soil for several
48 years until the presence of a suitable host plant (see Perry et al., 2018 for a review).

49 Management of cyst nematode populations is generally realised by chemical
50 nematicides, cultural practices and the use of resistant cultivars. However, due to their impacts
51 on human health and environment, several nematicides have now been withdrawn from the
52 market, such as Vydate® 10g banned in December 2020 in UK. These regulatory changes leave
53 many sectors with few effective solutions and encourage private companies and academic
54 research to develop new environmentally friendly strategies. Among them, one may be very
55 challenging and corresponds to the development of natural biocontrol products that induce the
56 suicide hatch of the J2s in the absence of the host plant. The “suicide hatching” concept has
57 already been explored and some authors proposed to use it to decrease population of parasites
58 in the soil such as parasitic weeds (Zwanenburg et al., 2016), pathogens (Balendres et al., 2016;
59 Rashid et al., 2013) or nematodes (Devine & Jones, 2000; Lettice & Jones, 2015). In cyst

60 nematodes, the perception of chemical cues released by the host plant is an essential prerequisite
61 to optimize nematode chances of successful infection through the synchronization of their life
62 cycle with the presence of the host plant. The suicide hatching strategy is thus a promoting
63 solution to control cyst nematode populations.

64 Unfortunately, biocontrol products are often less effective in the field than under
65 laboratory and greenhouse-controlled conditions. These performance differences may result
66 from the commercial formulation, unfavorable climatic conditions in the field and/or negative
67 potential interactions between the products and the soil environment (Le Mire et al., 2016; Nicot
68 et al., 2012; Parnell et al., 2016). This lack of efficiency can also be attributed to variations in
69 the pathogen response. Indeed, Bardin et al. (2013) revealed a wide variation in the reduction
70 of spore production among isolates of *Podosphaera xanthii*, responsible for the cucurbit
71 powdery mildew, when they soaked melon disk on an extract from *Fallopian sachalinensis*.
72 Also, Ali et al. (2014) indicated a susceptibility variation among pathovars of *Xanthomonas*
73 *campestris* to the *Cuscuta pedicellata* aqueous plant extracts. These results suggest a
74 susceptibility difference within pathogens against biocontrol agents or products. Predicting
75 these variations in pathogen responses is thus a very challenging question, little explored so far
76 in the framework of the development of biocontrol products.

77 In this way, the aim of this study was to try to predict the efficiency of root exudates,
78 which may be used to induce suicide hatching, among different populations of three nematode
79 species: *Heterodera schachtii*, *Globodera pallida* and *Heterodera carotae*. These three species
80 were chosen for their level of specialization; *Heterodera schachtii*, the sugar beet cyst
81 nematode, can parasitize a wide host range with many different plant families such as
82 Amaranthaceae, Brassicaceae, Polygonaceae and Solanaceae families (Turner & Subbotin,
83 2013), *Globodera pallida*, the potato cyst nematode, attacks the potato and others species of
84 *Solanum* such as tomato and eggplant (Sullivan et al., 2007) and *Heterodera carotae*, the carrot

85 cyst nematode, is highly specific and its development is restricted to the genera *Daucus* and
86 *Torilis* (Aubert, 1986). Our study follows the screening of a large range of root exudates, from
87 host and non-host plants, on the hatching of these three cyst nematode species (Ngala et al.,
88 2021). However, this screening used only one population per species and we have thus no idea
89 of the efficiency of root exudates according to the intra-specific diversity of nematodes. To
90 address this gap, we selected the most interesting root exudates and tested them on several
91 populations representative of the described spatial genetic structure of each species (Gautier et
92 al., 2019; Picard et al., 2007; Plantard et al., 2008), rather than choosing them randomly. Indeed,
93 for species with low dispersal capabilities, such as these nematode species, the neutral
94 evolutionary history could be correlated to phenotypic specificities. Then for each selected
95 population, both the hatching rate at the end of the hatching assay and the dynamic of hatching,
96 were analysed.

97

98 **2. MATERIALS AND METHODS**

99 *2.1 Nematode populations*

100 For *Heterodera schachtii*, six populations (Table 1), originated from Morocco (MAR C1),
101 Spain (ESP C2), France (AM2), Germany (GER C2), Italy (IT C2) and Ukraine (UKR C1),
102 were used and multiplied on the sugar beet cultivar Ardan (Fournet et al., 2018). Used cysts
103 were sized from 400 to 450 µm using sieves with the according meshes.

104 For *Globodera pallida*, five populations originated from Peru and members of the
105 genetic clades described by Picard et al. (2007) [clade I (P299 – Amantani 2), clade II (P240 –
106 Cusco 2), clade III (P212 – Andahuaylas 4), clade IV (P323 – Huancavelica) and clade V (P115
107 – Cajamarca)], one population from Chile (C3344) and five European populations from France
108 (Noirmoutier), Germany (Kalle), The Netherlands (Rookmaker), Scotland (Grown East Graigs)

109 and Switzerland (Chavornay) were used (Table 1). All populations were multiplied on the
110 potato cultivar Désirée. Used cysts were sized from 400 to 500 µm, except for C3344 and
111 Rookmaker with a diameter from 250 to 500 µm.

112 For *Heterodera carotae*, eight populations (Table 1) from Europe and members of the
113 genetic clusters described by Gautier et al. (2019), *i.e.* cluster 1 (0101 – FU – 1303 – ZAP) and
114 cluster 2 (2902 – Cre7 – 3001 – Hca02), were used and multiplied on the cultivar “Carottes
115 nantaises”. Used cysts were sized from 300 to 400 µm.

116 Cysts were stored at 5°C in moistened sand for *H. schachtii* and *H. carotae* and at 5°C
117 in a dry place for *G. pallida*.

118

119 2.2 Production of root exudates

120 For the three nematode species, plant root exudates were chosen according to Ngala et al. (2021)
121 and produced at the most efficient time (Table 2).

122 Potato root exudates were produced using 40 pregerminated tubers of potato cv Désirée
123 and put on a grid into a can with 10 L of tap water. In total, 400 tubers were used and three
124 weeks after an incubation in the dark at 20°C, root exudates were collected (*i.e.* all the solution
125 in the can), filtered at 0.20 µm and stored at -20°C.

126 For the production of root exudates from tomato cv Saint Pierre, broccoli cv Early
127 Purple Sprouting, sugar beet cv Acacia and brown mustard cv Aurea, two-and-a-half-weeks
128 after seedling in 65:20:15 Irish peat/black peat/perlite in a greenhouse at 21/17°C day/night
129 conditions with 16h photoperiod, seedlings were planted in a pot (1L) and grown in 54:40:6
130 Irish peat/sand/clay in the same greenhouse. Depending on plant species, root exudates were
131 produced at different times (Table 2) [as their activity is known to be influenced by the age of](#)
132 [plants in many species \(Masler & Perry, 2018\). Hence and to avoid any plant age effect in](#)

133 hatching assays, all leachates produced at different times and from all pots were pooled together
134 and stored at -20°C. For the production, each pot was saturated and was leached twice with 100
135 mL of tap water, 30 minutes apart. Then, the leachate was filtered at 0.14 µm.

136 Carrot root exudates were produced from carrot cv Touchon and cv Pusa kesar, two
137 weeks after seedling in Potgrond H 90 from Klasmann Deilmann in a climatic chamber at
138 20/18°C day/night and 85 to 90% of hygrometry conditions with 16h photoperiod, three
139 seedlings were planted in a pot (2L) and grown in Potgrond H 90 from Klasmann Kdeilmann
140 with sand in the same climatic chamber but at 70 to 80% of hygrometry. Root exudates were
141 produced during three weeks (at weeks 4, 5 and 6 after transplanting). For this, each pot was
142 saturated and was leached twice with 200 mL of borehole water, 30 minutes apart. Then, the
143 leachate was filtered at 1.6 µm. The leachate from all pots of the same species was pooled and
144 stored at -20°C.

145

146 2.3 Hatching assays

147 The hatching assays were conducted in a climatic chamber, in the dark, at 18°C for *Globodera*
148 *pallida* and *Heterodera carotae* and at 23°C for *Heterodera schachtii*. Root exudates from
149 potato, tomato and broccoli were tested on *G. pallida* populations, sugar beet, brown mustard
150 and broccoli on *H. schachtii* populations and the two carrots on *H. carotae* populations (see
151 Ngala et al., 2021). For this, 12-well plates (Costar®) with flat-bottomed wells, which contained
152 a sieve with 170 µm pores, were used. Each sample of root exudates was carbon dosed using a
153 FLASH 2000 CHNS/O Analysers (Thermo Scientific™) and standardised to 30 mg of carbon
154 per g of dry matter with autoclaved permuted water. Three cysts of each population for each
155 nematode species with 1.5 mL of root exudates were put per sieve and six replicates were
156 realized per population and root exudates. The number of hatched J2s was counted at days 2, 4,
157 10, 15, 30, 45 and 60 for *G. pallida* and *H. carotae* and at days 1, 2, 4, 10, 15 and 30 for *H.*

158 *schachtii* after the beginning of assays and at each count, root exudates were replaced with fresh
159 root exudates. At the end of the hatching experiment, cysts were crushed and the number of
160 unhatched viable J2s was counted, in order to calculate the hatching rate.

161

162 2.4 Data analysis

163 All statistical analyses were performed using the R software version 3.6.2 (R Development
164 Core Team, 2019). The hatching rate was calculated using the cumulative number of hatched
165 juveniles at the end of the experiment, but in order to consider the hatching dynamic in its
166 entirety, the Area Under the Hatching Curve (AUHC, see Fournet et al., 2018) was also
167 calculated for each replicate. The efficiency of root exudates on the hatching rate at the end of
168 the experiment and on the hatching dynamic (AUHC) for each cyst nematode species were
169 analysed by using generalized linear models (GLM) with binomial error and a logit link
170 function. A likelihood-ratio test was performed on the models as a type II analysis of variance
171 to test the significance of each term in the model and following by pairwise comparisons based
172 on estimate marginal means (“emmeans” and “CLD” functions, package “emmeans”) with the
173 false discovery rate (FDR) correction for p-values.

174

175 3. RESULTS

176 3.1 Influence of genetic diversity on the hatching rate

177 3.1.1 *Heterodera schachtii*

178 For the three root exudates (Fig. 1), there was a significant population effect with some
179 populations hatching better than others ($\chi^2=223.8$, $df=5$ for sugar beet cv Acacia; $\chi^2=720.2$,
180 $df=5$ for broccoli cv Early Purple Sprouting and $\chi^2=852.4$, $df=5$ for brown mustard cv Aurea,
181 and in all cases $P<0.0001$). However, and despite these significant effects, final hatching rates

182 were very high for all populations (superior to 85 %) except for the Spanish one (ESP C2) with
183 the brown mustard root exudate (Fig. 1).

184

185 3.1.2 *Globodera pallida*

186 For both tomato cv Saint Pierre and potato cv Désirée root exudates (Fig. 2) there was a
187 population effect on the final hatching rate ($\chi^2=945.2$, $df=10$ and $\chi^2=2361.0$, $df=10$
188 respectively, $P<0.0001$). Despite these significant effects, final hatching rates were very high
189 for all populations: between 90.4 and 99.6 % with tomato and between 85.6 and 99.5 % with
190 potato.

191 For the broccoli root exudate (Fig. 2), there was a strong population effect ($\chi^2=6058.8$,
192 $df=10$ and $P<0.0001$): all European populations were characterised by a medium final hatching
193 rate, between 53.1 to 77.4 %, while the French population and south American populations
194 were all characterised by a low final hatching rate, between 1.0 to 21.2 %.

195

196 3.1.3 *Heterodera carotae*

197 For both root exudates (Fig. 3), there was a population effect ($\chi^2=421.2$, $df=7$ and $\chi^2=760.6$,
198 $df=7$ for cv Touchon and cv Pusa Kesar respectively, in both case $P<0.0001$). However, and
199 despite these significant effects, all final hatching rates were very high and ranged from 67.9 to
200 96.7 %.

201

202 3.2 Influence of genetic diversity on the hatching dynamic

203 3.2.1 *Heterodera schachtii*

204 For the three tested root exudates, all populations exhibited the same hatching dynamic (*i.e.* for
205 a given root exudate, all population hatched at the same speed): $\chi^2=2.4$, $df=5$ and $P=0.790$ for
206 sugar beet cv Acacia; $\chi^2=3.1$, $df=5$ and $P=0.691$ for broccoli cv Early Purple Sprouting and
207 $\chi^2=4.6$, $df=5$ and $P=0.468$ for brown mustard cv Aurea, respectively (Table 3).

208

209 3.2.2 *Globodera pallida*

210 For the tomato root exudate (Table 4), despite a significant population effect on AUHC
211 ($\chi^2=34.5$, $df=10$ and $P=0.0001$), all populations except the population P115 from Peru exhibited
212 very close hatching dynamics.

213 For the potato root exudate (Table 4), there was a strong population effect on AUHC
214 ($\chi^2=133.7$, $df=10$ and $P<0.0001$) that revealed a strong difference in hatching dynamics
215 between European populations and the Chilean population that hatched primarily faster than
216 Peruvian populations.

217 For the broccoli root exudate (Table 4), the same pattern was observed with a strong
218 population effect ($\chi^2=1112.9$, $df=10$ and $P<0.0001$) associated to a strong difference in hatching
219 dynamic between some European populations that hatched faster and higher than all the
220 Peruvian populations, the population from Chile and the French population from Noirmoutier.

221

222 3.2.3 *Heterodera carotae*

223 The analysis of variance indicated some significant differences among populations for the
224 dynamic of hatching (AUHC) either for carrot cv Touchon ($\chi^2=54.0$, $df=7$ and $P<0.0001$) or
225 for carrot cv Pusa Kesar ($\chi^2=68.9$, $df=7$ and $P<0.0001$) (Table 5). However, these significant
226 differences were not due to the cluster membership.

227

228 4. DISCUSSION

229 The aim of this study was to test the efficiency of root exudates, expected to be used to stimulate
230 suicide hatching, against population diversity of three cyst nematode species. Here, we showed,
231 as expected, that root exudates from good host plants strongly stimulate the hatching with only
232 few differences between populations. However, we confirmed the surprising non-host plant
233 hatching stimulation recently shown for *G. pallida* by Ngala et al. (2021). We also highlighted
234 that such hatching stimulation is associated with a very contrasted behaviour between south
235 American and European populations.

236 For cyst nematodes, the hatching is the first essential step of their life cycle and is
237 conditioned, mostly, by the presence of the host plant. Among root exudates, three categories
238 of chemicals, which play a role in the hatching, have been highlighted: i) the hatching factors
239 (HF) such as glycinoclepin A which induce hatch of *Heterodera glycines* (Masamune et al.,
240 1982) or solanoclepin A for *G. rostochiensis* and *G. pallida* (Schenk et al., 1999; Tanino et
241 al., 2011), ii) hatching inhibitors (HI) which are produced before the HF during the root
242 development and iii) hatching stimulants (HS) for potentiating the impact of HF (Perry et al.,
243 2018). Some species, such as *Heterodera schachtii*, can hatch with a wide range of host as well
244 as in water unlike *Globodera* species that have a restricted host range to *Solanaceae* family
245 members and almost depend on host root exudates to hatch. As expected, *H. schachtii*, exhibited
246 a high hatching rate with the three root exudates tested (sugar beet, broccoli and brown mustard)
247 as the hatching in this species is more governed by specific climatic conditions (temperature,
248 humidity) than by root exudates (Fournet et al., 2018).

249 For *Globodera pallida*, root exudates from tomato and potato induce a very high
250 hatching for all populations with a hatching rate higher than 80%. However, Peruvian

251 populations exhibit a different hatching dynamic: they hatched slowly and at a lower final
252 hatching rate. This might be the result of the hatching assay temperature. Indeed, it was fixed
253 at 18°C while the temperature range for this species varied between 13 to 25 °C (Kaczmarek et
254 al., 2014) but with no certainty that 18°C correspond to the hatching optimum of each Peruvian
255 populations. Another hypothesis might be that European populations may be better adapted (*i.e.*
256 locally adapted) to European cultivars root exudates than Peruvian ones, resulting in the
257 observed differences in their respective final hatching rates.

258 As mentioned before, our most surprising result is obtained with broccoli root exudates.
259 Unexpectedly, broccoli being a non-host plant of *Globodera* species which belongs to the
260 Brassicaceae family, cv Early Purple Sprouting induced hatching, but only for European *G.*
261 *pallida* populations (except for the French population from Noirmoutier). There is a consensus
262 that all European populations originated from the south of Peru and were probably introduced
263 several times in Europe (Grenier et al., 2010; Hockland et al., 2012; Plantard et al., 2008) from
264 the same genetic pool. As a result, we could have expected an identical and positive response
265 of both Peruvian and European populations to broccoli root exudates. However, the *G. pallida*
266 clade I (in the south Peru) is the most genetically diverse one (Picard et al., 2007) and to explain
267 this contrasted behaviour, the most parsimonious hypothesis is that this clade encompasses both
268 populations receptive or not to Brassicaceae exudates. Thus, bottlenecks associated with
269 introduction events (stochastic) and environmental factors (deterministic) may have selected
270 the individuals that are receptive to broccoli root exudates in Europe. To test this hypothesis,
271 several cysts from different populations from the clade I have to be confronted to these broccoli
272 root exudates to highlight the presence of both behaviours at the population level. The
273 behaviour of the French population from Noirmoutier may be considered as a particular case,
274 possibly resulting either in a distinct introduction event from clade I (Plantard et al., 2008) or
275 in a specific and local evolutionary history. In addition to stimulating the hatching of potato

276 cyst nematodes, cruciferous species may also present other advantages. Indeed, several authors
277 (Aires et al., 2009; Lord et al., 2011; Ngala et al., 2015) suggested using brassica green manures
278 to control potato cyst nematodes as biofumigation practices. Brassicas contain glycosinolates,
279 which when hydrolysed by the myrosinase enzyme release several compounds including
280 nematicidal compounds such as isothiocyanates (Bones & Rossiter, 2006; Fahey et al., 2001).
281 Obviously, this surprising result will have to be confirmed, with other broccoli cultivars and
282 Brassicaceae species. First, it would be interesting to check if the same hatching results are
283 obtained using exudates from a wide collection of Brassicaceae species. Second, metabolomic
284 studies would identify hatching factors involved in this process (already described or new ones).

285 For *Heterodera carotae*, both root exudates ensure high hatching rate, between 68 to
286 97%, for all populations and there is also no effect of the genetic clusters highlighted by Gautier
287 et al. (2019). This is in line with the restricted host range of this species on plants belonging to
288 the *Daucus* and *Torilis* genera (Mugniery & Bossis, 1988). In addition, both carrots, cv Touchon
289 which is an European cultivar and cv Pusa Kesar, an Asian one (India), belonged to two
290 different genetic clusters (Baranski et al., 2012). In fact, cultivated carrots are separated into
291 two genetic groups, the Eastern group with accessions from the Middle East and Asia and the
292 Western one including accessions from Europe and America (Baranski et al., 2012; Grzebelus
293 et al., 2014; Iorizzo et al., 2013). This thus suggests that the hatching of *H. carotae* may be
294 independent of the genetic origin of carrots.

295 Ensuring the durability of control strategies is of a general and central interest in
296 agriculture, to prevent farmers from poor yield and strong economic losses, especially for new
297 biocontrol products for which there are few studies on the adaptive potential of pathogens.
298 Despite the observed significant differences among populations which may be related to local
299 adaptive process, host plant root exudates provide a strong percentage of hatching for the three
300 species. This means that, independently of any factor that may have a negative effect (*i.e.*

301 climate conditions that can affect hatching susceptibility...), biocontrol products based on the
302 use of host plant root exudates may be strongly efficient worldwide. Subject to confirmation,
303 this should not be the case for a non-host plant root exudate such as the broccoli for *G. pallida*:
304 the efficiency of the product would be strongly structured in space, according to the
305 evolutionary history of the nematode, leading to more or less predictable outcomes.

306

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311

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317

318 **AUTHORS' CONTRIBUTIONS**

319 CG and CP conducted the experiments according to a protocol elaborated by CG, JM, JCY,
320 ENO and SF. LR, CP and SF multiplied nematode populations used in this study. CG, JM and
321 SF analysed the data, realized the figures and wrote the text. All authors edited the paper and
322 approved the current version.

323

324 **CONFLICT OF INTEREST/COMPETING INTERESTS**

325 The authors declare that they have no conflict of interest.

326

327 **ETHICS APPROVAL**

328 The authors bear all the ethical responsibilities of this manuscript.

329

330 **CONSENT FOR PUBLICATION**

331 All authors have consented for this research to be published.

332

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433

434 **FIGURE LEGENDS**

435 **Fig. 1.** Mean of hatching percentage (n = 6) of *Heterodera schachtii* juveniles (mean values ±
436 SEM) at the end of the experiment (D30) for root exudates from sugar beet cv Acacia, broccoli
437 cv Early Purple Sprouting and brown mustard cv Aurea. Letters represent significant
438 differences between populations per root exudates according to a type II analysis of variance
439 (5% significance level).

440

441 **Fig. 2.** Mean of hatching percentage (n = 6) of *Globodera pallida* juveniles (mean values ±
442 SEM) at the end of the experiment (D60) for root exudates from broccoli cv Early Purple
443 Sprouting, potato cv Désirée and tomato cv Saint Pierre. The clade membership and
444 geographical location are indicated in the x-axis. Letters represent significant differences
445 between populations per root exudates according to a type II analysis of variance (5%
446 significance level).

447

448 **Fig. 3.** Mean of hatching percentage (n = 6) of *Heterodera carotae* juveniles (mean values ±
449 SEM) at the end of the experiment (D60) for root exudates from carrot cv Pusa Kesar and carrot
450 cv Touchon. The clade membership is indicated in the x-axis. Letters represent significant
451 differences between populations per root exudates according to a type II analysis of variance
452 (5% significance level).

453 **Table 1:** Nematode species, population code, sampling site and genetic clade membership.

Nematode species	Population code	Country	Genetic membership
<i>Heterodera schachtii</i>	AM2	France	-
	ESP C2	Spain	-
	UKR C1	Ukraine	-
	GER C2	Germany	-
	IT C2	Italy	-
	MAR C1	Morocco	-
<i>Heterodera carotae</i>	0101	France, Ain	Cluster 1
	FU	Switzerland	Cluster 1
	1303	France, Bouches du Rhône	Cluster 1
	ZAP	Italy	Cluster 1
	2902	France, Finistère	Cluster 2
	Cre7	France, Manche	Cluster 2
	3001	France, Gard	Cluster 2
	Hca02	France, Aisne	Cluster 2
<i>Globodera pallida</i>	P299	Peru, Amantani	Clade I
	P240	Peru, Cusco	Clade II
	P212	Peru, Andahuaylas	Clade III
	P323	Peru, Huancavelica	Clade IV
	P115	Peru, Cajamarca	Clade V
	C3344	Chile	-
	Noirmoutier	France	Clade I
	Kalle	Germany	Clade I
	Rookmaker	The Netherlands	Clade I
	Grown East Graigs	Scotland	Clade I
	Chavornay	Switzerland	Clade I

454

455 **Table 2:** Plant species, cultivar, weeks of production of root exudates, number of replicates
 456 used per plant species and nematode species associated for the hatching assay.

Plant species	Cultivar	Weeks of root exudate productions (after transplanting seedlings)	Number of replicates	Tested against nematode species
Tuber potato	Désirée	3	400	<i>Globodera pallida</i>
Tomato	Saint Pierre	2/3/4	120	<i>Globodera pallida</i>
Broccoli	Early Purple Sprouting	4/5	200	<i>Globodera pallida</i> <i>Heterodera schachtii</i>
Sugar beet	Acacia	4/5/6	100	<i>Heterodera schachtii</i>
Brown mustard	Aurea	4/5/6	120	<i>Heterodera schachtii</i>
Carrot	Touchon	4/5/6	78	<i>Heterodera carotae</i>
Carrot	Pusa kesar	4/5/6	78	<i>Heterodera carotae</i>

457

458 **Table 3:** Result of the Area Under the Hatching Curve (AUHC) (\pm Standard Errors of Mean)
 459 for *Heterodera schachtii* (n = 6) according to root exudates from sugar beet cv Acacia, broccoli
 460 cv Early Purple Sprouting and brown mustard cv Aurea. Letters represent significant
 461 differences between populations according to a type II analysis of variance (5% significance
 462 level).

Nematode species	Population code	Root exudates								
		Sugar beet (cv Acacia)			Broccoli (cv Early Purple Sprouting)			Brown mustard (cv Aurea)		
		AUHC	SEM		AUHC	SEM		AUHC	SEM	
<i>Heterodera schachtii</i>	AM2	27.56	0.33	a	27.49	0.13	a	27.24	0.28	a
	ESP C2	25.38	0.77	a	24.60	0.93	a	22.73	1.43	a
	UKR C1	25.14	0.78	a	27.88	0.27	a	27.07	0.48	a
	GER C2	26.63	0.16	a	27.11	0.20	a	26.68	0.49	a
	IT C2	25.01	0.81	a	25.07	0.61	a	26.26	0.55	a
	MAR C1	24.24	0.75	a	27.30	0.36	a	25.86	0.96	a

464 **Table 4:** Result of the Area Under the Hatching Curve (AUHC) (\pm Standard Errors of Mean)
 465 for *Globodera pallida* (n = 6) according to root exudates from tomato cv Saint Pierre, potato cv
 466 Désirée and broccoli cv Early Purple Sprouting. Letters represent significant differences
 467 between populations according to a type II analysis of variance (5% significance level).

Nematode species	Population code	Root exudates								
		Tomato (cv Saint Pierre)			Potato (cv Désirée)			Broccoli (cv Early Purple Sprouting)		
		AUHC	SEM		AUHC	SEM		AUHC	SEM	
<i>Globodera pallida</i>	CI I - P299	47.62	1.24	ab	37.01	2.11	c	3.68	2.51	de
	CI II - P240	50.59	1.13	ab	44.95	1.97	bc	9.06	1.84	c
	CI III - P212	47.90	2.59	ab	40.77	1.15	c	2.34	1.32	ef
	CI IV - P323	48.62	1.30	ab	40.78	2.34	c	2.93	0.56	def
	CI V - P115	43.25	1.85	b	36.00	2.43	c	0.47	0.25	f
	Chile	53.62	0.36	a	53.08	0.33	ab	5.04	1.32	cde
	France	52.78	0.21	a	50.84	0.36	ab	6.69	1.49	cd
	Germany	55.73	0.38	a	55.96	0.61	a	37.59	1.52	a
	The Netherlands	53.81	0.51	a	54.02	0.17	ab	26.13	1.60	b
	Scotland	53.36	0.65	a	51.36	1.49	ab	37.00	1.72	a
Switzerland	54.10	0.30	a	54.04	0.24	ab	33.56	3.35	ab	

468

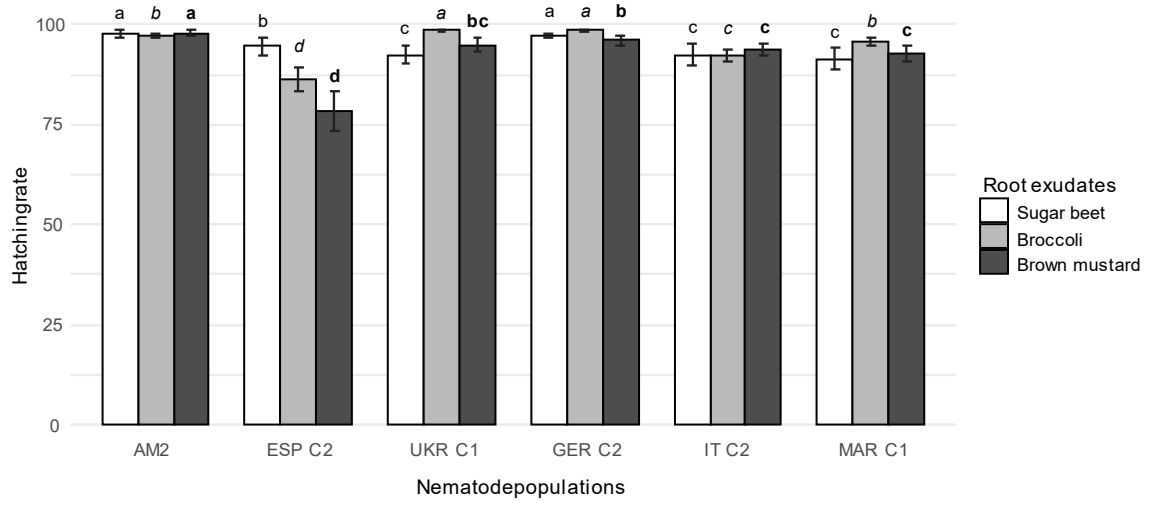
469

470 **Table 5:** Result of the Area Under the Hatching Curve (AUHC) (\pm Standard Errors of Mean)
 471 for *Heterodera carotae* (n = 6) according to root exudates from carrot cv Touchon and carrot
 472 cv Pusa Kesar. Letters represent significant differences between populations according to a type
 473 II analysis of variance (5% significance level).

474

Nematode species	Population code	Root exudates					
		Carrot (cv Touchon)			Carrot (cv Pusa kesar)		
		AUHC	SEM		AUHC	SEM	
<i>Heterodera carotae</i>	0101	34.94	1.68	a	40.08	0.86	a
	FU	29.88	2.59	abc	30.38	2.05	bcd
	1303	31.04	1.32	ab	38.69	0.66	ab
	ZAP	22.57	1.74	c	27.97	1.84	cd
	2902	30.79	1.21	ab	35.52	0.78	abc
	Cre7	38.20	1.22	a	42.34	1.32	a
	3001	33.56	0.99	a	38.93	1.21	a
	Hca02	23.98	2.02	bc	26.36	1.54	d

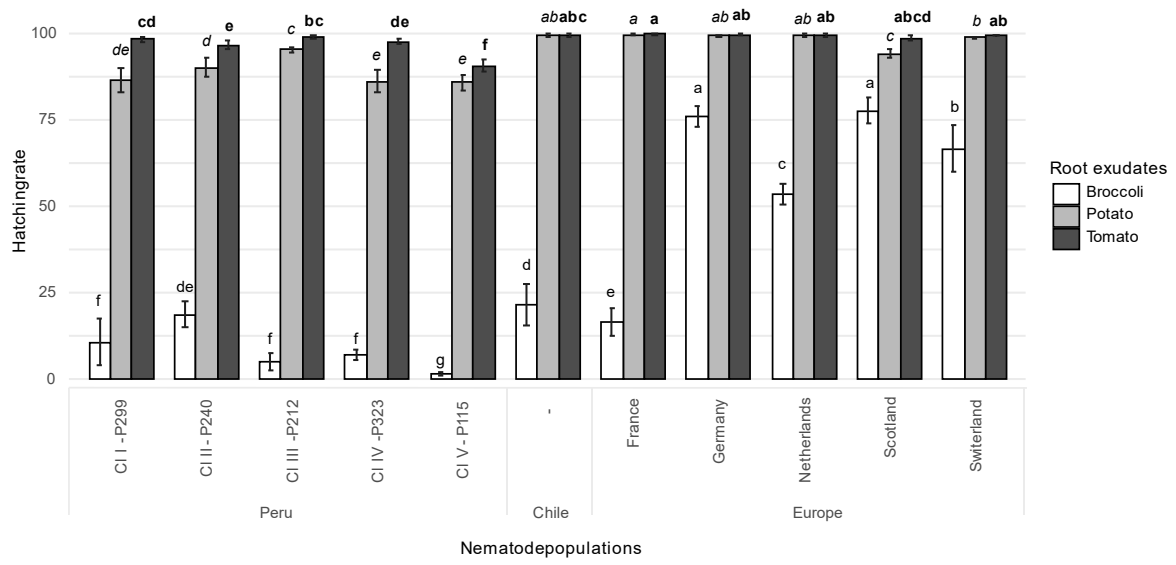
475 **Figure 1**



476

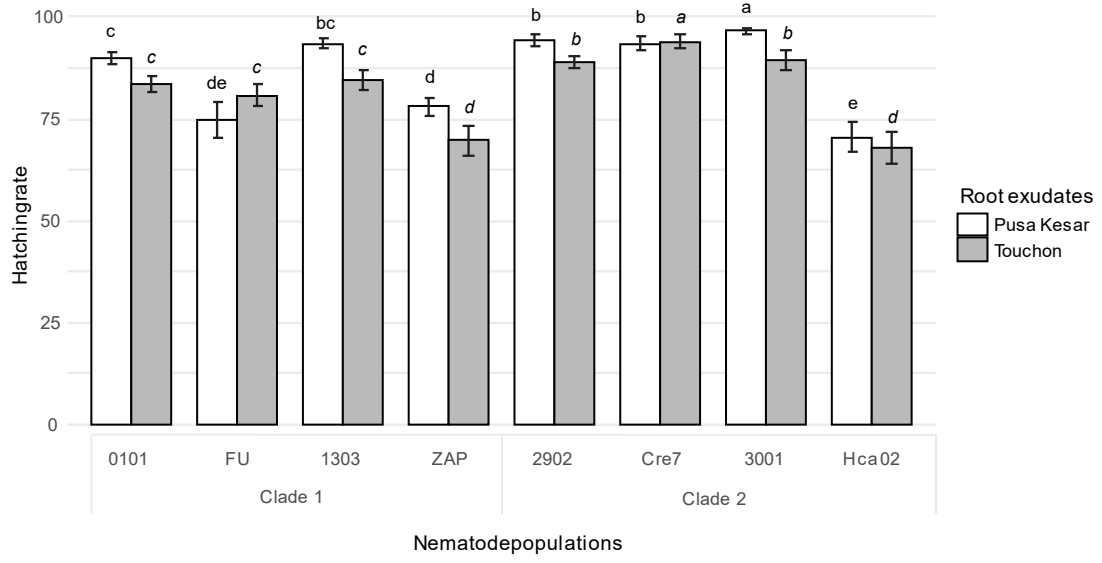
477

478 **Figure 2**



479

480 **Figure 3**



481