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1 **Reproductive efficiency of entomopathogenic nematodes as scavengers. Are they able to**
2 **fight for insect's cadavers?**
3
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13 **Abstract**

14

15 Entomopathogenic nematodes (EPNs) and their bacterial partners are well-studied insect
16 pathogens, and their persistence in soils is one of the key parameters for successful use as
17 biological control agents in agroecosystems. Free-living bacteriophagous nematodes (FLBNs) in
18 the genus *Oscheius*, often found in soils, can interfere in EPN reproduction when exposed to live
19 insect larvae. Both groups of nematodes can act as facultative scavengers as a survival strategy.
20 Our hypothesis was that EPNs will reproduce in insect cadavers under FLBN presence, but their
21 reproductive capacity will be severely limited when competing with other scavengers for the same
22 niche. We explored the outcome of EPN - *Oscheius* interaction by using freeze-killed larvae of
23 *Galleria mellonella*. The differential reproduction ability of two EPN species (*Steinernema*
24 *kraussei* and *Heterorhabditis megidis*), single applied or combined with two FLBNs (*Oscheius*
25 *onirici* or *Oscheius tipulae*), was evaluated under two different infective juvenile (IJ) pressure:
26 low (3 IJs/host) and high (20 IJs/host). EPNs were able to reproduce in insect cadavers even in
27 the presence of potential scavenger competitors, although EPN progeny was lower than that
28 recorded in live larvae. Hence, when a highly susceptible host is available, exploiting cadavers
29 by EPN might limit the adaptive advantage conferred by the bacteria partner, and might result in
30 an important trade-off on long-term persistence. Contrary to our hypothesis, for most of the
31 combinations, there were not evidences of competitive relationship between both groups of
32 nematodes in freeze-killed larvae, probably because their interactions are subject to interference
33 by the microbial growth inside the dead host. Indeed, evidences of possible beneficial effect of
34 FLBN presence were observed in certain EPN-FLBN treatments compared with single EPN
35 exposure, highlighting the species-specific and context dependency of these multitrophic
36 interactions occurring in the soil.

37

38 Key words: *Heterorhabditis megidis;* multitrophic interactions; *Oscheius onirici; Oscheius*
39 *tipulae;* scavenging; *Steinernema kraussei*

40 **1. Introduction**

41

42 Entomopathogenic nematodes (EPNs) are well-studied insect pathogens (Stock, 2015) and
43 important agents for the biological control of soil insect pests in agroecosystems (Denno et al.,
44 2008). This group of nematodes traditionally includes two phylogenetically distant families,
45 Steinernematidae and Heterorhabditidae (Blaxter et al., 1998), which share similarities in their
46 life cycles and behaviour as the result of convergent evolution (Poinar, 1993). For both families,
47 one stage of life cycle comprises a free-living stage called infective juvenile (IJ), which can
48 survive in the soil until it locates, penetrates and rapidly kills the host (48-72 hours) with the aid
49 of obligate bacterial partners, transmitted from one generation to another (Dillman et al., 2012).
50 A better understanding of EPN soil food web dynamics, particularly antagonistic
51 interactions, is critical to achieving a long-term EPN persistence in crops. The soil is a complex,
52 species-rich environment and thus, various organisms have the potential to influence the survival
53 and reproduction of EPNs. The survival of both naturally occurring IJs and those augmented for
54 biocontrol action, is affected by biotic as much as abiotic factors (Ishibashi & Kondo, 1987;
55 Griffin, 2015; Lewis et al., 2015). Moreover, introduced EPNs may alter the naturally occurring
56 microbiota (nematode fauna included) in the soil (Duncan et al., 2007; Ishibashi & Kondo, 1986;
57 Lewis et al., 2015). Nevertheless, as with most soil organisms, the natural habitats and behavioural
58 plasticity of EPNs are still poorly known. A better understanding of ecological associations in the
59 soil, such as competitive relationships and mutualism associations, is required to effectively use
60 EPNs as biological control agents in agroecosystems (Stuart et al., 2015).
61 Campos-Herrera et al. (2015a) studied the competition for the insect larva as resource (live
62 host) between two EPN species, *Heterorhabditis megidis* (Rhabditida: Heterorhabditidae) and
63 *Steinernema kraussei* (Rhabditida: Steinernematidae), and two free-living bacteriophagous
64 nematode (FLBN) species in the genus *Oscheius* (Rhabditida: Rhabditidae), *O. onirici* and *O.*
65 *tipulae*. The selection of these nematode species was based on their co-occurrence in field
66 experiments to evaluate the presence and activity of selected members of the nematode food web
67 (Campos-Herrera et al., 2015b). They observed that the interaction between these two different

68 groups of nematodes depended on the number of IJs in the initial inoculum and on the nematode
69 species combination. However, little attention was conferred to the *Oscheius* reproductive success
70 nor the prevalence of larvae allowing single or mixed progeny, to evaluate the full extent of the
71 competition.

72 The two species in the genus *Oscheius* used in previous experiments (Campos-Herrera et
73 al., 2015a) are hermaphroditic and easy to isolate from soil samples (Félix et al., 2001). *Oscheius*
74 *tipulae* was described initially as a saprophagous organism (Lam & Webster, 1971), insofar as
75 they are able to feed on insect cadavers and decaying organic matter. Sudhaus (1993) reported
76 that their use of cadavers can involve necromeny, process that implies to latch onto an insect, wait
77 for its death and then, exploit it to complete the life cycle (Sudhaus and Schulte, 1989). Although
78 Félix et al. (2001) suggested that *O. tipulae* is too common and ubiquitous to be associated with
79 the life cycle of a particular insect, other *Oscheius* spp. were reported to display necrometic
80 associations (Stock et al., 2005). Necromeny might be the intermediate evolutionary stage
81 between parasitism and entomopathogenicity (Dillman et al., 2012). In fact, entomopathogenic
82 behaviour has been ascribed to several species of nematodes in the genus *Oscheius* (Zhang et al.,
83 2008; Ye et al., 2010; Torres-Barragan et al., 2011), *O. onirici* included (Torrini et al. 2015).
84 Because of this possible transitional stage, the degree of entomopathogenic capability might differ
85 among populations of the same species. For example, contrary to the Italian isolate described by
86 Torrini et al. (2015), Swiss isolates did not exhibit entomopathogenic activity, but behaved as
87 facultative kleptoparasites that compete for insect cadavers killed by EPNs (Campos-Herrera et
88 al. 2015a).

89 Both EPN families had been considered as obligate parasites or pathogens of insects
90 (Poinar, 1979), although some evidence was reported early on the use of insect cadavers by EPNs
91 as a source of food and development (Jackson and Moore, 1968; Pye and Burman, 1978). Even
92 if EPNs have never been reported as scavenger organisms in nature, laboratory experiments had
93 shown that EPNs are able to colonise (San Blas et al., 2008) and produce offspring (San Blas &
94 Gowen, 2008; Půža & Mráček, 2010) in freeze-killed insects. San Blas & Gowen (2008) reported
95 evidences of EPN attraction to cadavers in olfactometer assays, and observed that certain species

96 can complete their life cycles when exposed to cadavers up to 240 hours-post-frozen old.
97 However, these studies were performed in absence of other possible competitors of the cadavers
98 as a resource.

99 Depending on the status of the available host (alive or dead), theoretically, the IJs can
100 follow the usual entomopathogenic development (live host) or act as facultative scavengers (dead
101 host). However, still is unknown to which extent each path will influence the net efficiency when
102 more naturalized conditions are considered, such as presence of other scavengers that can compete
103 for the cadaver. Various studies have addressed the EPN-FLBN interaction using live hosts
104 (Duncan et al., 2003; Campos-Herrera et al., 2012, 2015a); however, whether the nature of these
105 interactions could change using freeze-killed larvae instead of live larvae as hosts, and if EPNs
106 could still reproduce in cadavers under scavenger competition by FLBNs remains completely
107 unknown. We speculate that EPNs will be able to reproduce in insect cadavers under FLBN
108 presence, but with certain limitations than when following their entomopathogenic behaviour. In
109 addition, we expect that when EPN presence is restricted (low concentration), the reduction of
110 their efficiency by FLBN-co-occurrence will be magnified, in a species-specific, density-
111 dependent manner. Therefore, the aim of this study was to investigate the efficiency of EPN acting
112 as scavengers in the presence of other possible competitors, and to evaluate how the initial inocula
113 of EPN might contribute to modulate this interaction.

114

115 **2. Material and Methods**

116

117 *2.1. Nematode cultures*

118 The species of EPNs and FLBNs were selected on the basis of previous co-occurrence in
119 field experiments (Campos-Herrera et al., 2015b). In particular, we evaluated two EPN species
120 (*S. kraussei* OS population and *H. megidis* commercial, Andermatt Biocontrol AG), and two
121 FLBN species (*Oscheius onirici* MG-67 and *O. tipulae* MG-68). EPNs were cultured in larvae of
122 *Galleria mellonella* (Lepidoptera: Pyralidae) reared at University of Algarve (Portugal), IJs
123 recovered in tap water upon emergence, stored at 10-12 ºC, and used within 2 weeks of harvest

(Woodring & Kaya, 1988). FLBN species were mass-produced in Petri dishes containing a thin layer of 1.0% nutrient agar (NA, Fluka Analytical, Sigma–Aldrich), for 7–10 days at room temperature (20–22 ºC) in the dark (Campos-Herrera et al., 2015a). For each trial, several plates were rinsed in M9 buffer (Herrmann et al., 2006), producing a suspension of mainly juvenile nematodes with possibly some adults.

2.2. Scavenging behaviour of entomopathogenic nematodes and their competition with Oscheius *spp. for cadavers*

The EPN scavenger activity under FLBN competition was evaluated following the experimental design proposed by Campos-Herrera et al. (2015a), but using freeze-killed *G. mellonella* larvae as hosts. Briefly, we assigned one 24-well plate (Falcon Multiwell, 24 well Polystyrene, Corning Incorporated-Life Sciences, Duham, USA) per each of the 12 treatments considered per trial (Table 1). In each of the 16 wells per treatment, we added 1.0 g of sterile sand (neograd, Migros, Switzerland) and the suspension of nematodes/control adjusted to final volume of 200 µl/well. The concentration of FLBNs was a constant variable (500 nematodes per well, equivalent to 282.5 nematodes/cm^2), whereas the EPN density was adjusted to a low concentration (3 IJs per well each EPN species, hand-picked, equivalent to 1.7 IJs/cm^2) and a high concentration (20 IJs per well each EPN species, equivalent to 11.3 IJs/cm^2). Low numbers of IJs were applied in order to minimize the use of a model insect as *G. mellonella*, especially sensitive to infections by EPNs (Dutky et al., 1962). All the treatments (single application and combination) were inoculated at the same time, followed by the introduction of the freeze-killed host. After 4 days of incubation (21 ºC in the dark) all cadavers were thoroughly washed and placed individually in White traps (White, 1929). Nematode emergence was observed every 2-3 days over a period of 30 days, and final progeny (number of IJs and/or FLBNs, depending on the treatment) was counted 9-10 days after the onset of emergence. Both low and high EPN concentration experiments were performed at 2 different times, with freshly produced nematodes and hosts.

151 *2.3. Statistical analysis*

152 We analysed variables related to the EPN infectivity and reproduction as well as the EPN
153 impact on the FLBN activity. For EPNs, the variables were (i) frequency of larvae producing only
154 IJs (pure EPN emergences), (ii) frequency of larvae producing IJs (even when mixed with FLBN
155 emergences), and (iii) number of IJs produced per larvae. Similarly, for *Oscheius* spp. we
156 evaluated: (iv) frequency of larvae producing only *Oscheius* progeny (pure FLBN emergences),
157 (v) frequency of larvae producing *Oscheius* progeny (even when mixed with EPN emergences),
158 and (vi) number of *Oscheius* produced per larvae. Prior to statistical analysis, all variables
159 expressed as percentage were arcsine transformed, and quantitative variables were log (x + 1)
160 transformed. We confirmed that the data of the independent trials could be pooled by two ways
161 ANOVA, and thereafter, we employed t-student and one-way ANOVA for subsequent analysis
162 (SPSS 21.0, SPSS Statistics, SPSS Inc., Chicago, IL, USA). For each of the variables described
163 above, we consider the following factors: EPN species (*H. megidis, S. kraussei*), FLN species (*O.*
164 *tipulae, O. onirici),* the initial IJ concentration (low with 3 IJs, high with 20 IJs), and the
165 corresponding combinations. All data are presented as mean ± SEM of untransformed values.

166

167 **3. Results**

168

169 *3.1. Scavenging activity of entomopathogenic nematodes*

170 In general, irrespective of the EPN species studied (applied alone or combined with FLBN)
171 or the initial IJ inoculum (3 IJs or 20 IJs), *Oscheius* spp. presence does not affect the frequency
172 of larvae producing IJs as progeny (Fig. 1). The only exception was the combination of 3 IJs - *H.*
173 *megidis* and *O. onirici*, which recorded a significantly higher frequency of larvae producing IJs
174 (0.41 ± 0.09) compared with the single EPN application (0.13 ± 0.09, P = 0.040, Fig. 1A).
175 Differences in the initial IJ inoculum did not affect the larvae producing IJs in *H. megidis*
176 treatments. However, in the case of *S. kraussei*, in the low concentration experiment, the
177 frequency of larvae producing IJs was 0.06 ± 0.04 for all the treatments (EPNs single applied or
178 combined with *Oscheius* spp.), but this frequency increased in the high concentration experiment

179 to 0.25 ± 0.08 for single EPN application (P = 0.039), 0.31 ± 0.08 when combined with *O. onirici*
180 (P = 0.002), and 0.38 ± 0.09 when combined with *O. tipulae* (P = 0.010, Fig. 1B). Similarly, when
181 *H. megidis* and *S. kraussei* were combined, higher frequencies of larvae producing IJs were
182 observed in the high IJ inoculum than in the low concentration experiment, but this increase was
183 only significant (marginally) in the presence of *Oscheius* spp. (P = 0.075 when combined with *O.*
184 *onirici*; P = 0.049 when combined with *O. tipulae*, Fig. 1C). Number of IJs emerged per larva
185 was not affected neither by the presence of *Oscheius* spp. nor by the differences on the initial IJ
186 inoculum (Fig. 2).
187
188 *3.2. Scavenging activity of free-living bacterivorous nematodes*
189 The frequency of larvae producing FLBNs for *Oscheius* spp. single applications was not
190 different of these observed when combined with EPNs, for both low and high initial inoculum
191 (Fig. 3). Overall, differences on the initial IJ inoculum did not affect the frequency of larvae
192 producing FLBNs in pair-treatment comparison; however, some exceptions were observed when
193 *O. onirici* was involved. When the initial inoculum was increased from 3 IJs to 20 IJs, we observed
194 29% reduction in the incidence when combined with *H. megidis* (P = 0.012), and increased it by
195 10% when combined with *S. kraussei* (P = 0.083, Fig. 3A). Similarly, in some cases, the presence
196 of EPNs affected the number of *Oscheius* emerging per larva. Specifically, when *O. onirici* was
197 involved, a statistically significant reduction of FLBN emergence occurred when combined with
198 *H. megidis*. The EPN caused 32% reduction (P = 0.011) at the low concentration, and 44%
199 reduction (P = 0.001) in the high concentration experiment (Fig. 4A). When *O. tipulae* was
200 involved, statistically significant reduction of FLBN emergence only occurred in the high
201 concentration experiment, but for all treatments: 37% off for *H. megidis* (P = 0.001), 31% off for
202 *S. kraussei* (P = 0.001), and 50% off when both EPN species were combined (P = 0.001, Fig. 4B).
203 In pair-treatment comparison of the FLBN production between high and low initial IJ application,
204 the only significant reductions was observed when *Oscheius* spp. was combined with *S. kraussei*:
205 27% reduction in the case of *O. onirici* (P = 0.010) and 31% reduction in the presence of *O.*
206 *tipulae* (P = 0.018, Fig. 4).

207

208 **4. Discussion**

209

210 In agreement with our first hypothesis, EPNs were able to complete their life cycles in
211 insect cadavers even in the presence of potential scavenger competitors such as *Oscheius* spp. In
212 the study by San-Blas & Gowen (2008), EPN species differed in their scavenging ability in old
213 insect cadavers and fresh cadavers (24 h). Heterorhabditids were less successful in completing
214 their life cycles than steinernematids in old cadavers. Both San Blas & Gowen (2008) and Půža
215 & Mráček (2010) reported that IJs emerged from the majority of freshly freeze-killed *G.*
216 *mellonella* larvae, independently of the EPN species. In agreement with those studies, our results
217 did not reflect interspecific differences in the frequency of larvae producing IJs in single EPN
218 applications. However, in our experiments, considerably fewer cadavers supported IJ emergence
219 than the previous studies (San Blas & Gowen, 2008; Půža & Mráček, 2010). Without considering
220 the methodological differences among experiments, these differences are likely due to the reduced
221 starting IJ inocula: 3 IJs per larva (1.7 IJs/cm^2) or 20 IJs per larva (11.3 IJs/cm^2) in our
222 experiments, compared with 100 or 200 IJs per larva (12.6 IJs/cm^2 or higher) the earlier works.
223 Because few insect cadavers produced IJ offspring, our results should be viewed with caution;
224 nevertheless, in contrast to the findings by San Blas & Gowen (2008), the IJ production was, in
225 all cases, higher for *H. megidis* than for *S. kraussei*. The fact that the first generation adults of *H.*
226 *megidis* are hermaphroditic (Forst & Clarke, 2002; Stock, 2015) may help to partially explain its
227 biological advantage when initial IJ inocula were so limited, since *S. kraussei* needs the presence
228 of at least one female and one male to complete its life cycle and produce progeny. Additional
229 studies including more EPN species of both *Heterorhabditis* and *Steinernema* genera in
230 combination of different species of host (San Blas 2012; Půža & Mráček, 2010) are necessary to
231 establish whether there is a common predisposition for scavenging activity in each genus or if it
232 is species-specific and context dependent ecological scenario.

233 Our study revealed how exploiting cadavers by EPN might limit their final progeny,
234 highlighting the context-dependency (initial inoculum, host species) on the critical adaptive

235 advantage conferred by the bacteria partner, and hence, finding in the bacteria dynamic other
236 plausible reasons for these interspecific differences. For example, not all EPN species release
237 their symbiont bacteria within the same period of time after entering the insect´s hemocoel (Lewis
238 et al., 2015). *Steinernema glaseri* releases its symbiotic bacteria *Xenorhabdus poinarii*
239 (Enterobacteriales: Enterobacteriaceae) around 8 hours after entering the host hemocoel, whereas
240 *Heterorhabditis bacteriophora* requires just 30 minutes to release its own bacteria *Photorhabdus*
241 *luminescens* (Enterobacteriales: Enterobacteriaceae) (Wang et al. 1994). Upon release, symbiont
242 bacteria multiply rapidly, killing the host and producing antibiotics with antifungal and
243 antibacterial activities to obtain the ideal conditions for growth and reproduction of their
244 associated EPNs, protecting the specificity of the symbiosis by eliminating microbial competitors
245 (Boemare, 2002). A delay in the release of the symbiont bacteria in cadavers could benefit the
246 growth of the intestinal microflora already present on the dead host, which can be detrimental to
247 the best possible conditions for the establishment and development of the nematode-symbiotic
248 bacterium complex (Kaya, 2002). However, it remains unknown whether the EPNs release their
249 bacteria at the same time when acting as entomopathogens or scavengers. Growth by microbial
250 competitors could explain why, according to our results and supposition, both EPN species were
251 less skilful behaving like scavengers than performing as insect parasites (Campos-Herrera et al.,
252 2015a). Further investigations are required to unravel the extent to which the presence of
253 microbial competitors reduce the EPN progeny when acting as scavengers. Phylogenetic studies
254 support that entomopathogenic activity of *Heterorhabditis* and *Steinernema* nematodes is an
255 adaptation from ancestral trophic behaviour by FLBNs (Blaxter et al., 1998; Poinar, 1993).
256 Moreover, according to the dauer hypothesis, which holds that the similarities in physiology and
257 role of the dauer stage of free-living nematodes with the IJs of parasitic nematodes (Rogers and
258 Sommerville, 1963; Hawdon and Schad, 1991) suggest a pre-adaptation to parasitism (Crook,
259 2014; Hotez et al., 1993). Thus, facultative scavenging by EPNs could simply be a reminiscence
260 of its past as FLBNs. Additional studies are required to evaluate the impact of the hosts with
261 different degree of susceptibility to EPN attack might help understanding these context-dependent
262 scenarios (Půža & Mráček, 2010; San Blas et al., 2012).

263 Contrary to our expectations, the presence of *Oscheius* spp. did not affect much the EPN
264 reproductive ability when acting as scavengers. Perhaps the competitive pressure of exogenous
265 scavengers was lower, to the point of being negligible, compared with that exerted by the
266 endogenous bacterial growth. Indeed, in a few cases we observed an opposite trend to that
267 expected. In the low IJ inoculum experiment, the frequency of larvae producing IJs in the *H.*
268 *megidis* single application treatment was significantly lower than when combined with *Oscheius*
269 spp. Although the application of 3 IJs of amphimictic *S. kraussei* was too low for the successful
270 colonization of the nematode-bacterium complex into the cadaver, increasing to 20 IJs we
271 obtained a similar pattern as observed for 3 IJs-*H. megidis*. It seems plausible that if the symbiont
272 bacteria is able to settle within the insect's cadaver, but in too low amounts to compete against
273 hostile environment, the presence of bacteriophagous nematodes could assist EPN reproduction,
274 simply by feeding on other bacteria. Conversely, when the EPN-symbiont complex is able to
275 establish strongly (regardless whether the insect was killed or not by the EPN), other opportunists
276 such as *Oscheius* spp. did not seem to interfere much with EPN fitness, while their own fitness
277 was impaired. Such mechanisms could explain why FLBN production of *O. onirici* was
278 significantly lower when combined with *H. megidis* than with *S. kraussei*, while *O. tipulae*
279 reproductive success was significantly reduced for all treatments when initial inocula was
280 increased from 3 IJs to 20 IJs. Similar trends were observed when live hosts were exposed to
281 different EPN-*Oscheius* spp. combinations (Campos-Herrera et al., 2015a). Fewer larvae
282 produced FLBN progeny and fewer FLBNs emerged per larva when insects were killed by *H.*
283 *megidis* than by *S. kraussei* or the combination of both EPN species, especially at high inoculum
284 concentration (Supplementary data 1-3), when presumably the EPN-bacterium complex
285 competitive pressure was the highest for FLBNs.

286 Some evidences of competition by FLBNs towards EPNs were observed when live larvae
287 were used as hosts (Campos-Herrera et al., 2015a), but only under low EPN-bacteria complex
288 concentration conditions. In the current and previous studies, both laboratory experiments
289 (Campos-Herrera et al., 2015a) and bait field soil samples (Campos-Herrera et al., 2015b; Jaffuel

290 et al., 2016, 2017), recorded progeny of both heterorhabditids and steinernematids leaving the
291 same cadaver. However, Alatorre-Rosas & Kaya (1990) observed that, even if *Heterorhabditis*
292 and *Steinernema* dual infection occasionally occurred, and development of both EPN species
293 inside the insect cadaver is possible, their progeny eventually died. What may happen inside the
294 insect cadaver is an interspecific competition between the two different EPN species, probably
295 mediated by the symbiotic bacteria (Sicard et al., 2006), which would limit the final IJ production.
296 In general, if two *Steinernema* species co–infect an individual host, one species predominates in
297 the emerging progeny (Koppenhöfer et al., 1995; Půža & Mráček, 2009). Recently, *Steinernema*-
298 males were observed to physically injure and even kill both males and females of other
299 *Steinernema* species when competing for the same host (O'Callaghan et al., 2014; Zenner et al.
300 2014). Campos-Herrera et al. (2015a) expected that the FLBNs would take the advantage of the
301 EPN interspecific competition, which would result in a reduction of the final IJ production.
302 Effectively, the IJ outcome was lower when two EPN species were combined with *Oscheius* spp.
303 than in the treatment with two EPN species applied alone. This trade off could not been confirmed
304 when freeze-killed insect larvae were used as hosts. Production of IJs was also reduced, but only
305 in the high inoculum concentration treatments and too moderate to be significant. The low number
306 of larvae producing EPN offspring in these particular treatments could be insufficient to complete
307 an accurate statistical analysis, but it could also be that the competitive pressure of FLBNs is
308 much lower than that exerted on EPNs by endogenous bacterial growth.

309 Our study illustrates the complexity of the EPN fight for the cadaver under more naturalized
310 conditions. The results indicated that compared with the EPN traditional natural path
311 (entomopathogenic), scavenging activity is less productive in a highly susceptible host scenario.
312 It is plausible that the type of host (susceptible *versus* resistant to EPN attack) modulates this
313 interaction (Půža & Mráček, 2010), and hence, additional studies are recommended. The fight
314 between FLBN and EPN for the cadaver resources depends on species identity, and is modulated
315 by ecological context; for example, a low numbers of IJs were sufficient for *H. megidis* to
316 overcome the competition, whereas *S. kraussei* suffered strong competition even for higher initial
317 IJ inocumum. Also, it is plausible that the type of host (susceptible *versus* resistant to EPN attack)

318 modulates this interaction (San Blas et al., 2012; Půža & Mráček, 2010), and hence, additional
319 studies are recommended. In addition, EPN successful reproduction in the cadaver may
320 sometimes be more a question of bacterial competition than nematode interaction, and in this
321 scenario, the presence of FLBNs might alleviate the unfavourable bacterial conditions. Futures
322 studies might investigate the extent to which these patterns are consistent for species with various
323 life histories traits and behaviours, and particularly whether the presence of FLBN might be
324 beneficial under certain conditions. By addressing various ecological contexts of natural pressure,
325 we can better understand multitrophic interactions affecting EPNs, and we can identify key factors
326 modulating their efficiency and long-term persistence.

327

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338

339 **References**

340 Alatorre-Rosas, R., Kaya, H.K., 1990. Interspecific competition between entomopathogenic
341 nematodes in the genera *Heterorhabditis* and *Steinernema* for an insect host in sand. J.
342 Invertebr. Pathol. 55, 179–188.

343 Blaxter, M. L., De Ley, P., Garey, J. R., Liu, L. X., Scheldeman, P., Vierstraete, A., Vanfleteren,
344 R., Mackey, L. Y., Dorris, M., Frisse, L. M., Vida, J. T., Thomas, W. K., 1998. A molecular
345 evolutionary framework for the phylum Nematoda. Nature 392, 71–75.

346 Boemare, N., 2002. Biology, taxonomy and systematics of *Xenorhabdus* and *Photorhabdus*. In:
347 Gaugler, R. (Ed.), Entomopathogenic Nematology. CABI Publishing, Wallingford, UK,
348 pp. 35–56.
349 Campbell, J.F., Lewis, E.E., Stock, S.P., Nadler, S., Kaya, H.K., 2003. Evolution of host search
350 strategies in entomopathogenic nematodes. J. Nematol. 35, 142–145.
351 Campos-Herrera, R., El-Borai, F.E., Duncan, L.W., 2012. Wide interguild relationships among
352 entomopathogenic and free–living nematodes in soil as measured by real time qPCR. J.
353 Invertebr. Pathol. 111, 126–135.
354 Campos-Herrera, R., Půža, V., Jaffuel, G., Blanco-Pérez, R., Čepulytė Rakauskienė, R., Turlings,
355 T.C.J., 2015a. Unraveling the intraguild competition between *Oscheius spp.* nematodes and
356 entomopathogenic nematodes: implications for their natural distribution in Swiss
357 agricultural soils. J. Invertebr. Pathol. 132, 216-227.
358 Campos-Herrera, R., Jaffuel, G., Chiriboga, X., Blanco-Pérez, R., Fesselet, M., Půža, V.,
359 Mascher, F., Turlings, T.C.J., 2015b. Traditional and molecular detection methods reveal
360 intense interguild competition and other multitrophic interactions associated with native
361 entomopathogenic nematodes in Swiss tillage soils. Plant Soil 389, 237–255.
362 Crook, M., 2014. The dauer hypothesis and the evolution of parasitism: 20 years on and still going
363 strong. Int. J. Parasitol. 44(1), 1–8.
364 Denno, R. F., Gruner, D. S., & Kaplan, I., 2008. Potential for entomopathogenic nematodes in
365 biological control: A meta–analytical synthesis and insights from trophic cascade theory.
366 J. Nematol. 40(2), 61–72.
367 Dillman, A.R., Chaston, J.M., Adams, B.J., Ciche, T.A., Goodrich-Blair, H., Stock, S.P.,
368 Sternberg, P.W., 2012. An entomopathogenic nematode by any other name. PLoS
369 Pathogens 8, e1002527.
370 Duncan, L.W., Dunn, D.C., Bague, G., Nguyen, K., 2003. Competition between
371 entomopathogenic and free–living bacterivorous nematodes in larvae of the weevil
372 *Diaprepes abbreviatus*. J. Nematol. 35, 187–193.
373 Duncan, L.W., Graham, J.H., Zellers, J., Bright, D., Dunn, D.C., El-Borai, F.E., Porazinska, D.L.,

374 2007. Food web responses to augmenting the entomopathogenic nematodes in bare and
375 animal manure–mulched soil. J. Nematol. 39, 176–189.
376 Dutky, S.R., Thompson, J.V., Cantwell, G.E., 1962. A technique for mass rearing the greater wax
377 moth (Lepidoptera: Galleriidae). Proc. Entomol. Soc. Wash. 64, 56–58.
378 Félix, M.-A., Vierstraete, A., Vanfleteren, J., 2001. Three biological species closely related to
379 *Rhabditis (Oscheius) pseudodolichura* Körner in Osche, 1952. J. Nematol. 33, 104–109.
380 Forst, S.F., Clarke, D., 2002. Bacteria-nematode symbiosis. In: Gaugler, R. (Ed.),
381 Entomopathogenic Nematology. CABI Publishing, Wallinford, UK, pp. 57–77.
382 Griffin C.T., 2015. Behaviour and Population Dynamics of Entomopathogenic Nematodes
383 Following Application. In: Campos- Herrera, R. (Ed.), Nematode Pathogenesis of Insects
384 and Other Pests. Series: Sustainability in Plant and Crop Protection (Ciancio, A. Series
385 Ed.). Springer International Publishing, Switzerland, pp. 57–95.
386 Hawdon J.M., Schad G.A., 1991. Albumin and a dialyzable serum factor stimulate feeding in
387 vitro by third stage larvae of the canine hookworm *Ancylostoma caninum*. J. Parasitol. 77,
388 587–591.
389 Herrmann, M., Mayer, W.E., Sommer, R.J., 2006. Nematodes of the genus *Pristionchus* are
390 closely associated with scarab beetles and the Colorado potato beetle in Western Europe.
391 Zoology 109, 96-108.
392 Hotez P., Hawdon J., Schad G.A., 1993. Hookworm larval infectivity, arrest and
393 amphiparatenesis: the *Caenorhabditis elegans* daf-c paradigm. Parasitol. Today 9, 23–26.
394 Ishibashi, N., Kondo, E., 1986. *Steinernema feltiae* (DD-136) and *S. glaseri*: persistence in soil
395 and bark compost and their influence on native nematodes. J. Nematol. 18, 310–316.
396 Ishibashi, N., Kondo, E., 1987. Dynamic of entomogenous nematode *Steinernema feltiae* applied
397 to soil with and without nematicide treatment. J. Nematol. 19, 404–412.
398 Jackson, G.J., Moore, G.E., 1968. Infectivity of nematodes, *Neoaplectana* species, for the larva
399 of the weevil *Hylobius* pales, after rearing in species isolation. J. Invertebr. Pathol. 14, 194–
400 198.
401 Jaffuel, G., Blanco-Pérez, R., Büchi, L., Mäder, P., Fliessbach, A., Charles, R., Degen, T.,

402 Turlings, T.C.J., Campos-Herrera, R., 2017. Effects of cover crops on the overwintering
403 success of entomopathogenic nematodes and their antagonists. Appl. Soil Ecol. 114, 62–
404 73.
405 Jaffuel, G., Mäder, P., Blanco-Pérez, R., Chiriboga, X., Fliessbach, A., Turlings, T.C.J., Campos-
406 Herrera, R., 2016. Prevalence and activity of entomopathogenic nematodes and their
407 antagonists in soils that are subject to different agricultural practices. Agric. Ecosyst.
408 Environ. 230, 329-340.
409 Kaya, H.K., 2002. Natural enemies and other antagonists. In: Gaugler, R. (Ed.),
410 Entomopathogenic Nematology. CABI Publishing, Wallingford, UK, pp. 189–203.
411 Koppenhöfer, A.M., Kaya, H.K., Shanmugam, S., & Wood, G.L., 1995. Interspecific competition
412 between steinernematid nematodes within an insect host. J. Invertebr. Pathol. 66, 99–103.
413 Lam, A.B.Q., Webster J.M., 1971. Morphology and biology of *Panagrolaimus tipulae* n. sp.
414 (Panagrolaimidae) and *Rhabditis (Rhabditella) tipulae* n. sp. (Rhabditidae), from
415 leatherjacket larvae, *Tipula paludosa* (Diptera: Tipulidae). Nematologica 17:201–212.
416 Lewis, E.E., Hazir, S., Hodson, A., Gulcu, B., 2015. Trophic Relationships of Entomopathogenic
417 Nematodes in Agricultural Habitats. In: Campos- Herrera, R. (Ed.), Nematode
418 Pathogenesis of Insects and Other Pests. Series: Sustainability in Plant and Crop Protection
419 (Ciancio, A. Series Ed.). Springer International Publishing, Switzerland, pp. 139–164.
420 O'Callaghan, K.M., Zenner, A.N.R.L., Hartley, C.J., & Griffin, C.T., 2014. Interference
421 competition in entomopathogenic nematodes: Male *Steinernema* kill members of their own
422 and other species. Int. J. Parasitol. 44, 1009–1017.
423 Poinar, G.O., 1979. Nematodes for Biological Control of Insects. CRC Press, Inc., Boca Raton,
424 Florida, p. 249.
425 Poinar, G.O., 1993. Origins and phylogenetic relationships of the entomophilic rhabditids,
426 *Heterorhabditis* and *Steinernema*. Fundam. Appl. Nematol. 16, 333–338.
427 Půža, V., Mráček, Z., 2009. Mixed infection of *Galleria mellonella* with two entomopathogenic
428 nematodes (Nematoda: Rhabditida) species: *Steinernema affine* benefits from the presence
429 of *Steinernema kraussei*. J. Invertebr. Pathol. 102, 40–43.

430 Půža, V., Mráček, Z., 2010. Does scavenging extend the host range of entomopathogenic
431 nematodes (Nematoda: Steinernematidae)? J. Invertebr. Pathol. 104, 1–3.
432 Pye, A.E., Burman, M., 1978. *Neoaplectana carpocapsae*: infection and reproduction in large
433 pine weevil larvae, *Hylobius abietis*. Exp. Parasitol. 46, 1–11.
434 Rogers W.P., Sommerville R.I., 1963. The infective stage of nematode parasites and its
435 significance in parasitism. Adv. Parasitol. 1, 109–177.
436 San-Blas, E., Gowen, S.R., 2008. Facultative scavenging as a survival strategy of
437 entomopathogenic nematodes. Int. J. Parasitol. 38, 85–91.
438 San-Blas, E., Gowen, S.R., Pembroke, B., 2008. Scavenging or infection? Possible host choosing
439 by entomopathogenic nematodes. Nematology 10, 251–259.
440 San Blas, E., Pembroke, B., Gowen, S.R., 2012. Scavenging and infection of different hosts by
441 *Steinernema carpocapsae*. Nematropica 42, 123-130.
442 Sicard, M., Hinsinger, J., Le Brun, N., Pages, S., Boemare, N., & Moulia, C., 2006. Interspecific
443 competition between entomopathogenic nematodes (*Steinernema*) is modified by their
444 bacterial symbionts (*Xenorhabdus*). BMC Evol. Biol., 6, 68.
445 Stock, S.P., 2015. Diversity, Biology and Evolutionary Relationships. In: Campos- Herrera, R.
446 (Ed.), Nematode Pathogenesis of Insects and Other Pests. Series: Sustainability in Plant
447 and Crop Protection (Ciancio, A. Series Ed.). Springer International Publishing,
448 Switzerland, pp. 3–27.
449 Stock, S.P., Caicedo, A.M., Calatayud, P.A., 2005. *Rhabditis (Oscheius) colombiana* n. sp.
450 (Nematoda: Rhabditida), a necromenic associate of the subterranean burrower bug
451 *Cyrtomenus bergi* (Hemiptera: Cydnidae) from the Cauca Valley, Colombia. Nematology
452 7, 363–373.
453 Stuart, R.J., Barbercheck, M.E., Grewal, P.S., 2015. Entomopathogenic nematodes in the soil
454 environment: distributions, interactions and the influence of biotic and abiotic factors. In:
455 Campos- Herrera, R. (Ed.), Nematode Pathogenesis of Insects and Other Pests. Series:
456 Sustainability in Plant and Crop Protection (Ciancio, A. Series Ed.). Springer International
457 Publishing, Switzerland, pp. 96–137.

458 Sudhaus, W., 1993. Redescription of *Rhabditis (Oscheius) tipulae* (Nematoda: Rhabditidae)
459 associated with leatherjackets, larvae of *Tipula paludosa* (Diptera: Tipulidae).
460 Nematologica 39, 234–239.
461 Sudhaus, W., Schulte, F., 1989. *Rhabditis (Rhabditis) necromena sp*. n. (Nematoda: Rhabditidae)
462 from South Australian Diplopoda with notes on its siblings *R. myriophil*. Nematologica 35,
463 15–24.
464 Torres-Barragan, A., Suazo, A., Buhler, W.G., Cardoza, Y.J., 2011. Studies on the
465 entomopathogenicity and bacterial associates of the nematode *Oscheius carolinensis*. Biol
466 Contr. 59, 123–129.
467 Torrini, G., Mazza, G., Carletti, B., Benvenut, C., Roversi, P.F., Fanelli, E., de Luca, F., Trocoli,
468 A., Tarasco, E., 2015. *Oscheius onirici* sp. n. (Nematoda: Rhabditidae): a new
469 entomopathogenic nematode from an Italian cave. Zootaxa 3937, 533–548.
470 Wang, Y., Gaugler, R., & Cui, L. (1994). Variations in immune response of *Popillia japonica* and
471 *Acheta domesticus* to *Heterorhabditis bacteriophora* and *Steinernema* species. J. Nematol.
472 26, 11–18.
473 White, G.F., 1929. A method for obtaining infective nematode larvae from cultures. Science 66,
474 22-303.
475 Woodring, J.L., Kaya, H.K., 1988. Steinernematid and Heterorhabditid nematodes: a handbook
476 of biology and techniques. Southern Cooperative Series Bulletin 331. Arkansas
477 Agricultural Experiment Station, Arkansas.
478 Ye, W.M., Torres-Barragan, A., Cardoza, Y.J., 2010. *Oscheius carolinensis* n. sp (Nematoda:
479 Rhabditidae), a potential entomopathogenic nematode from vermicompost. Nematology
480 12, 121–135.
481 Zenner, A.N.R.L., O'Callaghan, K., & Griffin, C.T., 2014. Lethal fighting in nematodes is
482 dependent on developmental pathway: Male–male fighting in the entomopathogenic
483 nematode *Steinernema longicaudum*. PloS One, 9, e89385.
484 Zhang, C., Liu, J., Xu, M., Sun, J., Yang, S., An, X., Gao, G., Lin, M., Lai, R., He, Z., Wu, Y.,
485 Zhang, K., 2008. *Heterorhabditidoides chongmingensis* gen. nov., sp. nov. (Rhabditida:

486 Rhabditidae), a novel member of the entomopathogenic nematodes. J. Invertebr. Pathol.
487 98, 153–168.

488 **Figure legends**

489

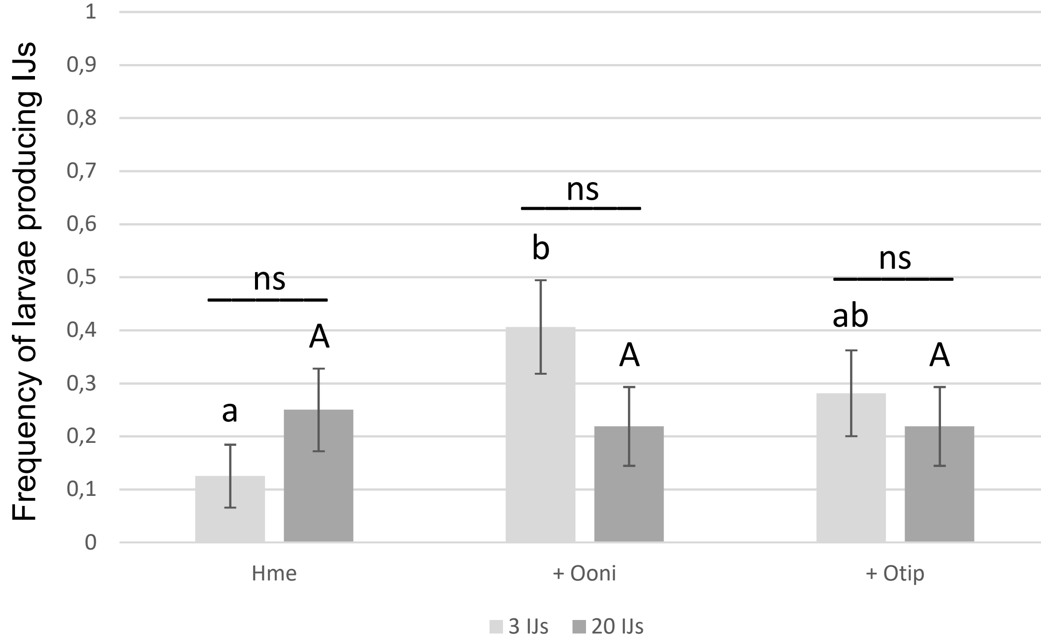
490 **Fig. 1.** Frequency of frozen-killed larvae producing infective juveniles (IJs), including when they
491 are mixed with *Oscheius* spp. emergences. **A.** Addition of either 3 infective juveniles (IJs)
492 or 20 IJs of *Heterorhabditis megidis* (Hme) alone or in combination of *Oscheius onirici*
493 (Ooni) or *Oscheius tipulae* (Otip). **B.** Addition of either 3 IJs or 20 IJs of *Steinernema*
494 *kraussei* (Skr) alone or in combination of Ooni or Otip. **C.** Addition of either 3 infective
495 juveniles (IJs) or 20 IJs of Hme and Skr mixed, alone or in combination of Ooni or Otip.
496 Letters indicate significant differences among treatments (One-way ANOVA, $P < 0.05$).
497 Pair-treatment compassion between initial inoculum is represented with lines above the
498 columns (Student's t-test (t): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, no significant). Data
499 are average ± SEM.

500 **Fig. 2.** Number of infective juveniles (IJs) produced per frozen-killed larva. **A.** Addition of either
501 3 infective juveniles (IJs) or 20 IJs of *Heterorhabditis megidis* (Hme) alone or in combination
502 of *Oscheius onirici* (Ooni) or *Oscheius tipulae* (Otip). **B.** Addition of either 3 IJs or 20 IJs of
503 *Steinernema kraussei* (Skr) alone or in combination of Ooni or Otip. **C.** Addition of either 3
504 infective juveniles (IJs) or 20 IJs of Hme and Skr mixed, alone or in combination of Ooni or
505 Otip. Letters indicate significant differences (One-way ANOVA, $P < 0.05$). Pair-treatment
506 compassion between initial inoculum is represented with lines above the columns (Student's
507 t-test (t): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, no significant). Data are average ± SEM.

508 **Fig. 3.** Frequency of frozen-killed larvae producing free-living bacteriophagous nematodes
509 (FLBNs), including when they are mixed with infective juvenile (IJ) emergence. **A.** Addition
510 of 500 *Oscheius onirici* (Ooni) by single application (sum of low and high concentration
511 experiments represented in the first column) or in combination of either *Heterorhabditis*
512 *megidis* (Hme), *Steinernema kraussei* (Skr), or Hme and Skr mixed, in high and low
513 concentration experiments. **B.** Addition of 500 *Oscheius tipulae* (Otip) by single application
514 (sum of low and high concentration experiments represented in the first column) or in

combination of either Hme, Skr, or both mixed, in high and low concentration experiments. Letters indicate significant differences (One-way ANOVA, $P < 0.05$). Pair-treatment compassion between initial inoculum is represented with lines above the columns (Student's t-test (t): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, no significant). Data are average ± SEM.

Fig. 4. Number of free-living bacteriophagous nematodes (FLBNs) produced per frozen-killed larva. **A.** Addition of 500 *Oscheius onirici* (Ooni) by single application (sum of low and high concentration experiments) or in combination of either Heterorhabditis megidis (Hme), *Steinernema kraussei* (Skr), or Hme and Skr mixed, in high and low concentration experiments. **B.** Addition of 500 *Oscheius tipulae* (Otip) by single application (sum of low and high concentration experiments) or in combination of either Hme, Skr, or both mixed, in high and low concentration experiments. Letters indicate significant differences (One-way ANOVA, $P < 0.05$). Pair-treatment compassion between initial inoculum is represented with lines above the columns (Student's t-test (t): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, no significant). Data are average ± SEM.



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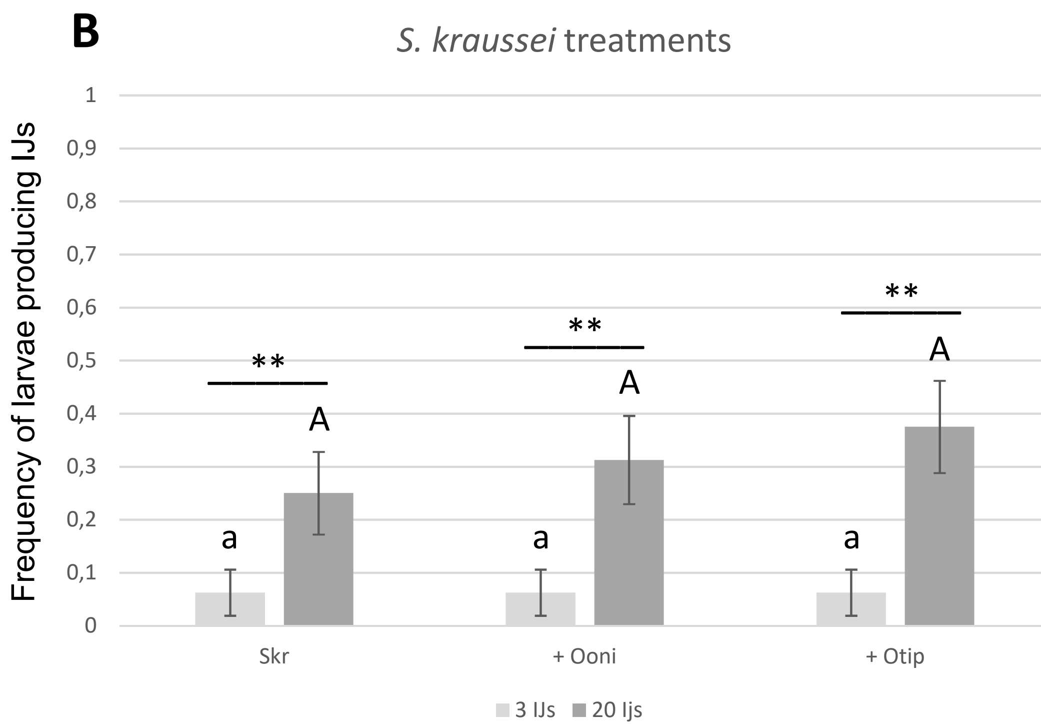

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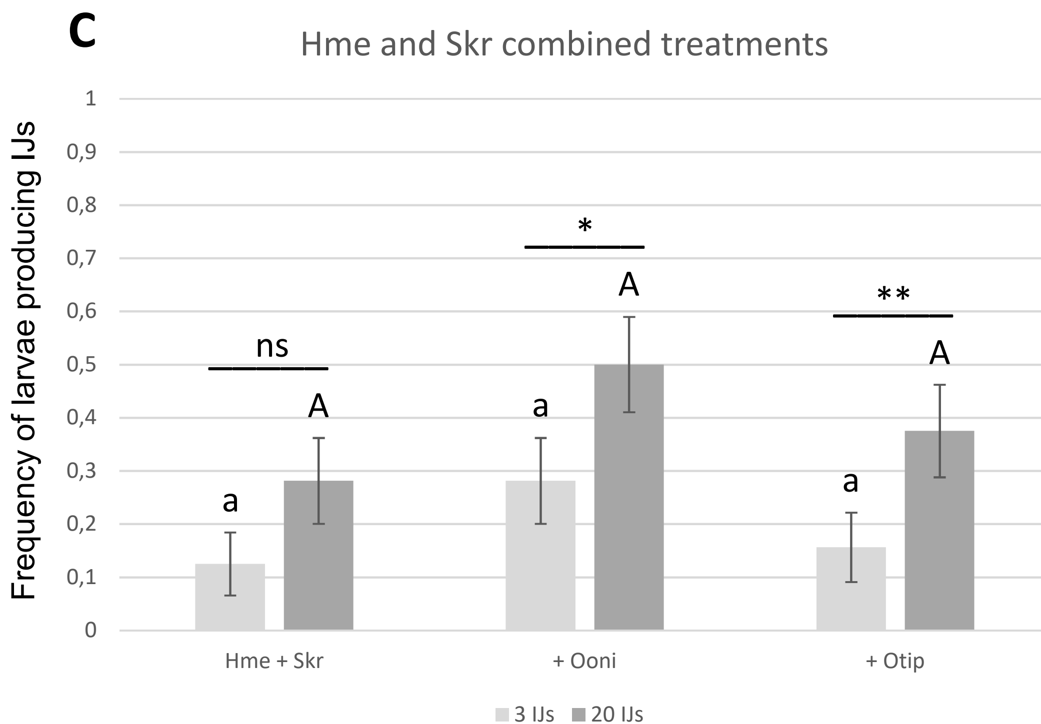
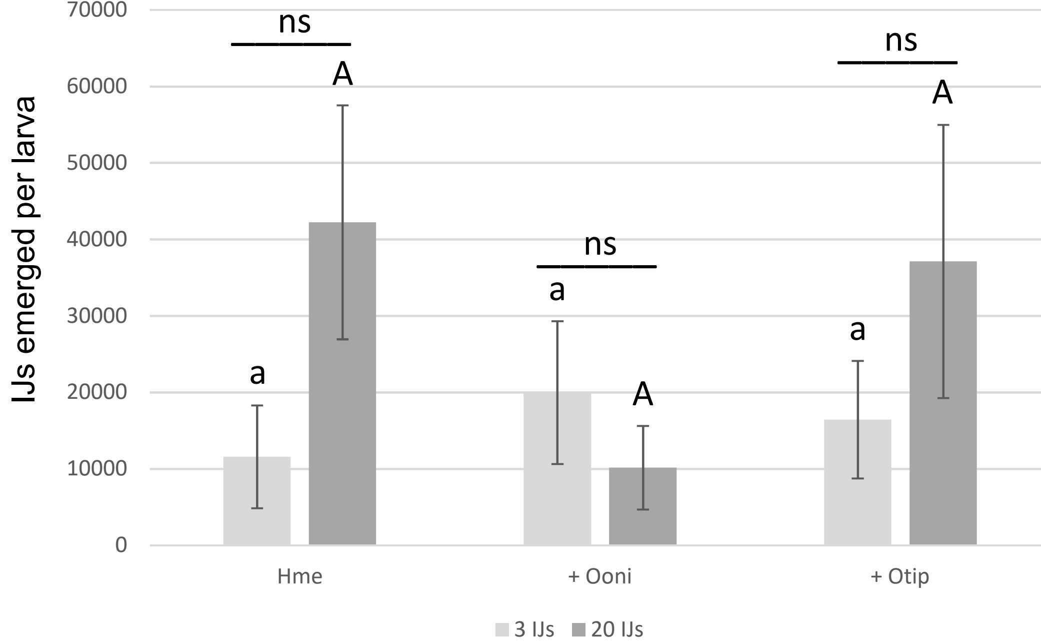
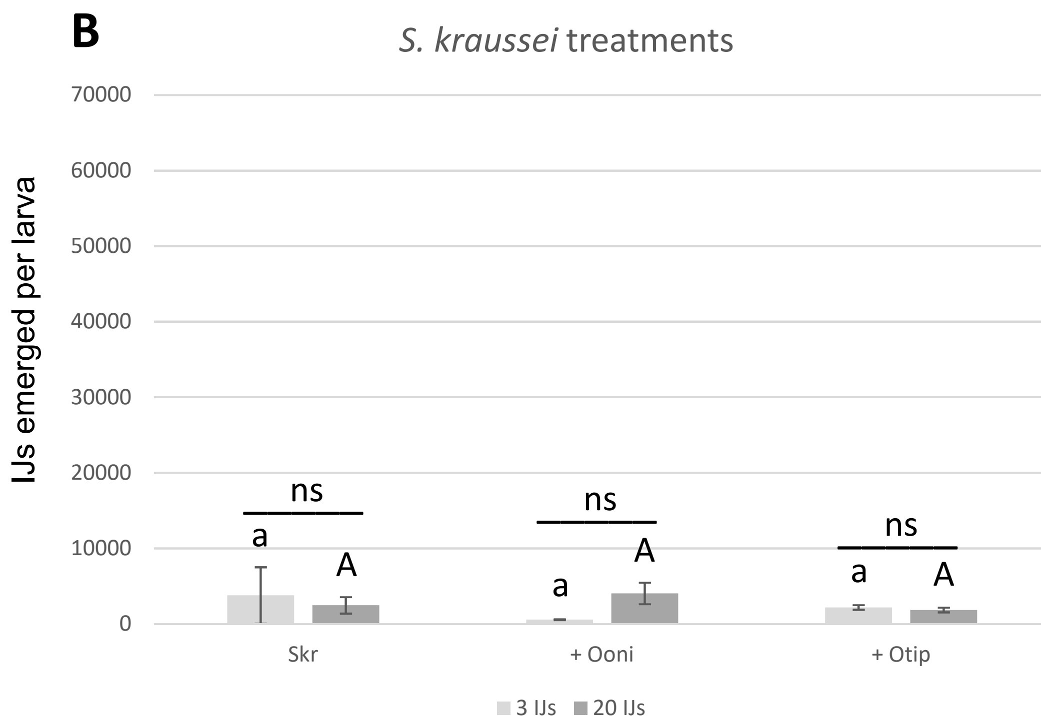
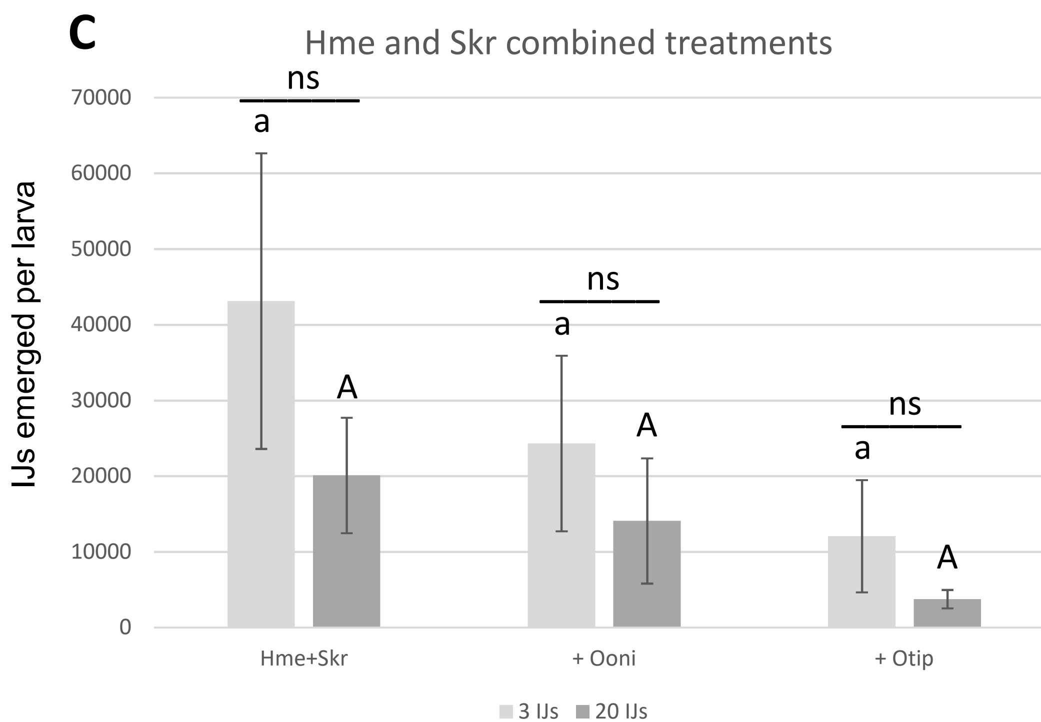
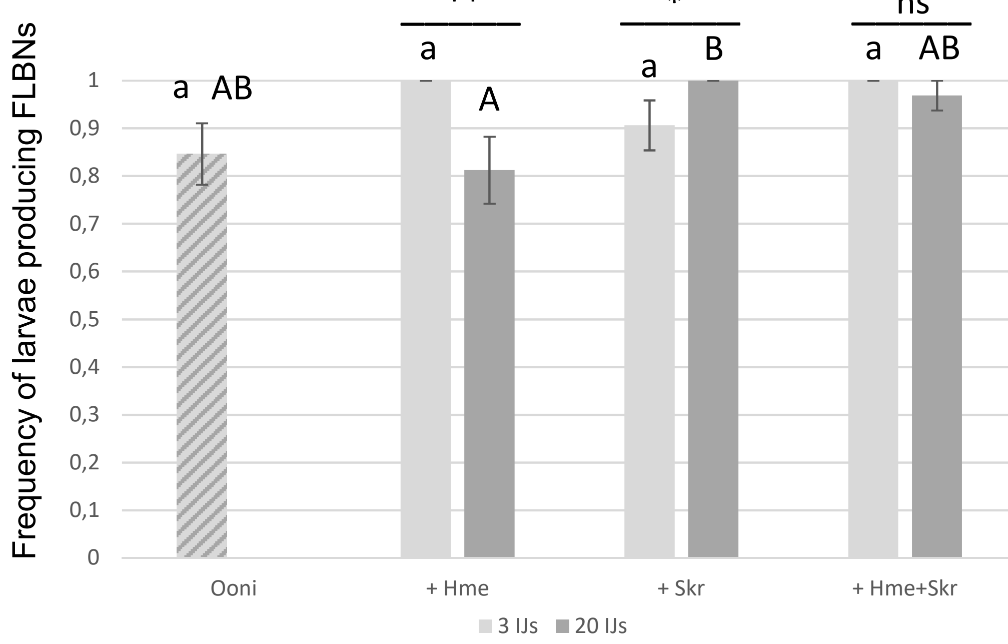
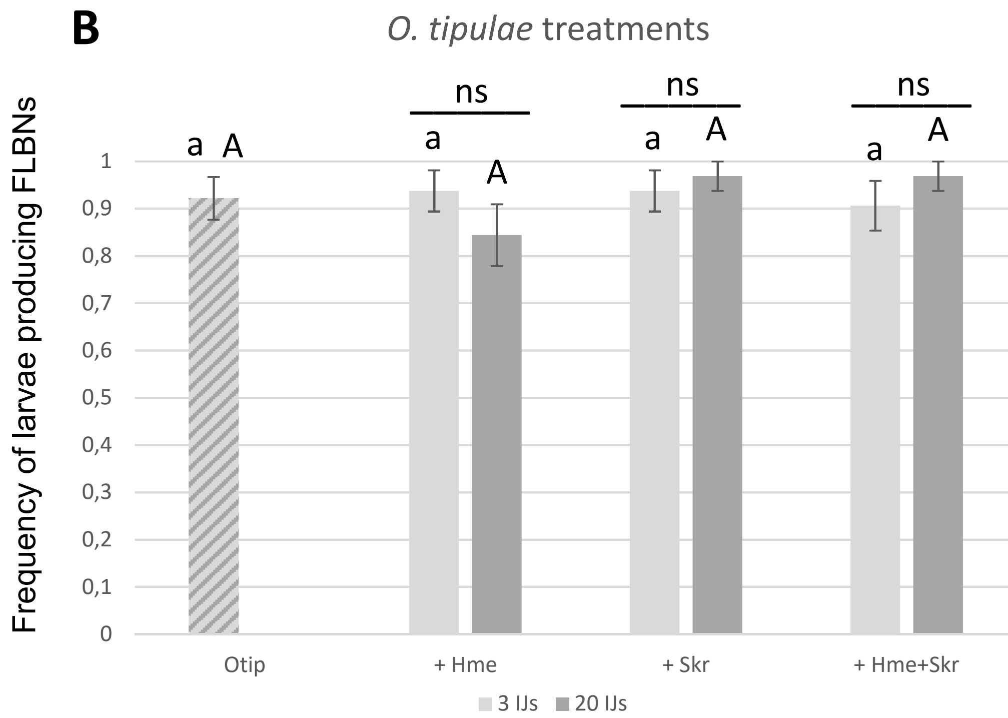


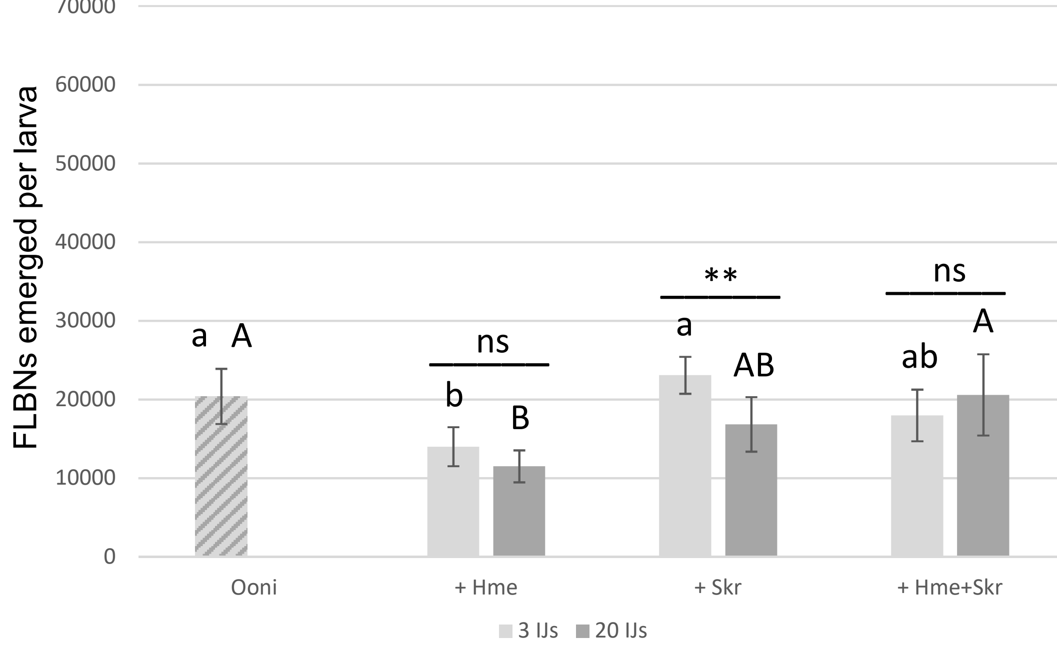

IJs emerged per larva
70000
60000
50000
40000
30000
20000
10000
0
ns
A
a
ns
a
A
ns
A
a
Hme
+ Ooni
+ Otip
3 IJs
20 IJs


B
S. kraussei treatments
IJs emerged per larva
70000
60000
50000
40000
30000
20000
10000
0
ns
a
A
ns
A
a
ns
a
A
Skr
+ Ooni
+ Otip
3 IJs
20 IJs


C
Hme and Skr combined treatments
IJs emerged per larva
70000
60000
50000
40000
30000
20000
10000
0
ns
a
A
ns
a
A
ns
a
A
Hme+Skr
+ Ooni
+ Otip
3 IJs
20 IJs

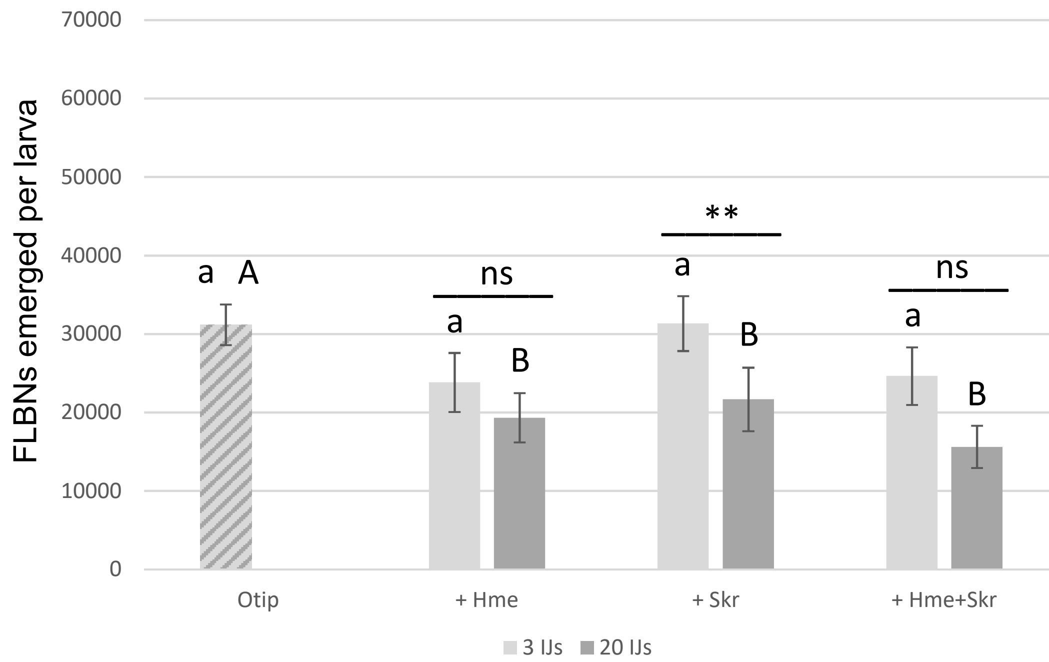

Frequency of larvae producing FLBNs
ns
a AB
a
A
a
B
a
AB
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Ooni
+ Hme
+ Skr
+ Hme+Skr
3 IJs
20 IJs


B
O. tipulae treatments
Frequency of larvae producing FLBNs
ns
ns
ns
a A
a
A
a
A
a
A
1
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
Otip
+ Hme
+ Skr
+ Hme+Skr
3 IJs
20 IJs



B

O. tipulae treatments

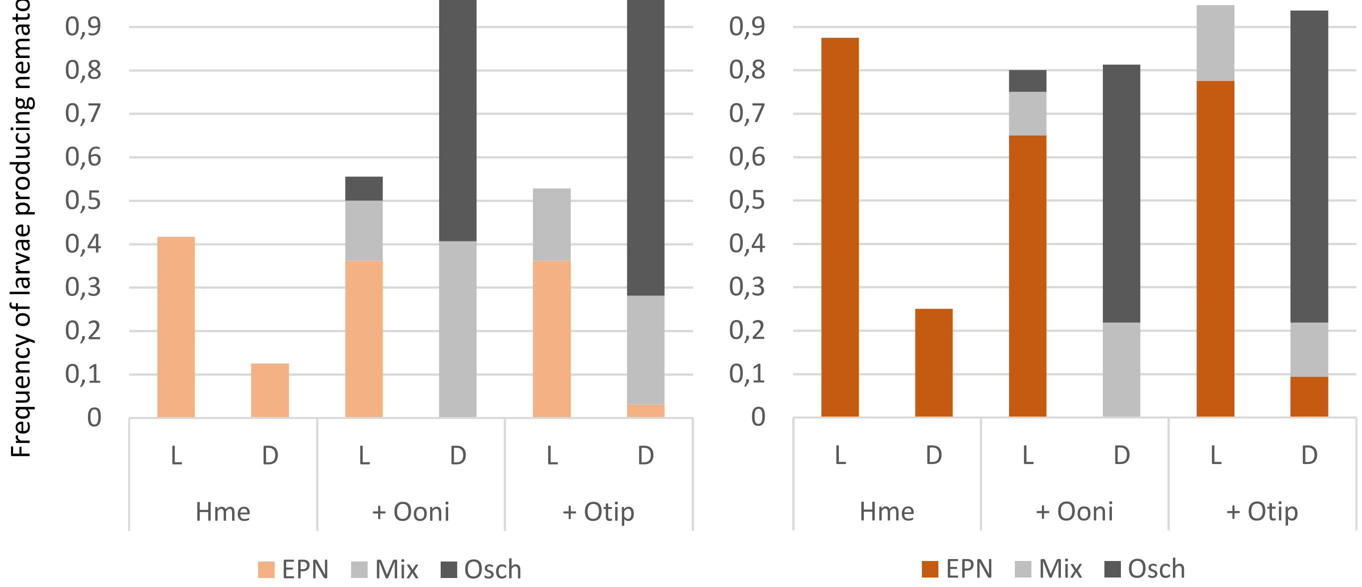


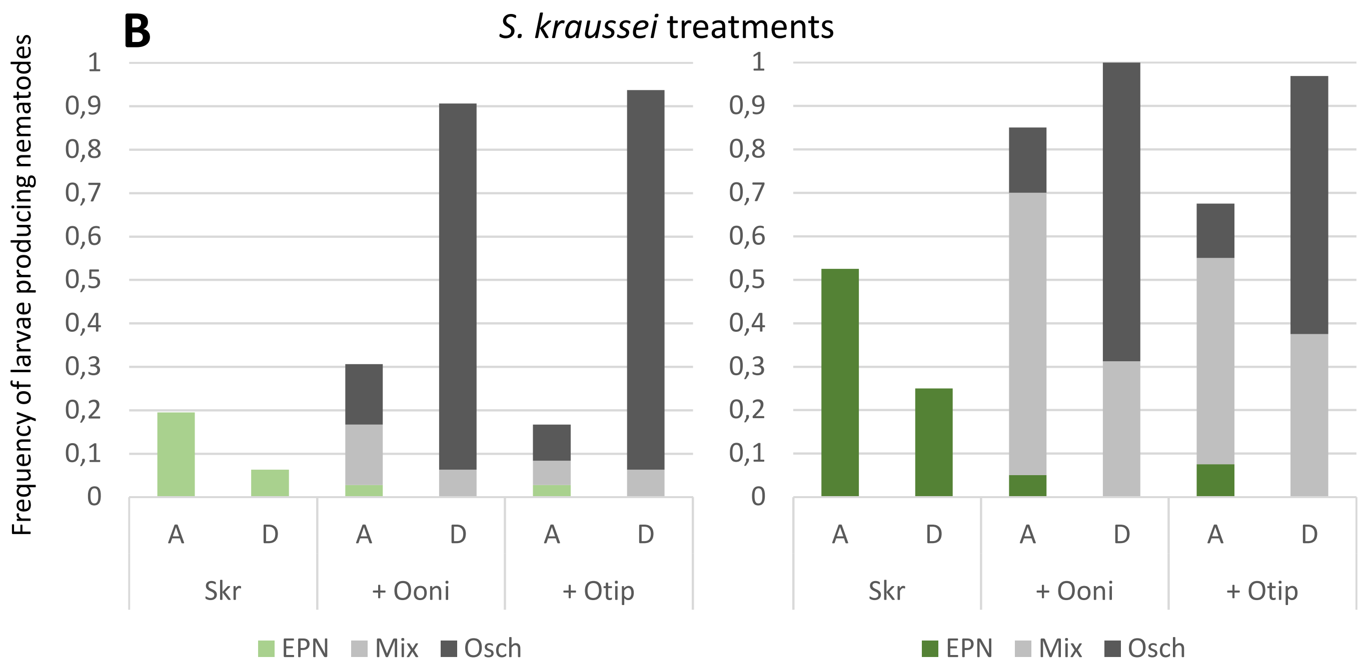
1 Table 1. Experimental design of the experiment to evaluate the scavenging behaviour of entomopathogenic nematodes (EPN) and their competition with

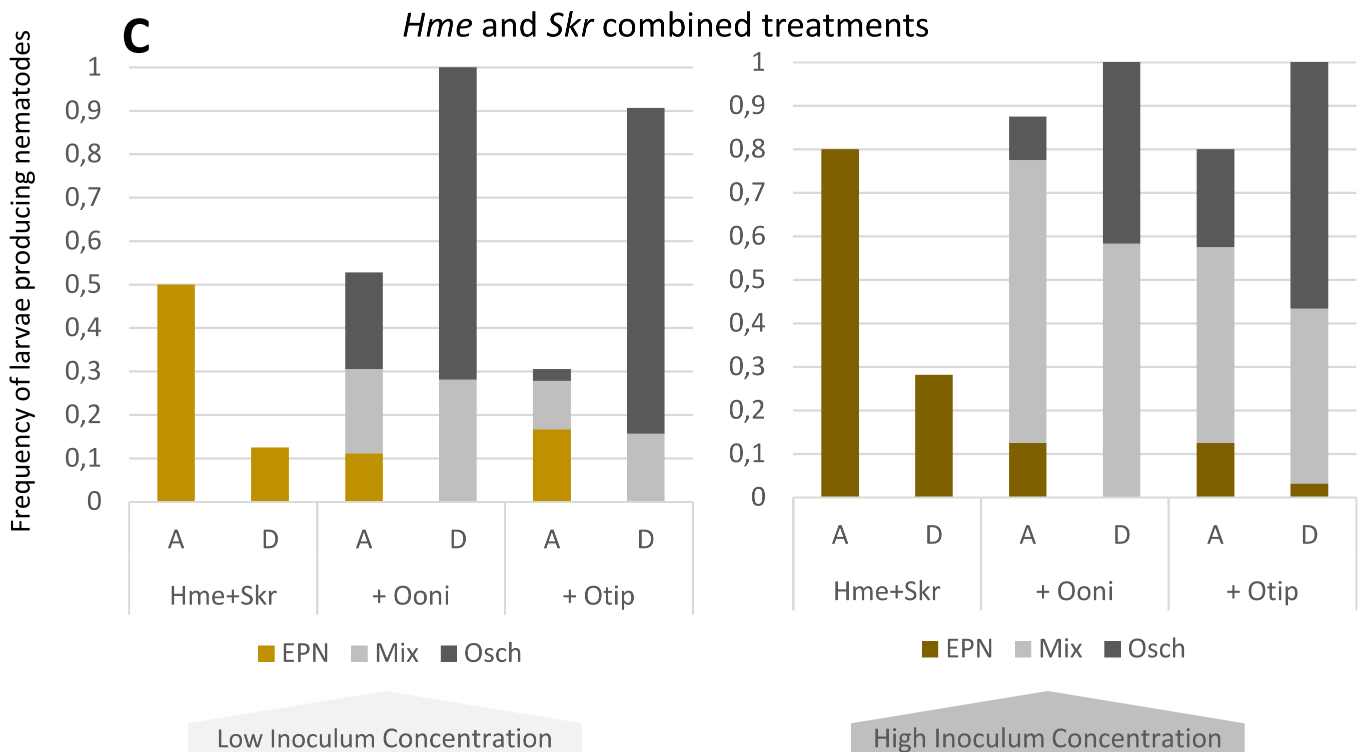
2 *Oscheius* spp.

Treatment code	EPN species	*Oscheius* species	EPN applied (IJs)/well	*Oscheius* applied/well
Hme	*H. megidis*	-	3 or 20	-
Skr	*S. kraussei*	-	3 or 20	-
Ooni	-	*O. onirici*	-	500
Otip	-	*O. tipulae*	-	500
Hme + Ooni	*H. megidis*	*O. onirici*	3 or 20	500
Hme + Otip	*H. megidis*	*O. tipulae*	3 or 20	500
Skr + Ooni	*S. kraussei*	*O. onirici*	3 or 20	500
Skr + Otip	*S. kraussei*	*O. tipulae*	3 or 20	500
Hme + Skr	*H. megidis + S. kraussei*	-	3 + 3 or 20 + 20	-
Hme + Skr + Ooni	*H. megidis + S. kraussei*	*O. onirici*	3 + 3 or 20 + 20	500
Hme + Skr + Otip	*H. megidis + S. kraussei*	*O. tipulae*	3 + 3 or 20 + 20	500
Control	-	-	-	-

3







Supplementary data 1. Frequency of larvae producing nematode progeny in the following categories: only infective juvenile (IJ) emergences, only free-living bacteriophagous nematode (FLBN) emergences, and both kind of nematodes mixed. Comparative of the competition experiments using live (L) or dead (D) insect larvae as host. **A.** Addition of either 3 infective juveniles (IJs) or 20 IJs of *Heterorhabditis*

1 **Supplementary data 2.** Differences between entomopathogenic nematode (EPN) reproduction ability between live and freeze-killed insects used as hosts. Data from the live
2 host were taken from Campos-Herrera et al. (2015a). Treatments: *Heterorhabditis megidis* (Hme) applied alone or in combination of *Oscheius onirici* (Ooni) or *Oscheius*
3 *tipulae* (Otip), *Steinernema kraussei* (Skr) applied alone or in combination of Ooni or Otip, and Hme and Skr mixed, applied alone or in combination of Ooni or Otip; for
4 initial inoculum (No. IJs) of 3 and 20 infective juveniles (IJs) (data are average ± SEM).

Treatment		Frequency of larvae producing any IJs		Frequency of larvae producing only IJs		Number of IJs produced per larvae	
Species	No. IJs	Live Larvae	↑/↓	Live Larvae	↑/↓	Live Larvae	↑/↓
Hme	3	0,42 ± 0,04	↓70%	0,42 ± 0,04	↓70%	70622 ± 14835	↓84%
Hme + Ooni	3	0,50 ± 0,09	↓19%	0,36 ± 0,08	↓100%	57346 ± 15610	↓65%
Hme + Otip	3	0,53 ± 0,08	↓47%	0,36 ± 0,08	↓91%	91220 ± 16612	↓82%
Skr	3	0,19 ± 0,07	↓68%	0,19 ± 0,07	↓68%	34635 ± 20664	↓89%
Skr + Ooni	3	0,17 ± 0,06	↓63%	0,03 ± 0,03	↓100%	450 ± 174	↑25%
Skr + Otip	3	0,08 ± 0,05	↓25%	0,03 ± 0,03	↓100%	2264 ± 799	↓3%
Hme + Skr	3+3	0,50 ± 0,09	↓74%	0,50 ± 0,09	↓74%	96296 ± 20227	↓55%
Hme + Skr + Ooni	3+3	0,31 ± 0,08	↓10%	0,11 ± 0,05	↓100%	44708 ± 20160	↓46%
Hme + Skr + Otip	3+3	0,28 ± 0,08	↓43%	0,17 ± 0,06	↓100%	48123 ± 29369	↓75%
Hme	20	0,88 ± 0,05	↓71%	0,88 ± 0,05	↓71%	167493 ± 11857	↓75%
Hme + Ooni	20	0,75 ± 0,07	↓71%	0,65 ± 0,08	↓100%	147310 ± 13091	↓93%
Hme + Otip	20	0,95 ± 0,04	↓77%	0,78 ± 0,07	↓88%	155303 ± 13577	↓76%
Skr	20	0,53 ± 0,08	↓52%	0,53 ± 0,08	↓52%	25387 ± 5225	↓90%
Skr + Ooni	20	0,70 ± 0,07	↓55%	0,05 ± 0,04	↓100%	15448 ± 4210	↓74%
Skr + Otip	20	0,55 ± 0,08	↓32%	0,08 ± 0,04	↓100%	14264 ± 3924	↓87%
Hme + Skr	20+20	0,80 ± 0,06	↓65%	0,80 ± 0,06	↓65%	59914 ± 10947	↓66%
Hme + Skr + Ooni	20+20	0,78 ± 0,07	↓36%	0,13 ± 0,05	↓100%	50996 ± 13077	↓72%
Hme + Skr + Otip	20+20	0,55 ± 0,08	↓31%	0,13 ± 0,05	↓77%	22413 ± 8553	↓83%

5 [a] Relative increment (↑) or decrease (↓) of the frequency of larvae producing any IJ (mixed or not with FLBNs) for freeze-killed larvae respect live larvae used as hosts.

6 [b] Relative increment (↑) or decrease (↓) of the frequency of larvae producing only IJs (no mixed with FLBNs) for freeze-killed larvae respect live larvae used as hosts.

7 [c] Relative increment (↑) or decrease (↓) of the number of IJs emerged per larva for freeze-killed larvae respect live larvae used as hosts.

1 **Supplementary data 3.** Differences on the free-living bacteriophage nematode (FLBN) reproduction ability between live and freeze-killed insects used as hosts. Data from
2 the live host were taken from Campos-Herrera et al. (2015a). Treatments: *Oscheius onirici* (Ooni) applied in combination of *Heterorhabditis megidis* (Hme), *Steinernema*
3 *kraussei* (Skr) or Hme and Skr mixed, and *Oscheius tipulae* (Otip) applied in combination of Hme, Skr or Hme and Skr mixed; for initial inoculum (No. IJs) of 3 and 20
4 infective juveniles (IJs) (data are average ± SEM).

		Frequency of larvae producing any FLBNs		Frequency of larvae producing only FLBNs		Number of FLBNs produced per larva	
Species	No. IJs	Live Larvae	↑/↓	Live Larvae	↑/↓	Live Larvae	↑/↓
Ooni + Hme	3	0,19 ± 0,07	↑414%	0,06 ± 0,04	↑969%	34375 ± 16883	↓53%
Ooni + Skr	3	0,28 ± 0,08	↑226%	0,14 ± 0,06	↑508%	14899 ± 5562	↑60%
Ooni + Hme + Skr	3+3	0,42 ± 0,08	↑138%	0,22 ± 0,07	↑227%	17082 ± 5463	↑5%
Otip + Hme	3	0,17 ± 0,06	↑481%	0,00	↑∞%	1844 ± 449	↑1192%
Otip + Skr	3	0,14 ± 0,06	↑575%	0,08 ± 0,05	↑950%	13330 ± 5342	↑135%
Otip + Hme + Skr	3+3	0,14 ± 0,06	↑550%	0,03 ± 0,03	↑3650%	5310 ± 4194	↑364%
Ooni + Hme	20	0,15 ± 0,06	↑442%	0,05 ± 0,04	↑1088%	2312 ± 749	↑861%
Ooni + Skr	20	0,80 ± 0,06	↑25%	0,15 ± 0,06	↑358%	22029 ± 5292	↓48%
Ooni + Hme + Skr	20+20	0,75 ± 0,07	↑29%	0,10 ± 0,05	↑370%	20940 ± 4175	↓2%
Otip + Hme	20	0,18 ± 0,06	↑400%	0,00	↑∞	1393 ± 313	↑1288%
Otip + Skr	20	0,60 ± 0,08	↑41%	0,13 ± 0,05	↑375%	15645 ± 4099	↑39%
Otip + Hme + Skr	20+20	0,68 ± 0,08	↑43%	0,23 ± 0,07	↑174%	31011 ± 9544	↓50%

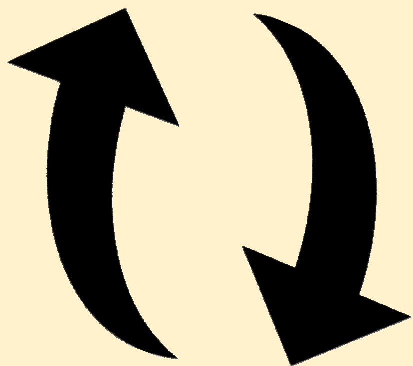
5 [a] Relative increment (↑) or decrease (↓) of the frequency of larvae producing any FLBN (mixed or not with IJs) for freeze-killed larvae respect live larvae used as hosts.

6 [b] Relative increment (↑) or decrease (↓) of the frequency of larvae producing only FLBNs (no mixed with IJs) for freeze-killed larvae respect live larvae used as hosts.

7 [c] Relative increment (↑) or decrease (↓) of the number of FLBNs emerged per larva for freeze-killed larvae respect live larvae used as hosts.

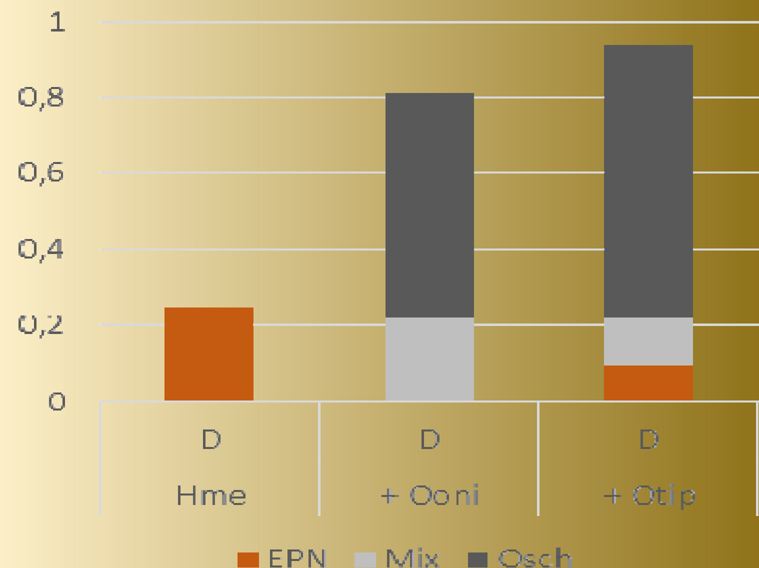
EPN – Bacteria Complex

Exogenous FLBNs



Dead Insect Larvae as Host

Low frequency of larvae producing IJs
High frequency of larvae producing FLBNs



1 **Research Highlights**

2

3 - Entomopathogenic nematodes (EPNs) co-occur with free-living nematodes (FLNs) in soils
4 - EPNs were able to reproduce in insect cadavers in the presence of scavenger FLNs
5 - EPN reproductive success is lower when acting as scavengers
6 - Using cadavers by EPNs might limit the advantage conferred by the bacteria partner
7 - Scavenging EPN-FLN interaction is species-specific and context dependency