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SUMMARY

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

Keywords: Biocontrol - *Globodera pallida – Heterodera schachtii – Heterodera cartoae –* Suicide hatching

1. INTRODUCTION

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

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

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

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

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

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

2. MATERIALS AND METHODS

2.1 Nematode populations

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

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

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

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

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

2.2 Production of root exudates

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

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

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

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

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

2.3 Hatching assays

The hatching assays were conducted in a climatic chamber, in the dark, at 18°C for *Globodera pallida* and *Heterodera carotae* and at 23°C for *Heterodera schachtii*. Root exudates from potato, tomato and broccoli were tested on *G. pallida* populations, sugar beet, brown mustard and broccoli on *H. schachtii* populations and the two carrots on *H. carotae* populations (see Ngala et al., 2021). For this, 12-well plates (Costar®) with flat-bottomed wells, which contained a sieve with 170 µm pores, were used. Each sample of root exudates was carbon dosed using a FLASH 2000 CHNS/O Analysers (Thermo Scientific™) and standardised to 30 mg of carbon per g of dry matter with autoclaved permuted water. Three cysts of each population for each nematode species with 1.5 mL of root exudates were put per sieve and six replicates were realized per population and root exudates. The number of hatched J2s was counted at days 2, 4, 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 (χ^2=223.8, df=5 for sugar beet cv Acacia; χ^2=720.2,
180 df=5 for broccoli cv Early Purple Sprouting and χ^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

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

3.1.2 Globodera pallida

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

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

3.1.3 Heterodera carotae

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

3.2 Influence of genetic diversity on the hatching dynamic

3.2.1 Heterodera schachtii

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

3.2.2 Globodera pallida

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

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

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

3.2.3 Heterodera carotae

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

4. DISCUSSION

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

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

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

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

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

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

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

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

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

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AUTHORS' CONTRIBUTIONS

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

CONFLICT OF INTEREST/COMPETING INTERESTS

The authors declare that they have no conflict of interest.

ETHICS APPROVAL

The authors bear all the ethical responsibilities of this manuscript.

CONSENT FOR PUBLICATION

All authors have consented for this research to be published.

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FIGURE LEGENDS

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

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

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

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

Nematode species	Population code	Country	Genetic membership
Heterodera schachtii	AM2	France	-
	ESP C2	Spain	-
	UKR C1	Ukraine	-
	GER C2	Germany	-
	IT C2	Italy	-
	MAR C1	Morocco	-
Heterodera carotae	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
Globodera pallida	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	*Globodera pallida*
Tomato	Saint Pierre	2/3/4	120	*Globodera pallida*
Broccoli	Early Purple Sprouting	4/5	200	*Globodera pallida* *Heterodera schachtii*
Sugar beet	Acacia	4/5/6	100	*Heterodera schachtii*
Brown mustard	Aurea	4/5/6	120	*Heterodera schachtii*
Carrot	Touchon	4/5/6	78	*Heterodera carotae*
Carrot	Pusa kesar	4/5/6	78	*Heterodera carotae*

457

458 **Table 3:** Result of the Area Under the Hatching Curve (AUHC) (± 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	
Heterodera schachtii	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) (± 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)			Root exudates: Potato (cv Désirée)			Root exudates: Broccoli (cv Early Purple Sprouting)		
		AUHC	SEM		AUHC	SEM		AUHC	SEM	
Globodera pallida	Cl I - P299	47.62	1.24	ab	37.01	2.11	c	3.68	2.51	de
	Cl II - P240	50.59	1.13	ab	44.95	1.97	bc	9.06	1.84	c
	Cl III - P212	47.90	2.59	ab	40.77	1.15	c	2.34	1.32	ef
	Cl IV - P323	48.62	1.30	ab	40.78	2.34	c	2.93	0.56	def
	Cl 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

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

Nematode species	Population code	Root exudates					
		Carrot (cv Touchon)			Carrot (cv Pusa kesar)		
		AUHC	SEM		AUHC	SEM	
Heterodera carotae	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**

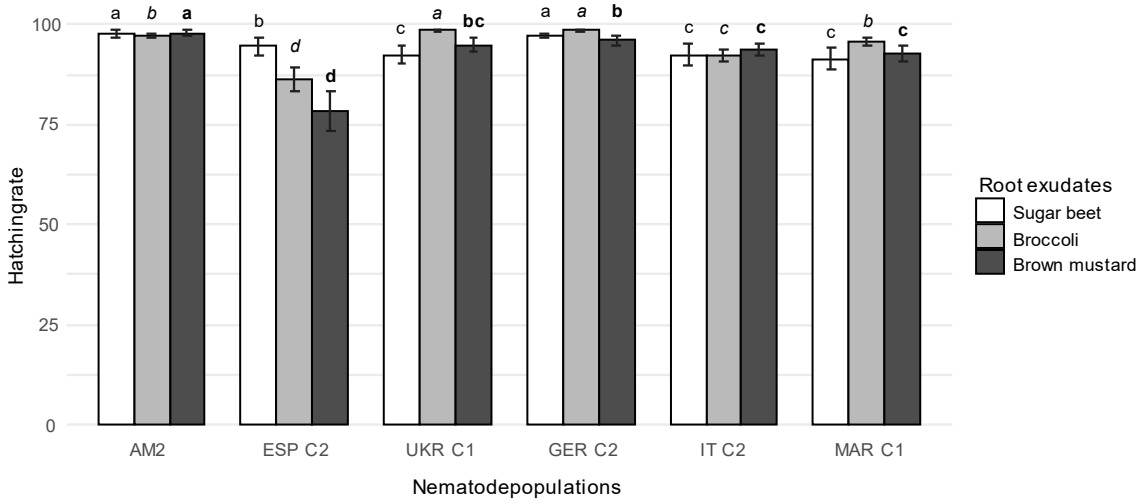

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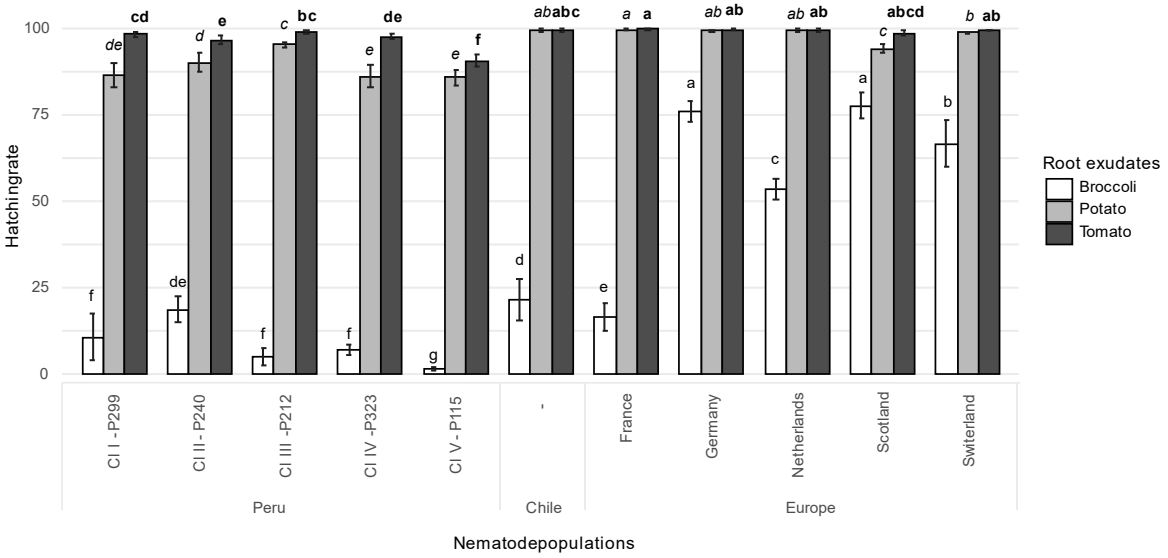
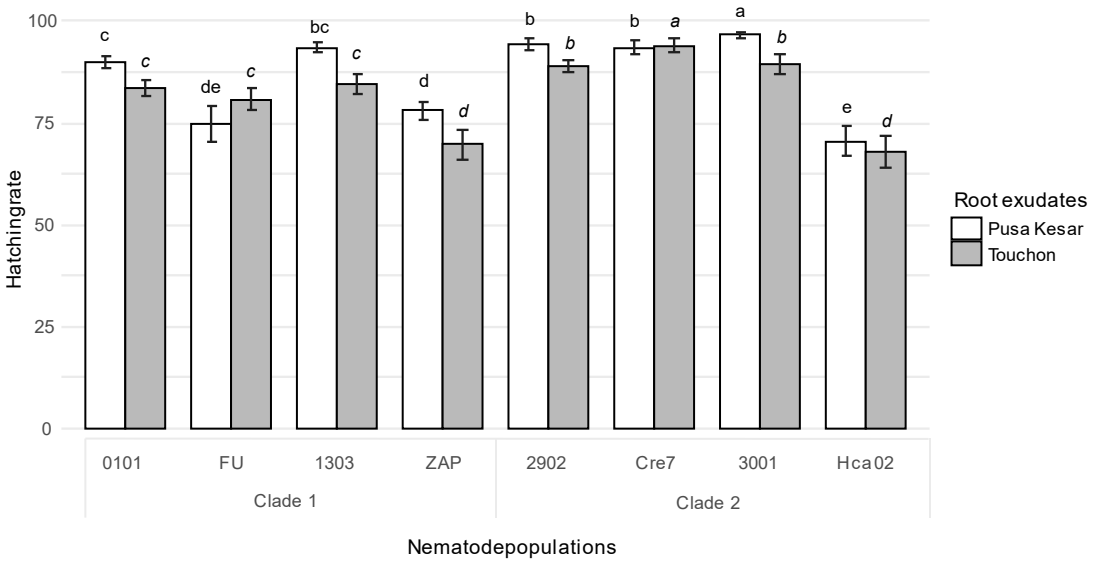
Figure 2


Hatching rate
100
75
50
25
0
Root exudates
Broccoli
Potato
Tomato
CI I - P299
CI II - P240
CI III - P212
CI IV - P323
CI V - P115
-
France
Germany
Netherlands
Scotland
Switerland
Peru
Chile
Europe
Nematodepopulations

480 **Figure 3**



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