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Ahmed, KSD, Stephens, C, Bistline-East, A, Williams, CD, Mc Donnell, RJ, Carnaghi, M, Huallacháin, D and Gormally, MJ

**Biological control of pestiferous slugs using *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae): Larval behavior and feeding on slugs exposed to *Phasmarhabditis hermaphrodita* (Schneider, 1859)**

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### Article

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1 **Biological control of pestiferous slugs using *Tetanocera elata* (Fabricius) (Diptera:**  
 2 **Sciomyzidae): Larval behavior and feeding on slugs exposed to *Phasmarhabditis***  
 3 ***hermaphrodita* (Schneider, 1859)**

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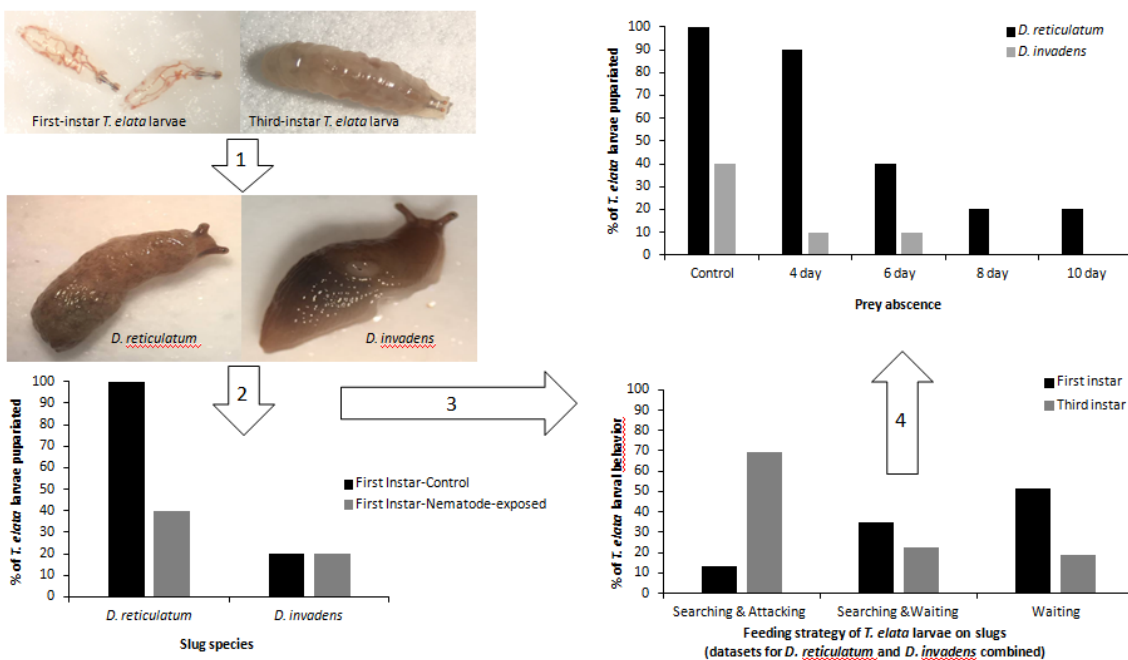
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17 **Highlights**

- 18 • *T. elata* larval survival outcomes depend on selected prey slug species.  
 19 • Successful pupariation in *T. elata* is reduced for neonate/third instar larvae fed on  
 20 nematode-exposed slugs.  
 21 • In the absence of prey up to four days post egg hatching, 90% of *T. elata* neonate  
 22 larvae pupariate successfully.  
 23 • “Waiting” and “Search & Attack” are the preferred strategies adopted by neonate and  
 24 third instar *T. elata* larvae respectively.



## 26 Abstract

27 While the larval stage of *Tetanocera elata* (Diptera: Sciomyzidae) is a known parasitoid and  
28 predator of pestiferous slugs, its biology and predatory behaviour as well as its interaction  
29 with slug parasitic nematodes requires further investigation. In this study, survival of larvae  
30 fed from the neonate stage on *Deroceras reticulatum* Müller (a previously known prey  
31 species) was significantly greater ( $p = 0.023$ ) than for larvae fed on *Deroceras invadens* Reise  
32 with 100% and 40% survival respectively. However, when fed solely on *D. reticulatum* which  
33 were previously exposed to *P. hermaphrodita*, only 20% of neonate larvae pupariated  
34 successfully. Ninety percent of neonate larvae maintained without food for the first four  
35 days and subsequently fed on *D. reticulatum* pupariated successfully although this  
36 decreased to below 50% for  $\geq 6$  days without food. Predatory third instar *T. elata* larvae  
37 appeared to select nematode-exposed *D. reticulatum* over non-exposed slugs with the  
38 continued feeding on nematode-exposed slugs also reducing the chances of successful  
39 pupariation by 25%. Records of maximum egg-laying by laboratory-reared female adults  
40 were greater (487 eggs) than previously recorded for field caught adults (373). The  
41 implications of these results for the potential use of *T. elata* as a biological control agent of  
42 pestiferous slugs are discussed.

43 **Key words:** Biological control, pestiferous slug, *Phasmarhabditis hermaphrodita*,  
44 Sciomyzidae, *Tetanocera elata*

45

## 46 1. Introduction

47 Pestiferous slugs cause damage to a diverse range of agricultural and horticultural crops  
48 throughout the world (Askary, 2010; Askary et al., 2017, Cordoba et al., 2018; Fritz et al.,  
49 2001; South, 2012; Speiser et al., 2001) primarily by feeding directly on the plant (Ester and  
50 Trul, 2000; Frank, 1998; Gould, 1961; Jaskulska et al., 2017) and/or as vectors of plant  
51 pathogens (Hasan and Vago, 1966). This results in significant economic losses to growers  
52 (Willis et al., 2006).

53 The grey field slug, *D. reticulatum* Müller, which is native to Europe but now has a  
54 worldwide distribution, is one of the most serious pestiferous slugs particularly across  
55 Europe (Godan, 1983; Tulli et al., 2009). It is also considered as one of the most important  
56 pestiferous slugs in Ireland and the UK (Tan and Grewal, 2001) due to its widespread  
57 distribution and its ability to survive in a range of habitats (Wilson et al., 1993). Another  
58 pestiferous slug, *D. invadens* Reise, Hutchinson, Schunacket Schlitt, 2011 (syn. *D.*  
59 *panormitanum* (Lessona et Pollonera, 1882)) originated in southern Italy but has been  
60 introduced to many countries over the last century (Hutchinson et al., 2014). *D. invadens*,  
61 considered one of the most important non-native slug pests to UK agriculture (Williams et  
62 al., 2010), was first detected in Ireland in the 1950s and it is now widespread in all but  
63 extreme western districts (Anderson, 2016a).

64 Molluskicides (generally in the form of methiocarb or metaldehyde and iron-based slug  
65 pellets) have been one of the most common methods of control for pestiferous slugs, with  
66 France spending €45 million per year (Howlett, 2012) and the UK spending more than £1.5  
67 million per year on slug pellets alone (Williams et al., 2010). However, the decision by the  
68 EU in 2014 to ban methiocarb (European Commission, 2014;2015) due to its toxic effects on

69 non-target organisms (Jones, 2014); concerns regarding the occurrence of metaldehyde in  
70 drinking water (Busquets et al., 2014; Kay and Grayson, 2014); and more recently reports  
71 that ferric phosphate pellets can have negative effects on other soil fauna (Castle et al.  
72 2017) necessitate the development of alternative pestiferous slug control strategies.

73 Biological control offers an alternative approach to chemical pest control. Augmentative  
74 biological control is currently applied on more than 30 million ha globally, with Europe being  
75 the largest commercial market for invertebrate biological control agents (van Lenteren *et*  
76 *al.*, 2018). The slug-parasitic nematode *P. hermaphrodita* is the only commercial nematode  
77 biological control agent available today in Europe as an alternative to chemical  
78 molluscicides. It is available under the trade name Nemaslug® and is sold in 15 different  
79 European countries primarily for the control of *D. reticulatum* (Pieterse et al., 2017a,  
80 2017b). While it is a welcome addition to pestiferous slug control, some laboratory studies  
81 have shown that these nematodes kill only approximately 60% of *D. reticulatum* when  
82 applied directly onto the body of the slug or onto the soil, with efficacy likely to be even  
83 lower under field conditions. In addition, other pestiferous slug species appear to be less  
84 susceptible (Dankowska, 2006; Rae et al., 2008).

85 An alternative potential biological control agent of pestiferous slugs is the marsh/shade fly  
86 *T. elata*. Knutson *et al.* (1965) were the first to demonstrate that while first and second  
87 instar parasitoid larvae of *T. elata* appeared to feed only on the pestiferous slug species *D.*  
88 *reticulatum* and *D. laeve*, predatory third instars could feed on a range of slug species. Since  
89 then, Hynes *et al.* (2014a; 2014b and 2014c) have contributed significantly to our knowledge  
90 of the biology of *T. elata* with regard to adult oviposition rates and longevity (using  
91 predominantly field-caught adults) and temperature effects on egg development (2014a),  
92 and the duration of the larval stages (2014b). Hynes *et al.* (2014c) also undertook  
93 preliminary larval feeding behavior studies. Nevertheless, the biology of *T. elata* still needs  
94 to be understood in a systematic manner to exploit this species against different pestiferous  
95 slugs.

96 The aims of this study are to:

- 97 1. Quantify the fecundity of laboratory-eclosed female *T. elata* to inform the design of  
98 potential mass cultures of the species (previous oviposition studies by Knutson *et al.*  
99 [1965], Beaver [1973] and Hynes *et al.* [2014a] used predominantly field-caught adults).
- 100 2. Determine whether the obligate parasitoid first instar life stage of *T. elata* can feed and  
101 survive to pupal stage on a pestiferous slug species (i.e. *D. invadens*) not previously  
102 documented as a potential host for *T. elata*.
- 103 3. Assess the impacts of *P. hermaphrodita*-exposed slugs on the development of neonate *T.*  
104 *elata* larvae to determine the potential for both control agents to be used synergistically  
105 for pestiferous slug control.
- 106
- 107 4. Determine, using prey-choice experiments, the susceptibility of slugs exposed to *P.*  
108 *hermaphrodita* to predation by third instar *T. elata* larvae.
- 109
- 110 5. Assess the effects of the absence of prey over different periods on neonate larval  
111 development and survival.
- 112

113 6. Investigate the feeding behaviors of first and third instar larvae to inform our  
114 understanding of how *T. elata* larvae locate pestiferous slugs (second instar larvae  
115 develop inside the first slug host and, therefore, do not exhibit predatory behavior).

116

## 117 **2. Materials and methods:**

### 118 **2.1 Field collections and maintenance of laboratory cultures of *T. elata***

119 Adult *T. elata* flies were collected from extensive, low input grasslands in Co. Galway in the  
120 west of Ireland (lat: 53.289750, long: -9.066056) using a sweep net and pooter on July 12<sup>th</sup>  
121 and 23<sup>rd</sup> (2017). Captured flies were sexed, paired as female-male couples, and maintained  
122 in the laboratory, in covered glass jars (11.6 X 6 cm) containing water and food consisting of  
123 a mixture of honey and brewer's yeast in a 3:1 ratio (Hynes et al., 2014a). The number of  
124 eggs laid per day was recorded and eggs were transferred to Petri dishes (55 mm X 15 mm)  
125 containing filter paper on top of damp cotton wool. Parafilm® (Bemis Parafilm M™) was  
126 used to seal the Petri dishes, which were then kept at room temperature (~19-20°C) until  
127 egg hatching.

### 128 **2.2 Slug collections and maintenance**

129 *D. reticulatum* and *D. invadens* were collected using slug metric traps (de Sangosse Pont du  
130 Casse, France) placed on grasslands at a number of locations in Co. Galway (lat: 53.277083,  
131 long: -9.062333 and 53.279833, -9.056778). Slugs collected in the field were maintained in  
132 the laboratory in plastic boxes (16.5 x 11.4 x 5 cm) on damp tissue covered with filter paper  
133 on which a piece of carrot was placed. Boxes were cleaned every 3-4 days at which stage  
134 food was replaced and filter paper changed, and any dead slugs were removed from the  
135 box. Slug cultures were maintained for at least 21 days post-collection, after which they  
136 were examined to ensure none of the slugs displayed symptoms of naturally-occurring  
137 infections of the nematode *P. hermaphrodita* or other congeneric malacophagous species  
138 (Carnaghi et al., 2017). For those experiments which investigated the impact of nematode-  
139 exposed slugs on *T. elata* behavior, predation and subsequent development, it was essential  
140 that field-collected slugs were deemed to be nematode-free before experiments  
141 commenced.

### 142 **2.3 *T. elata* neonate larval development and survival on control slugs and slugs exposed to** 143 ***P. hermaphrodita***

144 Using the procedures of Carnaghi *et al.* (2017), each slug was exposed to a uniform  
145 suspension of *P. hermaphrodita*. For every suspension used, three subsamples of similar  
146 volumes were created and the number of moving nematodes in each subsample were  
147 counted under a stereo microscope. The average number of nematodes per subsample was  
148 then calculated to ensure that 30 nematodes/cm<sup>2</sup> (approx.) were applied directly to the  
149 mantle of each slug using a micropipette (see Carnaghi et al., 2017 for details). Two days  
150 later, a neonate *T. elata* larva was placed directly on the mantle of each of 11 nematode-  
151 exposed *D. reticulatum* and 11 nematode-exposed *D. invadens* slugs. Similarly, a neonate *T.*  
152 *elata* larva was placed directly on the mantle of each of 10 control (nematode-free) *D.*  
153 *reticulatum* and 10 *D. invadens* slugs. *T. elata* larval development and survival were  
154 subsequently recorded until pupariation or death.

## 155 **2.4 Effect of absence of prey on neonate *T. elata* larval development and survival**

156 Preliminary experiments using 50 neonate larvae (maintained in batches of 10 -12) were  
157 placed in Petri dishes containing a damp cotton pad covered by filter paper and maintained  
158 without prey to determine neonate larval survivorship in the absence of food. Based on the  
159 results of these experiments the impact of the absence of prey for 4, 6, 8 and 10 days on the  
160 subsequent number of slugs killed and the duration of *T. elata* life-cycle stages was recorded  
161 using the pestiferous slug species *D. reticulatum* and *D. invadens*. Ten replicates were  
162 conducted for each slug species and for each period without food (total of 100 experiments  
163 including controls) and these were monitored until larvae pupariated or died before  
164 pupariation.

## 165 **2.5 Comparison of *P. hermaphrodita* -exposed and control slugs (*D. reticulatum*) on third 166 instar *T. elata* prey choice**

167 Feeding trials of third instar larvae on nematode-exposed versus control *D. reticulatum*  
168 were undertaken to determine whether nematode-exposed slugs were more or less  
169 susceptible to predation by third instar *T. elata* larvae. Slugs ( $n=11$ ) of similar weights were  
170 distinguished from each other using colored elastomers (blue for exposed slugs and orange  
171 for control) injected just below surface of the foot of the slug (Mc Donnell and Gormally,  
172 2011). One exposed and one control slug were then placed together with one third instar *T.*  
173 *elata* larva in individual Petri dishes which were sealed with Parafilm® to prevent the slugs  
174 escaping. Larval food choice was recorded, and subsequent slugs provided thereafter  
175 reflected the initial food choice (i.e. a control or nematode exposed slug). This process  
176 continued until the larvae pupariated or died before pupariation.

## 177 **2.6 Larval feeding behavior**

178 While the experiments described in 2.4 (above) examined the effects of absence of prey on  
179 *T. elata* development and survival, further experiments were undertaken to examine the  
180 feeding behavior of larvae after different durations without prey. With this in mind, the  
181 attacking and feeding behavior of neonate and third instar *T. elata* larvae were observed  
182 using *D. reticulatum* and *D. invadens*. The latter was selected since, to date, the first (and  
183 second) instar of *T. elata* has been recorded as a known obligate parasitoid of *D. reticulatum*  
184 and *D. laeve* only (Knutson et al., 1965). Trials began on 14<sup>th</sup> November 2017 using neonate  
185 larvae hatched from eggs collected from laboratory-reared, second generation adults.  
186 Separate behavioral experiments using *D. reticulatum* and *D. invadens* were conducted by  
187 exposing neonate *T. elata* larvae to a slug on the day of hatching, 3 days after hatching, and  
188 6 days after hatching. In each case, 10 replicates for each slug/*T. elata* larval treatment were  
189 conducted resulting in 60 replicates in total with each slug being weighed before the  
190 experiments commenced. Each replicate was directly observed for up to a maximum of 8  
191 hours and larval behavior categorized according to Hynes *et al.* (2014c). In cases where  
192 larvae did not feed during the 8-hour observation period and had not fed by the following  
193 morning, new experiments were set up using fresh slugs and new *T. elata* larvae to ensure  
194 the completion of 60 successful feeding experiments in total.

195 Feeding behavior trials with third instar *T. elata* specimens were also undertaken using  
196 larvae which had been fed on either *D. reticulatum* only or *D. invadens* only from the time of  
197 hatching. Once the larvae reached third instar, they were not fed for 3-8 days to maximize  
198 the chances that larvae would demonstrate predatory behavior (Hynes et al., 2014c). Fifteen

199 replicates for *D. invadens* using larvae previously fed on *D. invadens* only and 12 replicates  
200 for *D. reticulatum* using larvae previously fed on *D. reticulatum* only were used for this trial.  
201 Third instar trials took place in a darkened room and were recorded using the “Nightshot”  
202 setting on a SONY Handycam FDR – AX33. This permitted direct observations in darkened  
203 conditions which would be more similar to field situations. Using this approach, however,  
204 was not possible for first instar behavioral trials due to their small size.

## 205 **2.7 Statistical analyses**

206 While *T. elata* occurs widely in the Palaearctic region, it has a patchy distribution because of  
207 which it can sometimes be challenging to find specimens in large quantities in the field.  
208 Nevertheless, where there were sufficient numbers of replicates in this study, statistical  
209 comparisons between the treatments were undertaken in SPSS (IBM, SPSS Statistics v. 24)  
210 using non-parametric Mann–Whitney *U* tests. The Spearman’s rank correlation coefficient  
211 was used to determine the correlations between the treatments. Statistical differences in  
212 larval feeding behaviors were predicted using a chi square test. All data were analyzed at  
213 the  $P < 0.05$  standard level of significance.

214

## 215 **3. Results**

### 216 **3.1 Egg-laying by laboratory-reared *T. elata***

217 The mean number of eggs laid per female was 291.4 eggs ( $\pm 50.5$  SE) (Fig. 1) with a total of  
218 1,457 eggs laid over a period of 38 days by five paired individuals that emerged from  
219 laboratory-reared pupae. The mean pre-oviposition period was 5.2 days ( $\pm 0.73$  SE) and the  
220 total number of eggs laid by individual females ranged from 207 to 487 eggs (Fig. 1), with  
221 the numbers laid each day ranging from 1 to 46 eggs per female. The mean duration of the  
222 egg stage ( $n = 31$ ) at laboratory/room temperature was 9.3 days ( $\pm 0.17$ ). No significant  
223 correlations were found between the age at which the males died, and total number of eggs  
224 laid by the females ( $R = 0.82$ ,  $P = 0.089$ ,  $n = 5$ ) nor between the age at which the females  
225 died and the number of eggs laid ( $R = 0.5$ ,  $P = 0.391$ ,  $n = 5$ ).

### 226 **3.2 *T. elata* neonate larval development and survival on control slugs and slugs exposed to 227 *P. hermaphrodita***

228 This experiment demonstrated that neonate *T. elata* larvae were able to feed successfully  
229 on another slug species (i.e. *D. invadens*) apart from *D. reticulatum* and *D. laeve* (as  
230 previously described by Knutson *et al.* [1965]). In larvae reared on control slugs, the  
231 pupariation rate was, however, significantly ( $U = 20$ ,  $P = 0.023$ ) less when larvae fed on *D.*  
232 *invadens* only (just four out of 10 pupariated successfully) compared to *D. reticulatum* only  
233 (all 10 larvae pupariated successfully) (Fig. 2). In addition, the duration of the larval stage  
234 was significantly longer ( $U = 2$ ,  $P = 0.008$ ) for *D. invadens*-reared larvae than for *D.*  
235 *reticulatum*-fed larvae (Table 1). Despite this, there were no significant differences between  
236 slug species tested regarding the weight of slug killed per larva ( $U = 15.5$ ,  $P = 0.53$ ) and  
237 puparial weight ( $U = 11$ ,  $P = 0.24$ ) (Table 1).

238 Neonate survivorship to puparial stage was less successful where *D. reticulatum* was  
239 exposed to *P. hermaphrodita* with only two out of 11 (18.2%) larvae pupariating successfully  
240 compared to 100% successful pupariation using control slugs (Fig. 2a). There was little



241 difference in mean larval period and mean pupal weight between control and nematode-  
242 exposed *D. reticulatum*, although mean slug weight killed per larva was almost 40% greater  
243 for larvae reared on control versus nematode exposed *D. reticulatum* (Table 1). Similar poor  
244 pupariation rates (Fig. 2b) and low slug weights killed per larva were observed for neonate  
245 larvae fed on *D. invadens* exposed to the nematodes, in addition to which mean larval  
246 period was considerably reduced in nematode-exposed *D. invadens* compared to controls  
247 (Table 1).

### 248 **3.3 Comparison of availability of *P. hermaphrodita* nematode-exposed and control slugs** 249 **(*D. reticulatum*) on third instar *T. elata* prey choice**

250 When offered the choice of either control *D. reticulatum* or *D. reticulatum* exposed to  
251 nematodes, third instar larvae chose to feed more frequently on nematode-exposed slugs  
252 than control slugs ( $\chi^2 (n = 11) = 4.54, P = 0.033$ ). However, while the three larvae which  
253 initially selected a control slug (and were subsequently fed on control slugs only) pupariated  
254 successfully, only six of the eight larvae which initially selected a nematode-exposed slug  
255 (and were subsequently fed on nematode-exposed slugs only) pupariated successfully.

### 256 **3.4 Effect of absence of prey on neonate *T. elata* larval development and survival**

257 While more than 95% of neonate *T. elata* larvae ( $n = 50$ ) survived without prey for seven  
258 days (Fig. 3), by day 14 all larvae were dead. The impact of different duration periods  
259 without food post-egg-hatching on subsequent larval development showed that successful  
260 pupariation was significantly more frequent for neonate larvae without food for 4 days  
261 (subsequently fed on *D. reticulatum*) compared to neonate larvae maintained without food  
262 for the first 6 days ( $\chi^2 (n = 10) = 5.49, P = 0.019$ ) as well as 8 and 10 days respectively ( $\chi^2 (n =$   
263  $10) = 9.9, P = 0.0016$ , for both 8 and 10 days) (Fig. 4).

264 The pupariation success of larvae fed on *D. invadens* in comparison with *D. reticulatum* after  
265 four days was also significantly ( $\chi^2 (n = 10) = 12.8, P = 0.0003$ ) lower. Only 10% pupariated  
266 successfully after 4 and 6 days without food, and none pupariated after 8 and 10 days  
267 without food (Fig. 4). While it is difficult to draw firm conclusions due to larval mortalities,  
268 there appears to be little difference in mean slug weight killed per larva between controls  
269 and those larvae where food was absent at the neonate stage regardless of the slug species  
270 tested (Table 2).

### 271 **3.5 Larval feeding behavior**

272 Given that no obvious differences in feeding behavior were observed between larvae which  
273 were allowed to feed on both slug species immediately after hatching and those starved for  
274 3 and 6 days respectively (Appendix 1), these data were combined to give an overall picture  
275 of larval feeding behavior. An examination of the predation strategies adopted by *T. elata*  
276 larvae on *D. reticulatum* and *D. invadens* revealed significant differences between neonate  
277 ( $n = 60$ ) and third instar ( $n = 27$ ) larvae ( $\chi^2 = 18.67, df = 2, P < 0.05$ ) (Fig. 5). Over 50% of  
278 neonate larvae simply waited for slugs to pass nearby before attacking with 35% searching  
279 first followed by waiting, and 13.3 % actively searching and attacking slug prey. In  
280 comparison, 59.2% of third instar larvae actively searched and attacked slugs with 22.2 %  
281 actively searching and then waiting; and only 18.5 % simply waiting for a slug to pass by. The  
282 mode of attacking, latching on (position adopted by larva prior to immobilizing the slug  
283 [Hynes et al., 2014c]) and feeding also varied according to the larval stage (Fig. 6). While

284 there appeared to be little difference in the location on the slug attacked by neonate larvae,  
285 the majority (18 out of 27 - 66.7%) of third instar larvae attacked slug tails. The tail of the  
286 slug was also the most popular location for latching on by third instar larvae but was the  
287 least popular location for latching for neonate larvae. While the latero-ventral surface of the  
288 slug was the most frequently recorded feeding site for neonate larvae, there was no clear  
289 preference among third instar larvae. All the neonate larvae were subsequently found  
290 feeding under the slug mantle one day after the feeding trials commenced.

291

#### 292 **4. Discussion**

293 The number of eggs laid by laboratory-reared flies ranged from 207-487 eggs, similar to  
294 results reported by previous studies (Knutson et al., 1965; Beaver, 1973; and Hynes et al.,  
295 2014a). Knutson *et al.* (1965) suggested that repeated copulation is not necessary for  
296 fertilizing large numbers of eggs and this is supported by Hynes *et al.* (2014a), who recorded  
297 one field-caught female laying 166 eggs (44% of which subsequently hatched) without a  
298 male partner in the laboratory. However, in the current study, one of the females where the  
299 male partner survived longest, and which was observed pairing repeatedly, laid 487 eggs.  
300 The potential effects of repeated mating on egg production requires further investigation to  
301 ensure maximum egg production in large scale cultures for biological control programs.

302 Prior to commencing the behavior experiments, the survivorship of neonate *T. elata* larvae  
303 in the absence of prey was measured. Our results showed that larvae can survive without  
304 food for up to two weeks, although their ability to feed and survive on a host is greatly  
305 reduced after six days without food to below 40%. This contrasts with the findings of  
306 Knutson *et al.* (1965) who stated that neonate *T. elata* larvae die after four or five days  
307 without food. This could be due to their experiments being conducted in laboratories in  
308 continental Europe where late summer and early fall temperatures can be higher than in the  
309 west of Ireland, thereby increasing metabolic rates (Gormally, 1988). Knutson *et al.* (1965)  
310 also stated that *T. elata* neonates are host-specific to only *D. reticulatum* and *D. laeve*. While  
311 the results of our study demonstrate that *T. elata* neonate larvae can be reared on both *D.*  
312 *invadens* and *D. reticulatum*, larval survivorship to pupariation was greatly reduced on *D.*  
313 *invadens* compared to *D. reticulatum*. Additionally, larval development was significantly  
314 longer when larvae were reared on control *D. invadens* than on control *D. reticulatum*  
315 despite there being little difference in mean slug weight killed or final puparial weight  
316 between slug species. While this could be due to differences in the nutritional value (prey  
317 quality) between both control slugs, similar mean larval durations for *T. elata* larvae when  
318 fed on nematode-exposed *D. reticulatum* and *D. invadens* suggests that more than one  
319 factor may be at play and further experiments with more replicates are required to provide  
320 an answer for this.

321 This ability of neonate larvae to feed on a number of pest slug species makes *T. elata* more  
322 important as a biological control agent against pestiferous slugs. While the reasons for  
323 greater mortality of larvae feeding solely on *D. invadens* are yet unknown, third instar larvae  
324 in the wild would likely have a choice of other slug species on which to feed which could  
325 improve chances of successful pupariation but further experiments are required to address  
326 this question.

327 Of the three larval feeding behavior strategies observed (searching & attacking, searching &  
328 waiting, and waiting; see Hynes *et al.* (2014c)), more than half (51.6%) of neonate larvae  
329 exhibited the “waiting” response, while for third instar larvae, almost 60% of larvae  
330 displayed the “searching & attacking” response without any physical contact being made by  
331 the third instar larva with a slug. Foraging animals are typically classified as either “ambush”,  
332 in which they stay motionless for long periods of time waiting for their prey to pass by, or  
333 “cruise” foragers, searching actively for their prey (O’Brien *et al.*, 1990). We show here that  
334 *T. elata* displays different strategies during different life stages, which is supported by  
335 Knutson and Vala (2011) who have noted the labile nature of feeding behavior in  
336 Sciomyzidae in general. Since *T. elata* is an obligate parasitoid in its first and second larval  
337 stages, the neonate waiting response probably reflects general parasitoid behavior. This  
338 type of behavior has been observed in the juvenile stage of parasitoid wasps when in the  
339 vicinity of their hosts (Mohamad *et al.*, 2015). Waiting also suggests the behavior of a  
340 generalist parasitoid (as evidenced by neonate *T. elata* larvae feeding on more than one  
341 species of slug) since specialist parasitoids tend to actively search and find their hosts (Wang  
342 and Keller, 2002). The preference among third instar larvae reflects more the behavior of  
343 predators (searching & attacking) and this is supported by previous studies on third instar  
344 larval behavior by Hynes *et al.* (2014c). The latero-ventral region of the slug appears to be  
345 the preferred area for “latching” and “feeding” by neonate *T. elata* larvae while the slug tail  
346 region for both “attacking” and “latching” appear to be preferred by third instar larvae. No  
347 obvious trend was discernible for sites of “attack” for neonate larvae or sites of “feeding”  
348 for third instar. While Hynes *et al.* (2014c) studied the behavior of only limited numbers of  
349 third instar larvae, they found that the slug tail was also used for latching but was less  
350 popular than the latero-ventral surface of the slug. Their findings are in contrast with the  
351 results of the current study where the slug tail appeared to be the most preferred site for  
352 attacking and latching. Further investigation is required to explore contributing factors to  
353 variations in individual predatory strategies and to determine how “searching & attacking”  
354 can be more frequently encouraged as this would pose a clear advantage for an effective  
355 biological control agent when used by an active predator (Matthews and Matthews 2009).

356 Since many neonate larvae wait until a slug passes before attacking, it is vital to determine  
357 the length of time a neonate larva can survive before successfully obtaining its first slug  
358 host. Not surprisingly, neonate larvae reared on *D. invadens* had low survival rates up to  
359 pupariation (10%) after four and six days without food and none survived after eight and ten  
360 days without a host. In contrast, neonate larvae reared on *D. reticulatum* resulted in 90%,  
361 40%, 20%, and 20% survival up to pupariation after four, six, eight, and ten days  
362 (respectively) without a host. It is interesting to note a 90% survival rate after four days  
363 without a host for larvae reared on *D. reticulatum*, indicating a likely adaptation to facilitate  
364 the “waiting” behavior exhibited by many neonate larvae. This survival period is likely to be  
365 longer in the wild where temperatures would frequently be below those recorded in the  
366 laboratory where these experiments were conducted.

367 Given that *P. hermaphrodita* is currently the only commercially-available nematode  
368 biocontrol agent of slugs on the market (Askary *et al.*, 2017), the implications of using this in  
369 areas where *T. elata* is naturally-occurring is of interest. The key question here is whether  
370 both *T. elata* and *P. hermaphrodita* could be used in tandem as a part of an integrated pest  
371 management (IPM) approach or whether slugs exposed to *P. hermaphrodita* may have an  
372 adverse effect on *T. elata* larvae. The survivorship of neonate larvae to pupariation when

373 reared only on nematode-exposed *D. reticulatum* was just 20% compared to 100%  
374 survivorship for controls, while in neonates reared on similarly exposed *D. invadens*  
375 survivorship was also 20% in comparison to 40% for controls. The average number of slugs  
376 killed, larval period, and puparial weight by both control and nematode-exposed *D.*  
377 *reticulatum* did not show any major differences. Although mean larval duration was shorter  
378 and mean weight of slug killed per larva was less for nematode-exposed *D. invadens*, it is  
379 difficult to make concrete inferences for *D. invadens* given the low number of individuals  
380 which survived to pupariation ( $n = 2$ ).

381 Where third instar larvae were given the choice of feeding on either control or nematode-  
382 exposed *D. reticulatum*, they showed a preference for the nematode-exposed slugs. One  
383 possible explanation for this is that *P. hermaphrodita*-exposed slugs could be  
384 immunocompromised making them an easier target for *T. elata* larvae, but further research  
385 needs to be undertaken using recorded trials for review and confirmation of results to prove  
386 this definitively. Only 75% (six out of eight) of those third instar larvae which selected  
387 nematode-exposed slugs and were subsequently fed only nematode-exposed slugs  
388 pupariated successfully compared to successful pupariation for all third instar larvae fed on  
389 control *D. reticulatum*. The fact that third instar larvae may preferentially select nematode-  
390 exposed *D. reticulatum* over non-exposed individuals with a subsequent lower than  
391 expected outcome for successful pupariation may be of some concern. Nermuť *et al.* (2014)  
392 shows that *P. hermaphrodita* use slug tissue as a nutrient-rich source for reproduction and it  
393 is possible that this may affect the subsequent nutritional value for *T. elata* larvae. However,  
394 this experiment needs to be repeated with a greater number of replicates and where third  
395 instar larvae are permitted to choose between nematode-infected and non-infected slugs  
396 each time they attack a new slug. Limited numbers of larvae prevented such experiments  
397 being conducted in this study. Similarly, the no-choice feeding trials where the neonate  
398 larvae were given no choice other than to parasitize nematode-exposed slugs resulted in  
399 very low survivorship to pupariation (20%). This low survivorship could possibly be due to a  
400 deterrent effect by the nematode-bacteria complex which defends the host slug against  
401 being predated or scavenged by other organisms as a result of bacterial metabolites  
402 associated with the nematodes (Pechova & Foltan 2008; Foltan & Puza 2009). On the other  
403 hand, given the findings of Wilson *et al.* (1994) and Rae *et al.* (2010) that *P. hermaphrodita*  
404 has large amounts of associated bacteria, the possibility that they produce toxins that affect  
405 *T. elata* larvae directly requires further investigation.

406 In conclusion, our results show that the maximum number of eggs laid by laboratory-  
407 eclosed *T. elata* females is greater than previously recorded for field-caught females and  
408 that neonate larvae are capable of parasitizing slug species (i.e., *D. invadens*) other than the  
409 previously recorded *D. reticulatum* and *D. laeve*. Neonate larvae, which primarily exhibit a  
410 “waiting” strategy to find a slug host, can survive more than 10 days without food. However,  
411 larvae have a greater chance of reaching the puparial stage if they feed on *D. reticulatum*  
412 throughout the larval stage than if they feed on *D. invadens*. Results also indicate that while  
413 predatory third instar *T. elata* larvae appear to select nematode-exposed *D. reticulatum*  
414 over non-exposed slugs, continued feeding on nematode-exposed slugs during development  
415 reduces the chances of successful pupariation. While this study suggests that feeding on *D.*  
416 *invadens* and nematode-exposed slugs can reduce the chances of *T. elata* pupariating  
417 successfully, further work involving prey-choice experiments throughout the larval stage is  
418 required. This will be particularly important in predicting biotic factors (e.g. prey type) that

419 may determine shifts in *T. elata* populations in the field which consequently may affect  
420 pestiferous slug populations.

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425 **Declarations of interest:** None

426

#### 427 **References**

428 Anderson, R., 2016. *Deroceras (Deroceras) invadens* Reise, Hutchinson, Schunack & Schlitt  
429 2011. [Online]. Available at: <http://www.habitas.org.uk/molluscireland/species.asp?ID=73>  
430 (Accessed: 4<sup>th</sup> /February/2018).

431 Askary, T.H., 2010. Nematodes as biocontrol agents. In: Sociology, Organic Farming, Climate  
432 Change and Soil Science (Ed. E. Lichtfouse). Springer, Netherlands, pp. 347-378.

433 Askary, T.H., Nermuť, J., Ahmad, M.J., Ganai, M.A., 2017. Future thrusts in expanding the  
434 use of entomopathogenic and slug parasitic nematodes in agriculture. In: Biocontrol Agents:  
435 Entomopathogenic and Slug Parasitic Nematodes (eds. M.M.M. Abd-Elgawad, T.H. Askary  
436 and James Coupland). CAB International, Wallingford, UK, pp. 620-627.

437 Beaver, O., 1973. Egg laying studies on some British sciomyzid flies (Diptera: Sciomyzidae).  
438 *Hydrobiologia*, 43, 1-12.

439 Busquets, R., Kozynchenko, O. P., Whitby, R. L., Tennison, S. R., Cundy, A. B., 2014. Phenolic  
440 carbon tailored for the removal of polar organic contaminants from water: A solution to the  
441 metaldehyde problem? *Water Research* 61, 46-56.

442 Carnaghi, M., Rae, R., De Ley, I.T., Johnston, E., Kindermann, G., Mc Donnell, R.J., O'Hanlon,  
443 A., Reich, I., Sheahan J., Williams C.D., Gormally M.J., 2017. Nematode associates and  
444 susceptibility of a protected slug (*Geomalacus maculosus*) to four biological control  
445 nematodes. *Biological Control Science and Technology* 27:294-299.

446 Castle, G., Mills, G., Gravell, A., Jones, L., Townsend, I., Cameron, D., Fones, G., 2017. Review  
447 of the molluscicide metaldehyde in the environment. *Environmental Science: Water  
448 Research & Technology* 3(3): 415-428.

449 Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F., Partridge, L., 1995. Cost of mating in  
450 *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*  
451 373: 241-244.

452 Cordoba, M., Millar, J.G., Mc Donnell, R.J., 2018. Development of a High-Throughput  
453 Laboratory Bioassay for Testing Potential Attractants for Terrestrial Snails and Slugs. *J Econ  
454 Entomol* 111: 637-644.

455 Dankowska, E., 2006. Laboratory studies on the use of a nematode *Phasmarhabditis*  
456 *hermaphrodita* (Schneider) in slug control. *Folia Malacologica* 14.

457 Ester, A., Trul, R., 2000. Slug damage and control of field slug (*Deroceras reticulatum*  
458 (Müller)) by carvone in stored potatoes. *Potato Research* 43:253-261.

459 European Commission, 2014. Commission implementing regulation (EU) No 187/2014. The  
460 Official Journal of the European Union.

461 European Commission, 2015. Commission implementing regulation (EU) 2015/404. The  
462 Official Journal of the European Union.

463 Foltan, P., Puza, V., 2009. To complete their life cycle, pathogenic nematode-bacteria  
464 complexes deter scavengers from feeding on their host cadaver. *Behavioural Processes*. 80:  
465 76-79.

466 Frank, T., 1998. Slug damage and numbers of the slug pests, *Arion lusitanicus* and *Deroceras*  
467 *reticulatum*, in oilseed rape grown beside sown wildflower strips. *Agriculture, Ecosystems &*  
468 *Environment* 67:67-78.

469 Fritz, R.S., Hochwender, C.G., Lewkiewicz, D.A., Bothwell, S., Orians, C.M., 2001. Seedling  
470 herbivory by slugs in a willow hybrid system: developmental changes in damage, chemical  
471 defence, and plant performance. *Oecologia* 129:87-97.

472 Godan, D., 1983. *Pest slugs and snails. Biology and control.* Springer-Verlag.

473 Gormally, M. J., 1988. Temperature and the biology and predation of *Ilione albiseta*  
474 (Diptera: Sciomyzidae)—potential biological control agent of liver fluke. *Hydrobiologia*  
475 166(3): 239-246.

476 Gould, H., 1961. Observations on slug damage to winter wheat in East Anglia, 1957–1959.  
477 *Plant Pathology* 10:142-146.

478 Hasan, S., Vago, C., 1966. Transmission of *Alternaria brassicicola* by slugs. *Plant Disease*  
479 *Reporter* 50:764-767.

480 Howlett, S. A., 2012. Terrestrial slug problems: classical biological control and beyond. *CAB*  
481 *Reviews* 7 (051): 1-10.

482 Hutchinson, J., Reise, H., Robinson, D., 2014. A biography of an invasive terrestrial slug: the  
483 spread, distribution and habitat of *Deroceras invadens*. *NeoBiota* 23:17.

484 Hynes, T., Mc Donnell, R.J., Gormally, M.J., 2014a. Oviposition, adult longevity and  
485 temperature effects on the eggs of *Tetanocera elata* (Fab.) (Diptera: Sciomyzidae): a  
486 potential biological control agent for slugs. *Journal of Applied Entomology* 138:670-676.

487 Hynes, T., Mc Donnell, R.J., Kirsch, A., Dillon, R., O’Hora, R., Gormally, M.J., 2014b. Effect of  
488 temperature on the larval stage of *Tetanocera elata* (Diptera: Sciomyzidae) – Potential  
489 biological control agent of pestiferous slugs. *Biological Control* 74: 45-51.

490 Hynes, T., Giordani, I., Larkin, M., Mc Donnell, R.J., Gormally, M.J., 2014c. Larval feeding  
491 behaviour of *Tetanocera elata* (Diptera: Sciomyzidae): Potential biological control agent of  
492 pestiferous slugs. *Biological Control Science and Technology* 24:1077-1082.

493 Jaskulska, M., Kozłowski, J., Kozłowska, M., 2017. Susceptibility of field bean cultivars to slug  
494 damage. *Folia Malacologica* 25:273-280.

495 Jones, D., 2014. EU will ban methiocarb slug pellets after 19 Sept. [Online]. Available at:  
496 <http://www.fwi.co.uk/arable/eu-will-ban-methiocarb-slug-pellets-after-19-sept.htm>  
497 (Accessed: 1<sup>st</sup>/February/2018).

498 Kay, P., Grayson, R., 2014. Using water industry data to assess the metaldehyde pollution  
499 problem. *Water and Environment Journal* 28, 410-417.

500 Knutson, L., Stephenson, J., Berg, C., 1965. Biology of a slug-killing fly, *Tetanocera elata*  
501 (Diptera: Sciomyzidae). *Journal of Molluscan Studies* 36:213-220.

502 Knutson, L., Vala, J.C., 2011. The biology of snail-killing Sciomyzidae flies. Cambridge  
503 University Press.

504 Matthews, R.W., Matthews, J.R., 2009. Insect behavior. Springer Science & Business Media.

505 Mc Donnell, R.J., Gormally, M.J., 2011. Distribution and population dynamics of the Kerry  
506 Slug, *Geomalacus maculosus* (Arionidae). Irish Wildlife Manuals, No. 54. National Parks and  
507 Wildlife Service, Department of Arts, Heritage and the Gaeltacht, Dublin, Ireland: 1-93.

508 Mohamad, R., Wajnberg, E., Monge, J.P., Goubault, M., 2015. The effect of direct  
509 interspecific competition on patch exploitation strategies in parasitoid wasps. *Oecologia*  
510 177:305-315.

511 Nermuť, J., Půža, V., Mráček, Z., 2014. The effect of different growing substrates on the  
512 development and quality of *Phasmarhabditis hermaphrodita* (Nematoda: Rhabditidae).  
513 *Biocontrol Science and Technology* 24:1026-1038.

514 O'Brien, W.J., Browman, H.I., Evans, B.I., 1990. Search strategies of foraging animals.  
515 *American Scientist* 78: 152-160.

516 Pechova, H., Fontan, P., 2008. The parasitic nematode *Phasmarhabditis hermaphrodita*  
517 defends its slug host from being predated or scavenged by manipulating host spatial  
518 behaviour. *Behavioural Processes* 78: 416-420.

519 Pieterse, A., Malan, A., Ross, J., 2017a. Nematodes that associate with terrestrial molluscs as  
520 definitive hosts, including *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) and its  
521 development as a biological molluscicide. *Journal of Helminthology* 91:517-527.

522 Pieterse, A., Tiedt, L.R., Malan, A.P., Ross, J.L., 2017b. First record of *Phasmarhabditis*  
523 *papillosa* (Nematoda: Rhabditidae) in South Africa, and its virulence against the invasive  
524 slug, *Deroceras panormitanum*. *Nematology* 19:1035-1050.

525 Rae, R.G., Robertson, J.F., Wilson, M.J., 2008. Susceptibility and immune response of  
526 *Deroceras reticulatum*, *Milax gagates*, and *Limax pseudoflavus* exposed to the slug parasitic  
527 nematode *Phasmarhabditis hermaphrodita*. *J. Invertebrate Pathology* 97: 61-69.

528 Reise, H., Hutchinson, J., Schunack, S., Schlitt, B., 2011. *Deroceras panormitanum* and  
529 congeners from Malta and Sicily, with a re-description of the widespread pest slug as  
530 *Deroceras invadens* n. sp. *Folia Malacologica* 19:201.

531 South, A., 2012. Terrestrial slugs: biology, ecology, and control. Chapman & Hall, London.

532 Speiser, B., Glen, D., Piggott, S., Ester, A., Davies, K., Castillejo, J., Coupland, J., 2001. Slug  
533 damage and control of slugs in horticultural crops. Research Institute of Organic Agriculture

534 (FiBL). [Online]. Available at:  
535 [https://www.schneckenprofi.de/shops/schneckenprofi/downloads/slug\\_damage\\_and\\_cont](https://www.schneckenprofi.de/shops/schneckenprofi/downloads/slug_damage_and_control.pdf)  
536 [rol.pdf](https://www.schneckenprofi.de/shops/schneckenprofi/downloads/slug_damage_and_control.pdf) (accessed: 11<sup>th</sup>/June/2018).

537 Tan, L., Grewal, P.S., 2001. Infection behavior of the rhabditid nematode *Phasmarhabditis*  
538 *hermaphrodita* to the grey garden slug *Deroceras reticulatum*. Journal of Parasitology  
539 87:1349-1354.

540 Tulli, M.C., Carmona, D.M., López, A.N., Manetti, P.L., Vincini, A.M., Cendoya, G., 2009.  
541 Predación de la babosa *Deroceras reticulatum* (Pulmonata: Stylommatophora) por *Scarites*  
542 *anthracinus* (Coleoptera: Carabidae). Ecología Austral 19:55-61.

543 van Lenteren, J.C., Bolckmans, K., Köhl, J., Ravensberg, W.J., Urbaneja, A., 2018. Biological  
544 control using invertebrates and microorganisms: plenty of new opportunities. Biological  
545 Control 63:39-59.

546 Wang, X.G., Keller, M.A., 2002. A comparison of the host-searching efficiency of two larval  
547 parasitoids of *Plutella xylostella*. Ecological Entomology 27:105-114.

548 Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R., Varia, S., Lamontagne-  
549 Godwin, J., Thomas, S., Murphy, S., 2010. The economic cost of invasive non-native species  
550 on Great Britain. CABI Proj No VM10066:1-99.

551 Willis, J.C., Bohan, D.A., Choi, Y.H., Conrad, K.F., Semenov, M.A., 2006. Use of an  
552 individual-based model to forecast the effect of climate change on the dynamics,  
553 abundance, and geographical range of the pest slug *Deroceras reticulatum* in the UK. Global  
554 Change Biology 12:1643-1657.

555 Wilson, M., Glen, D., George, S., 1993. The rhabditid nematode *Phasmarhabditis*  
556 *hermaphrodita* as a potential biological control agent for slugs. Biological Control Science  
557 and Technology 3:503-511.

558 Wilson, M., Glen, D., Hughes, L., Pearce, J., Rodgers, P., 1994. Laboratory tests of the  
559 potential of entomopathogenic nematodes for the control of field slugs (*Deroceras*  
560 *reticulatum*) Journal of Invertebrate Pathology 64:182-187.

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571 **Table 1:** *T. elata* development from neonate to pupa when fed on either *D. reticulatum* or *D.*  
572 *invadens* using control slugs (Control) or slugs exposed to *Phasmarhabditis hermaphrodita*  
573 (*Ph*).

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575 **Table 2:** Comparison of neonate larval period to pupariation, mean number of slugs killed  
576 per larva and mean weights of slugs killed per larva feeding on either *D. reticulatum* or *D.*  
577 *invadens* \*.

578 \* While each experiment commenced with 10 larvae, n in each column represents the  
579 number of larvae which pupariated successfully.

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602 **Table 1**

<i>Tetanocera elata</i> development	<i>Deroceras reticulatum</i> (Mean $\pm$ SE)		<i>Deroceras invadens</i> (Mean $\pm$ SE)	
	Control <i>n</i> =10	<i>Ph</i> <i>n</i> = 2	Control <i>n</i> =4	<i>Ph</i> <i>n</i> = 2
Mean larval period (d)	38.20 $\pm$ 1.25	40 $\pm$ 3.00	51 $\pm$ 4.54	38. $\pm$ 5.00
Mean number of slugs killed / larva	8.30 $\pm$ 0.63	7.50 $\pm$ 0.50	9.75 $\pm$ 1.00	5.50 $\pm$ 0.50
Mean slug weight killed / larva (g)	0.27 $\pm$ 0.01	0.17 $\pm$ 0.01	0.28	0.17 $\pm$ 0.01
Mean pupal weight (g)	0.05	0.05	0.04	0.03

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605 **Table 2**

Starvation time (d)	0 (Control)		4		6		8		10	
	<i>D. reticulatum</i> n=10	<i>D. invadens</i> n=4	<i>D. reticulatum</i> n=9	<i>D. invadens</i> n=1	<i>D. reticulatum</i> n=4	<i>D. invadens</i> n=1	<i>D. reticulatum</i> n=2	<i>D. invadens</i> n=0	<i>D. reticulatum</i> n=2	<i>D. invadens</i> n=0
Mean larval period (d)	38.2±1.25	51±4.64	37±1.05	30.00	37.5±1.44	34.00	43.5±0.5	L. Died	37±1.00	L. Died
Mean number of slugs killed / larva	8.3±0.63	9.75±1.71	7.9±0.61	9.00	8.5±0.96	9.00	6.5±0.5	L. Died	10±3.00	L. Died
Mean slug weight killed / larva (g)	0.27±0.01	0.28±0.01	0.27±0.02	0.30	0.25±0.01	0.27	0.25±0.01	L. Died	0.23±0.02	L. Died

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607 **Figure 1:** Mean cumulative and cumulative number of eggs laid per day by 5 laboratory-  
608 reared *T. elata* adult females. The date each fly died is marked with an arrow (black = males;  
609 grey = females).

610 **Figure 2:** Survivorship of neonate larvae to puparial stage when fed solely on either (a) *D.*  
611 *reticulatum* or (b) *D. invadens* control ( $n = 10$ ) and nematode-exposed slugs ( $n = 11$ ).

612 **Figure 3:** Survivorship of neonate *T. elata* larvae in the absence of slug prey ( $n = 50$ ).

613 **Figure 4:** Impact of absence of prey from 4 to 10 days during the neonate larval stage on *T.*  
614 *elata* pupariation success ( $n = 10$ ).

615 **Figure 5:** Predatory strategies (Searching & Attacking [SA], Searching & Waiting [SW] and  
616 Waiting [W]) adopted by *T. elata* larvae ( $n = 60$  neonate larvae;  $n = 27$  third instar larvae) in  
617 the presence of slugs (*D. reticulatum* and *D. invadens* datasets combined).

618 **Figure 6:** Attacking, latching\* and feeding locations of (a) neonate and (b) third instar *T.*  
619 *elata* larvae on slugs (*D. reticulatum* and *Deroceras invadens* datasets combined) (black =  
620 head of slug; light grey = latero-ventral surface of slug; grey = tail of slug).

621 \*Latching: Position adopted by larva before immobilising the slug (after Hynes et al., 2014c)

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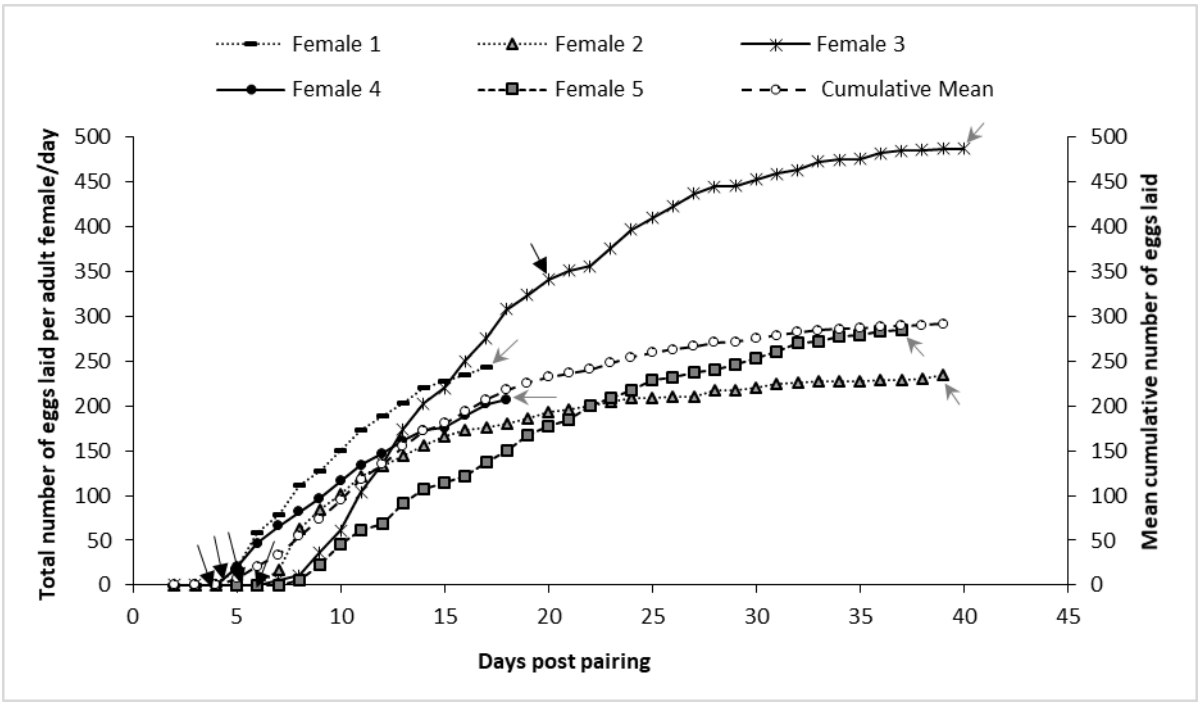
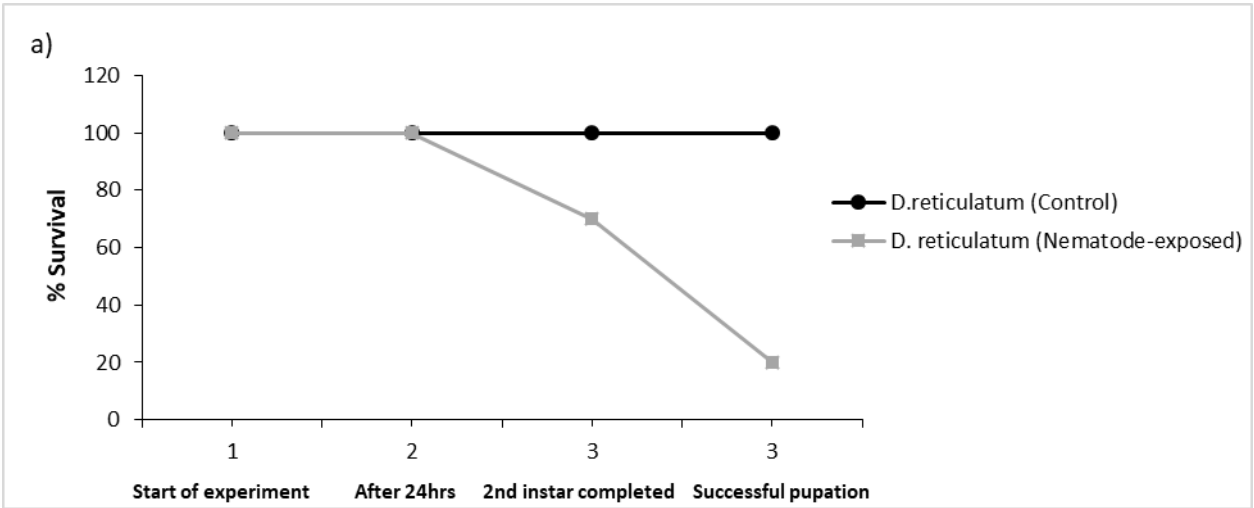


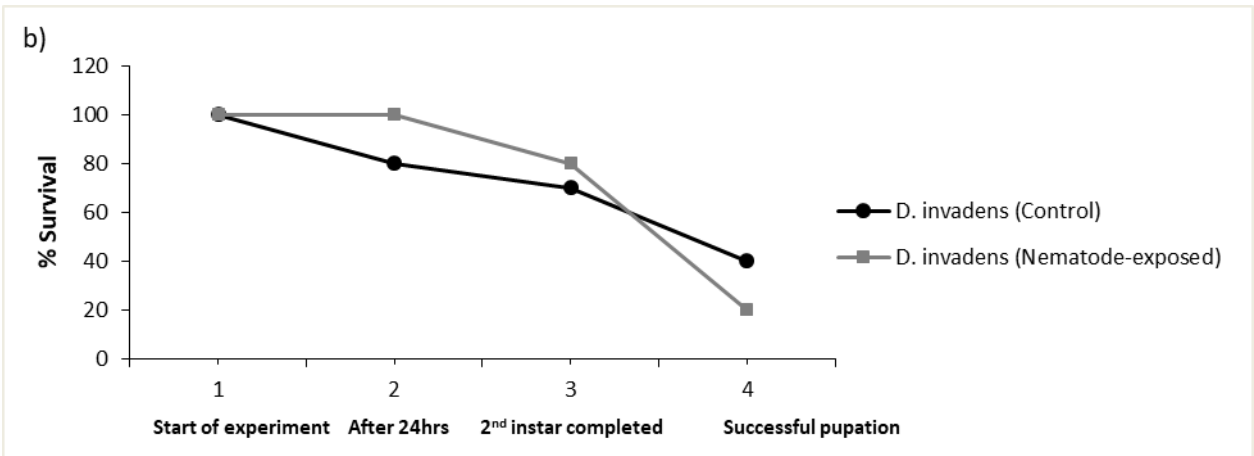
Figure 1



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670 **Figure 2a**

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673 **Figure 2b**

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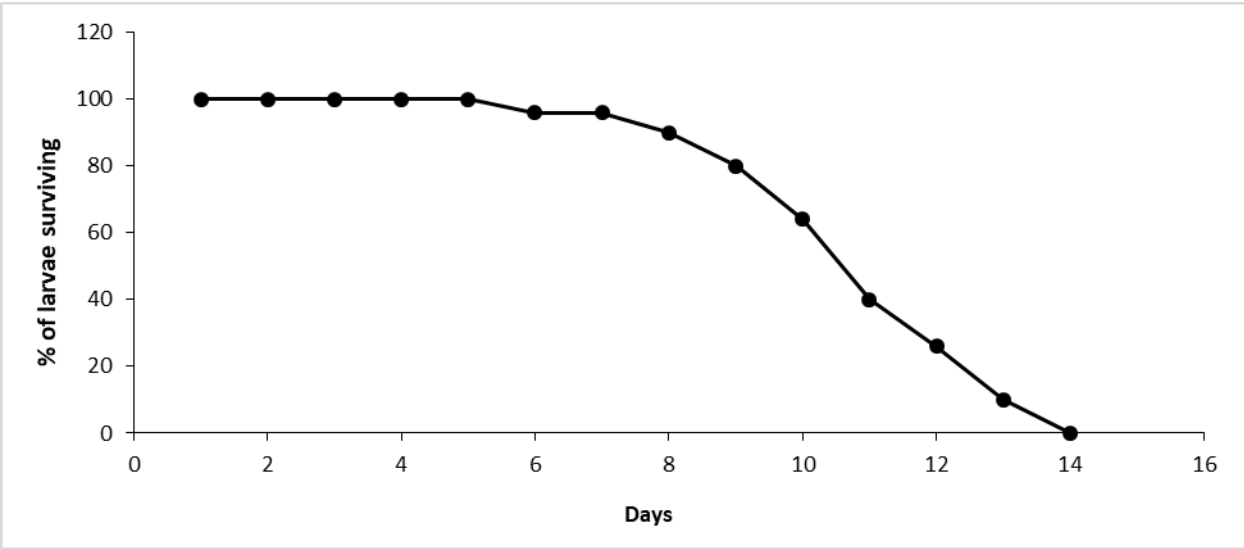
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685 **Figure 3**

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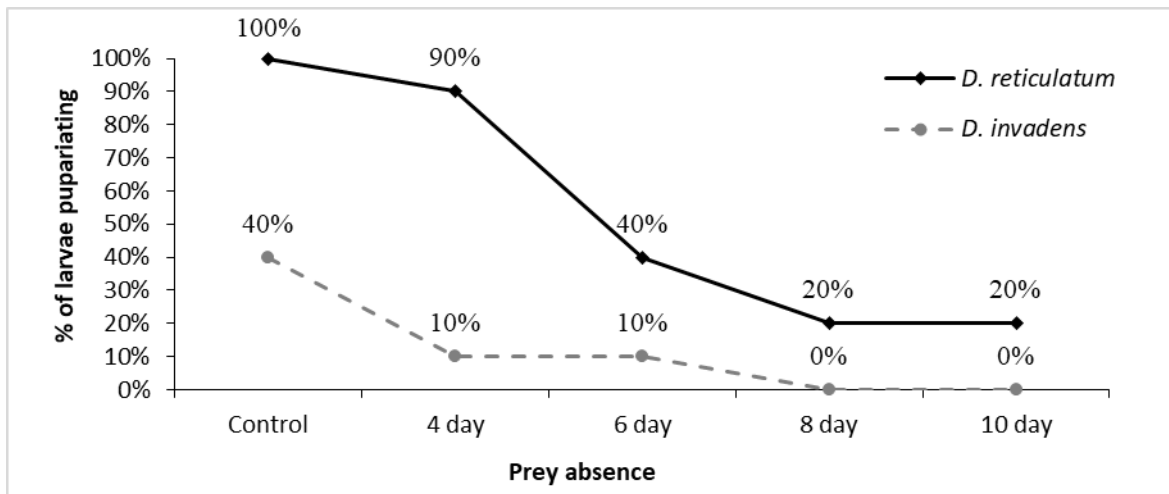
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704 **Figure 4**

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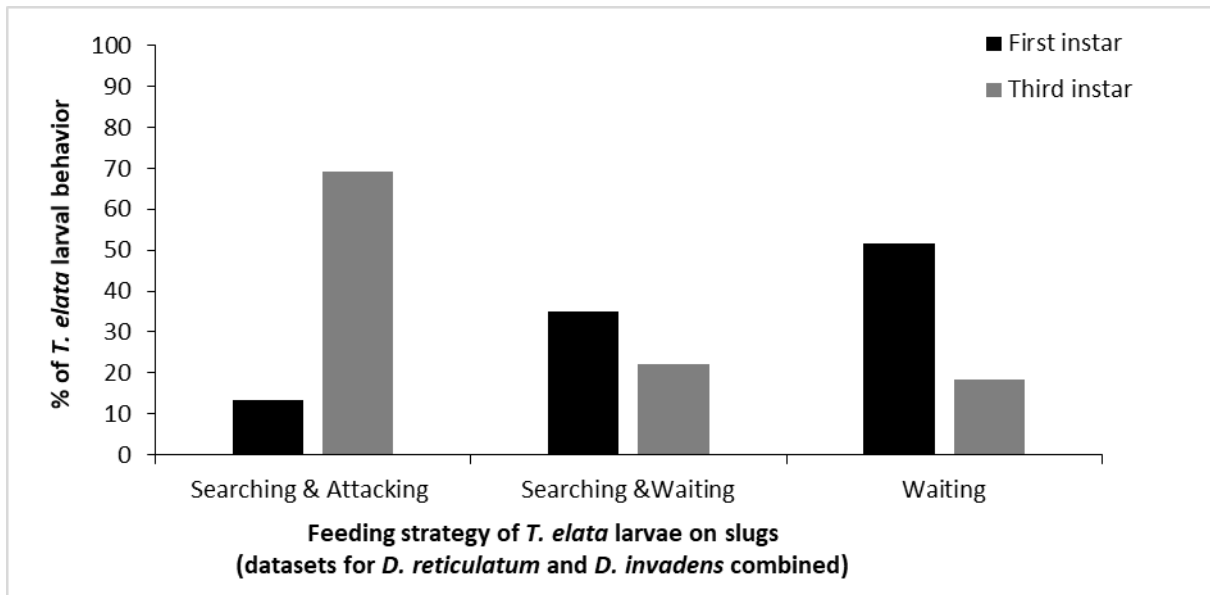
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722 **Figure 5**

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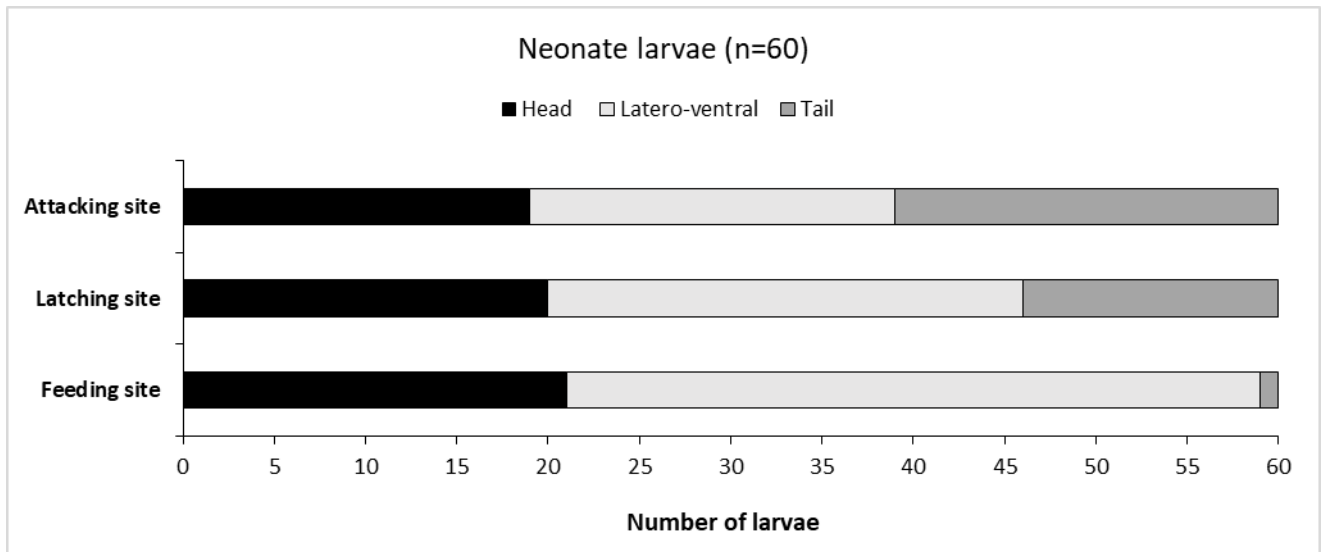
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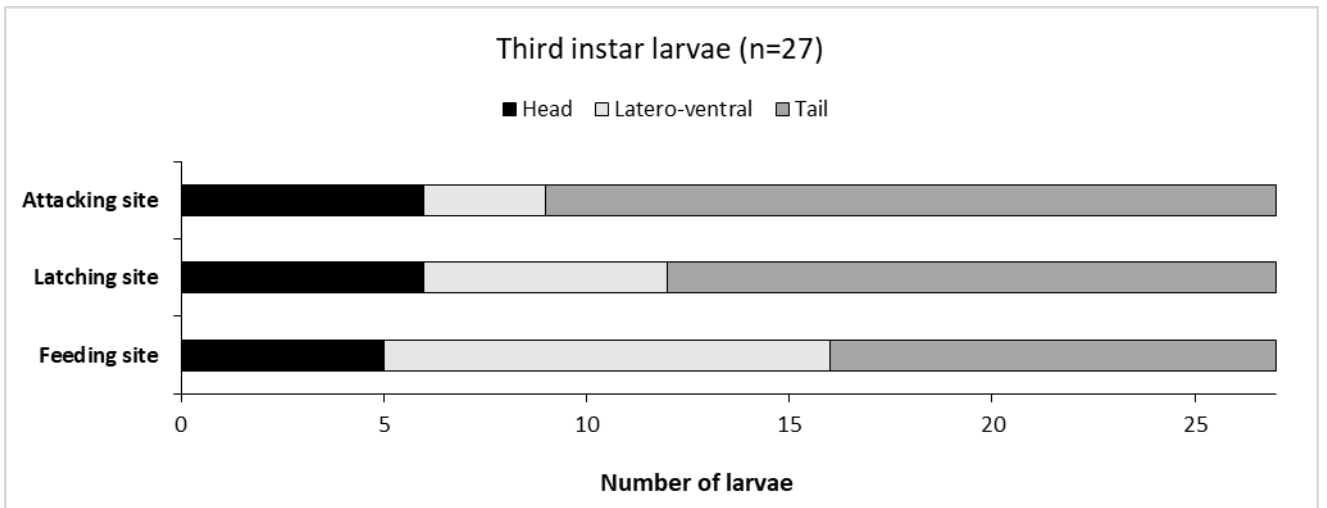
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742 **Figure 6 a**

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745 **Figure 6 b**

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752 Appendix 1: Larval behavior-neonate larval behavior (a-d); third instar larval behavior (e-h).

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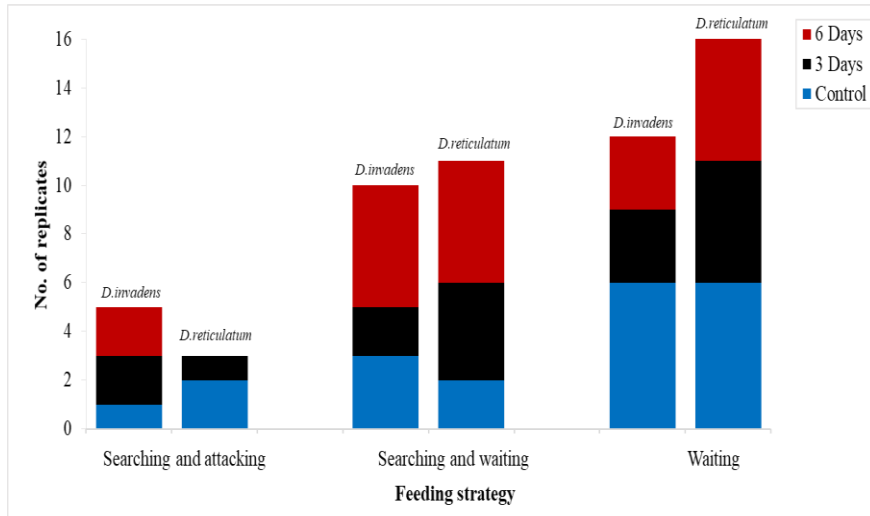
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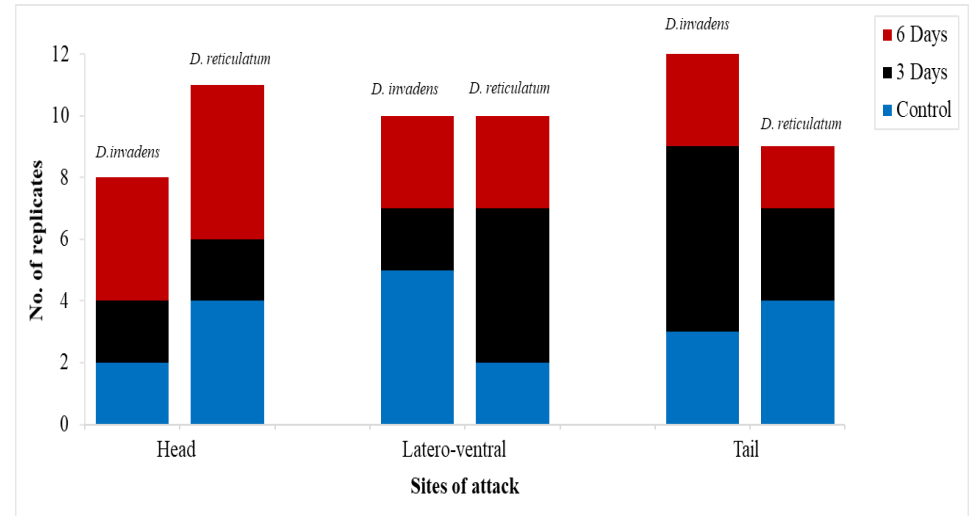
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763 a- Feeding strategy of neonate *T. elata* larvae on both slug species



764 b- Sites of neonate *T. elata* larvae attack on both slug species

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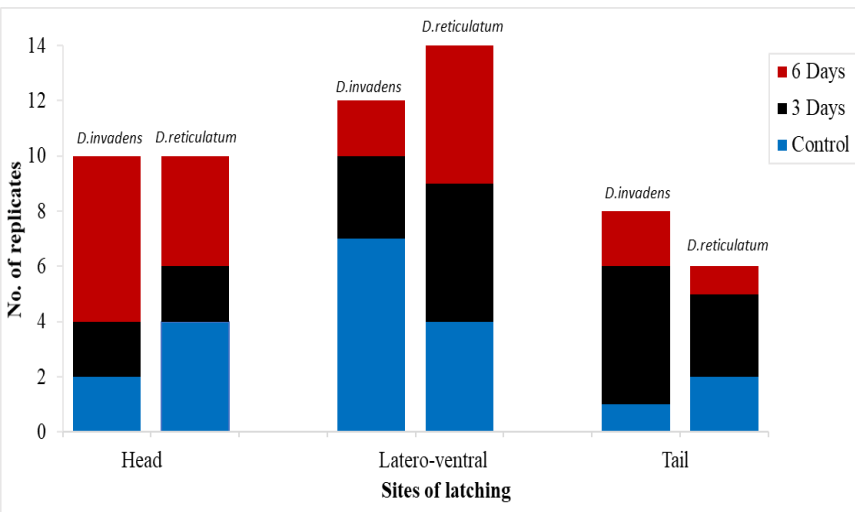
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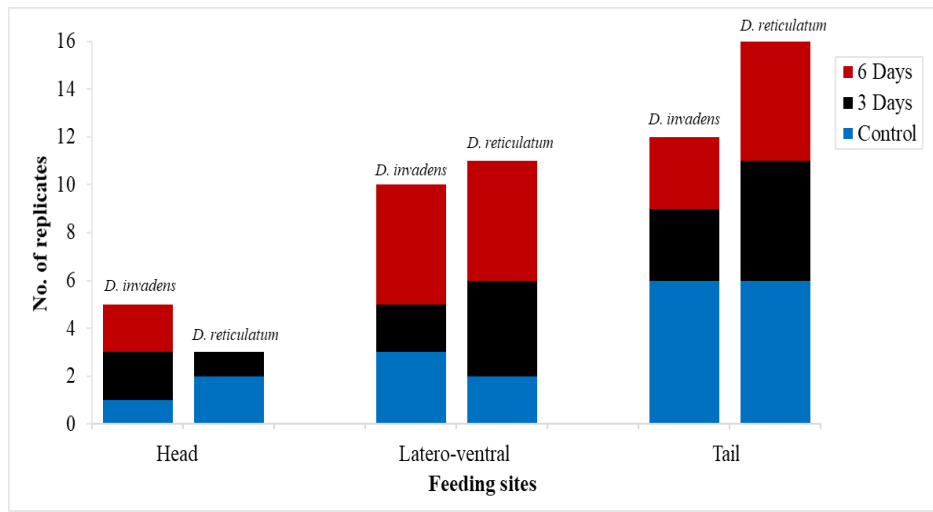
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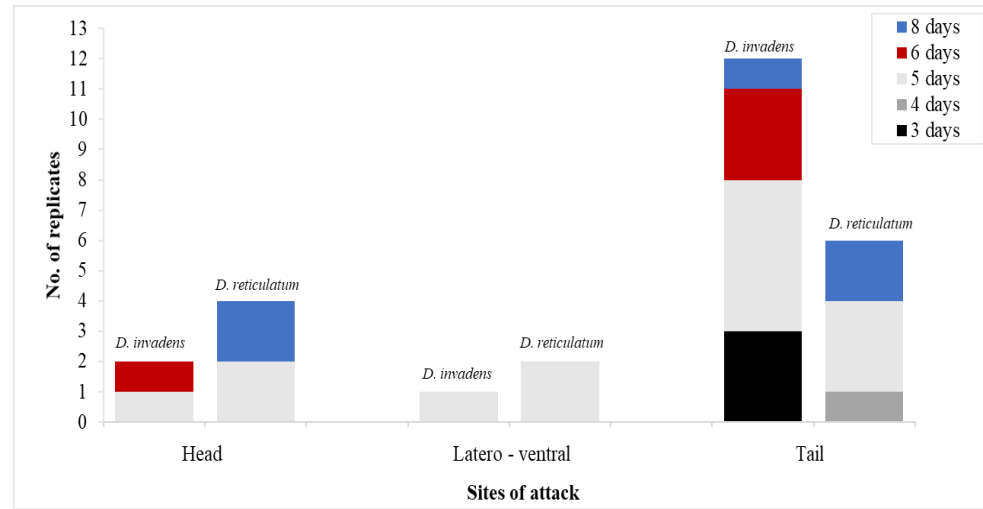
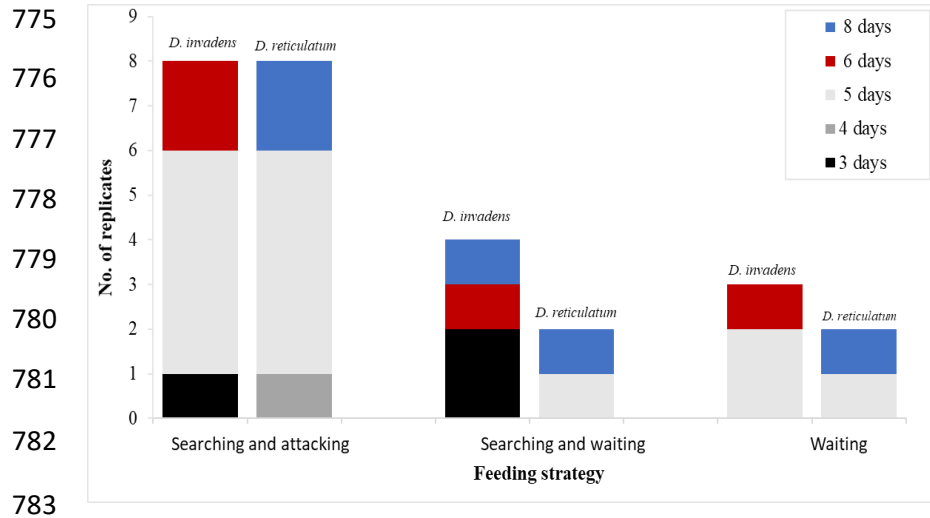
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774 c- Latching sites on both slug species by neonate *T. elata* larvae

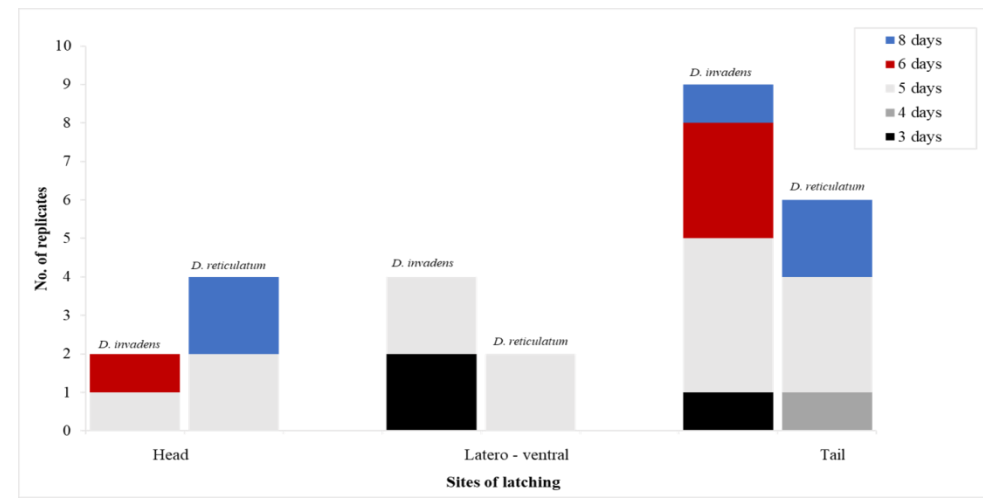
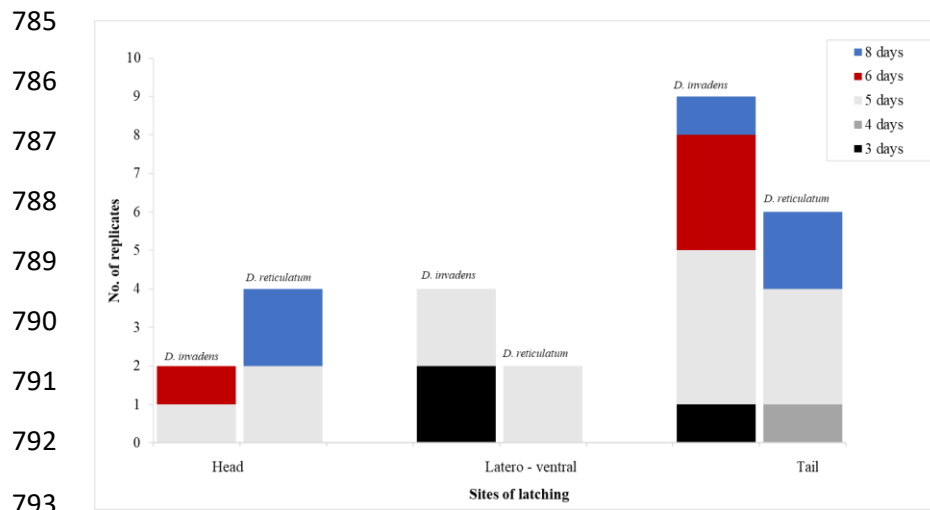


774 d- Feeding sites by neonate *T. elata* larvae on both slug species



784 e- Feeding strategy of third instar *T. elata* on both slug species

784 f- Sites of third instar *T. elata* attack on both slug species



794 g- Latching sites on both slug species by third instar *T. elata* larvae

794 h- Feeding sites by third instar *T. elata* larvae on both slug species