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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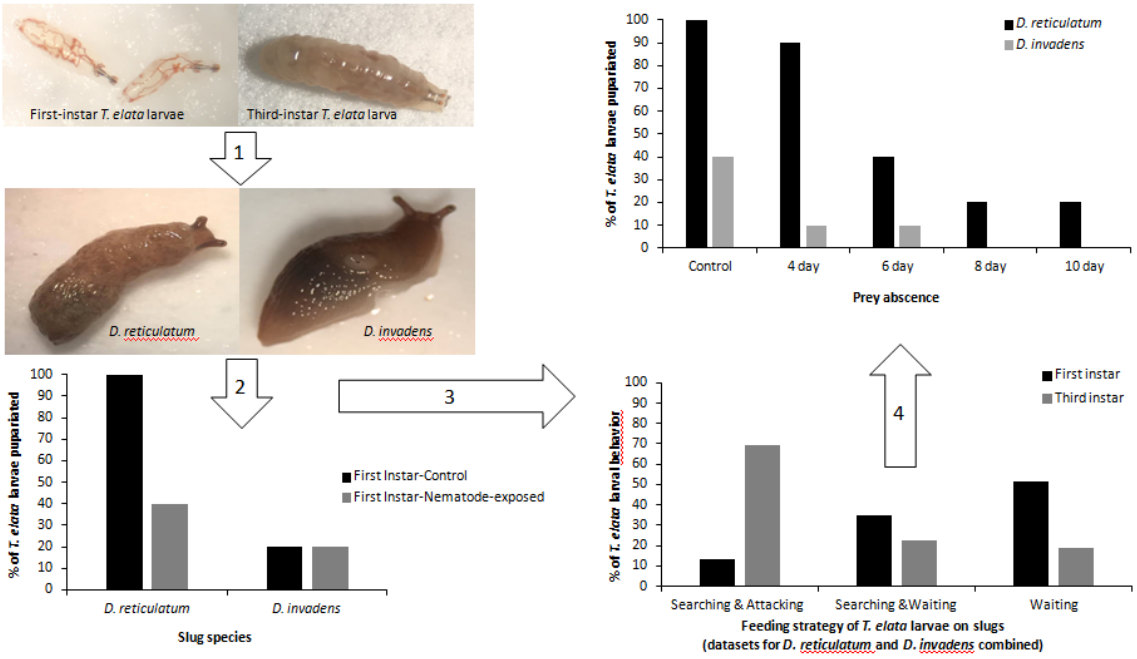
Highlights

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



Abstract

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

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

1. Introduction

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

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

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

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

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

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

The aims of this study are to:

1. Quantify the fecundity of laboratory-eclosed female *T. elata* to inform the design of potential mass cultures of the species (previous oviposition studies by Knutson *et al.* [1965], Beaver [1973] and Hynes *et al.* [2014a] used predominantly field-caught adults).

2. Determine whether the obligate parasitoid first instar life stage of *T. elata* can feed and survive to pupal stage on a pestiferous slug species (i.e. *D. invadens*) not previously documented as a potential host for *T. elata*.

3. Assess the impacts of *P. hermaphrodita*-exposed slugs on the development of neonate *T. elata* larvae to determine the potential for both control agents to be used synergistically for pestiferous slug control.

4. Determine, using prey-choice experiments, the susceptibility of slugs exposed to *P. hermaphrodita* to predation by third instar *T. elata* larvae.

5. Assess the effects of the absence of prey over different periods on neonate larval development and survival.

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

2. Materials and methods:

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

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

2.2 Slug collections and maintenance

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

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

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

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

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

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

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

2.6 Larval feeding behavior

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

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

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

2.7 Statistical analyses

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

3. Results

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

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

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

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

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

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

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

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

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

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

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

3.5 Larval feeding behavior

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

Acknowledgements

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Declarations of interest: None

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

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

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

602 **Table 1**

***Tetanocera elata* development**	***Deroceras reticulatum*** (Mean ± SE)		***Deroceras invadens*** (Mean ± SE)	
	Control *n*=10	***Ph*** *n*= 2	**Control** *n*=4	***Ph*** *n* = 2
Mean larval period (d)	38.20 ± 1.25	40 ± 3.00	51± 4.54	38.± 5.00
Mean number of slugs killed / larva	8.30 ± 0.63	7.50 ± 0.50	9.75 ± 1.00	5.50 ± 0.50
Mean slug weight killed / larva (g)	0.27 ± 0.01	0.17 ± 0.01	0.28	0.17 ± 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**	
	D. reticulatum *n*=10	*D. invadens* *n*=4	*D. reticulatum* *n*=9	D. *invadens* *n*=1	*D. reticulatum* *n*=4	D. *invadens* *n*=1	*D. reticulatum* *n*=2	D. *invadens* *n*=0	*D. reticulatum* *n*=2	D. *invadens* *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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Figure 1: Mean cumulative and cumulative number of eggs laid per day by 5 laboratory-reared *T. elata* adult females. The date each fly died is marked with an arrow (black = males; grey = females).

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

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

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

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

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

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

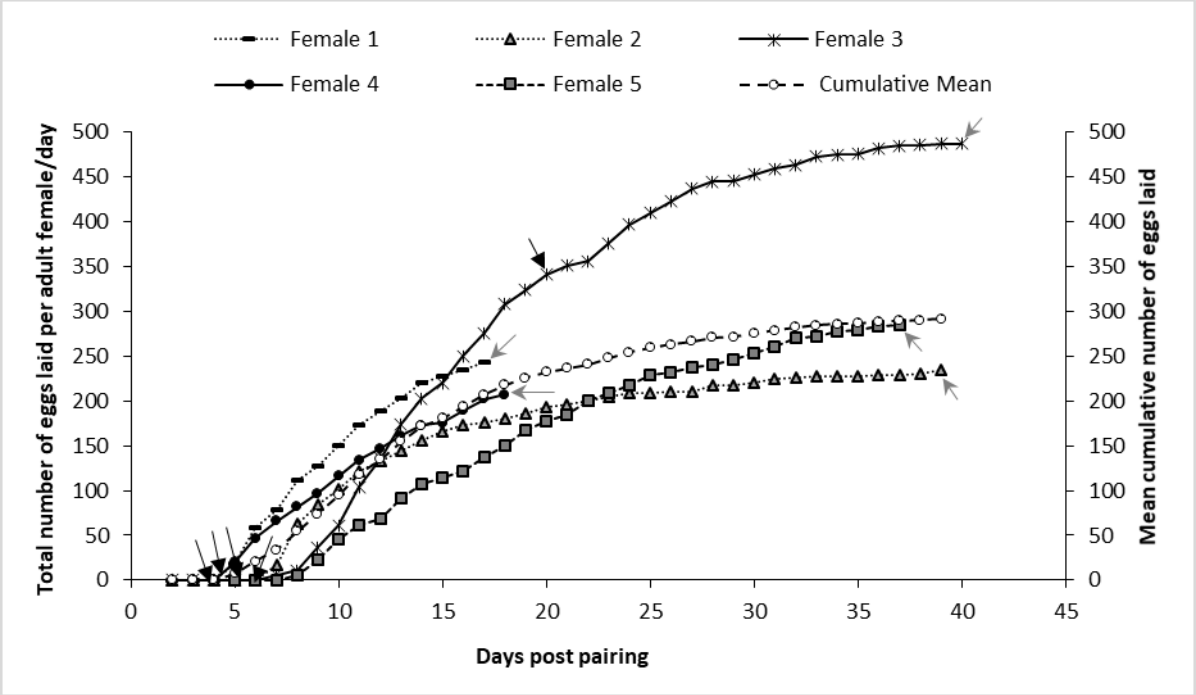
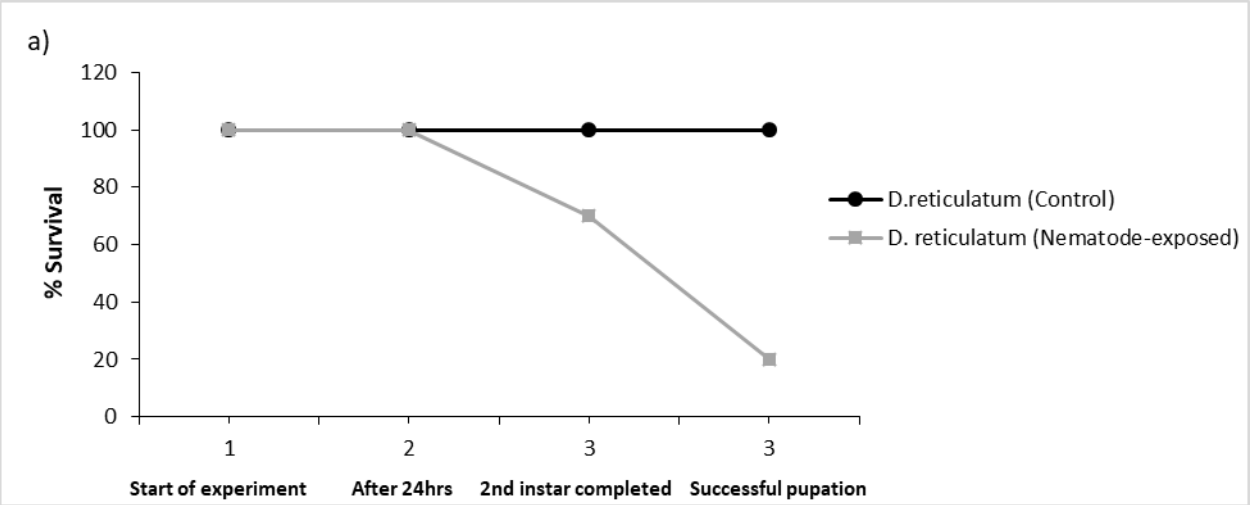

Female 1
Female 2
Female 3
Female 4
Female 5
Cumulative Mean
Total number of eggs laid per adult female/day
Mean cumulative number of eggs laid
Days post pairing
0
50
100
150
200
250
300
350
400
450
500
0
5
10
15
20
25
30
35
40
45

Figure 1



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670 **Figure 2a**

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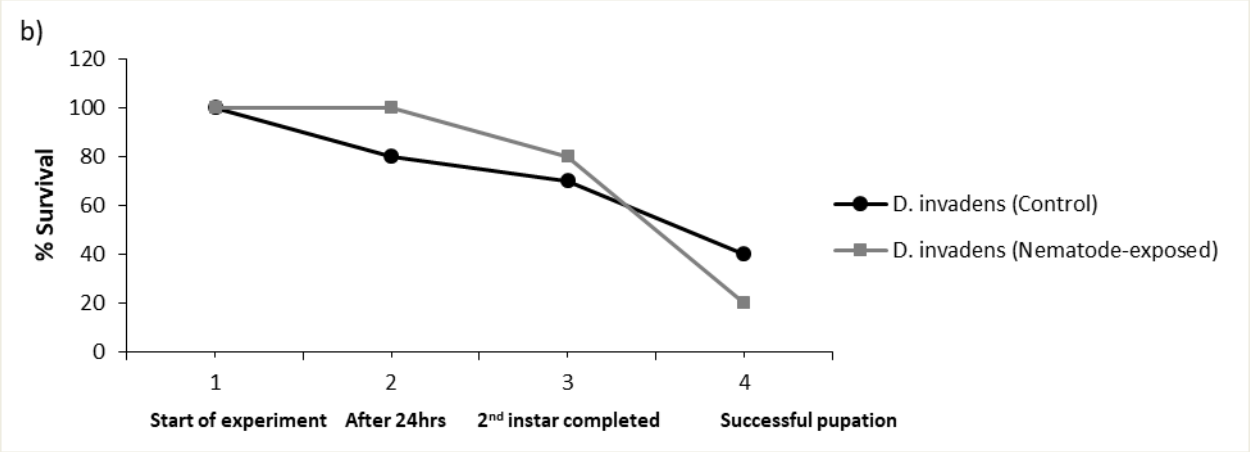

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673 **Figure 2b**

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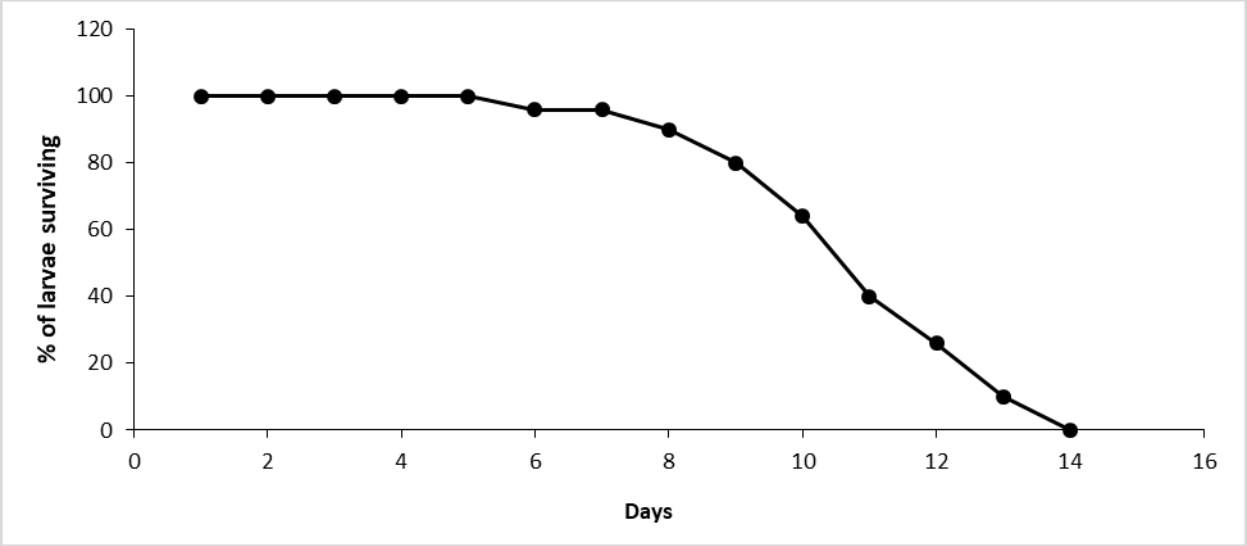


Figure 3

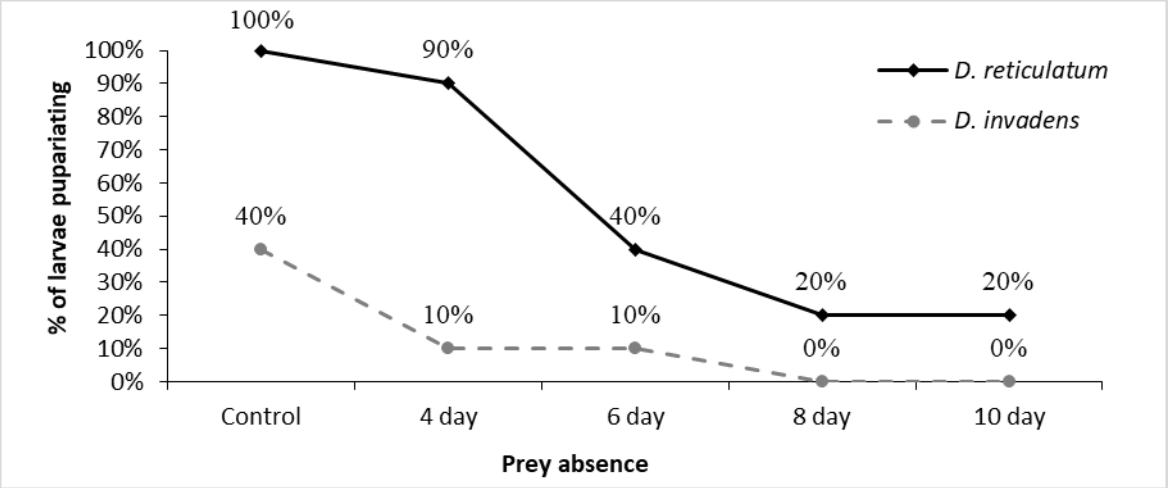

100%
90%
40%
40%
20%
20%
10%
10%
0%
0%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of larvae pupariating
Control
4 day
6 day
8 day
10 day
Prey absence
D. reticulatum
D. invadens

Figure 4

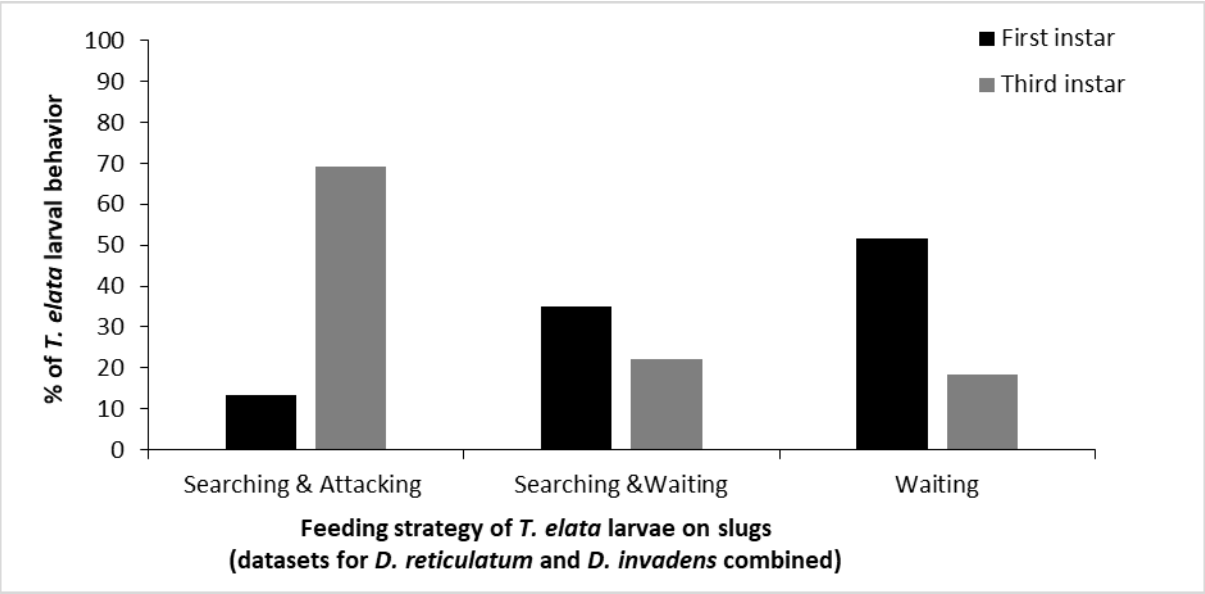

First instar
Third instar
% of T. elata larval behavior
100
90
80
70
60
50
40
30
20
10
0
Searching & Attacking
Searching &Waiting
Waiting
Feeding strategy of T. elata larvae on slugs
(datasets for D. reticulatum and D. invadens combined)

Figure 5

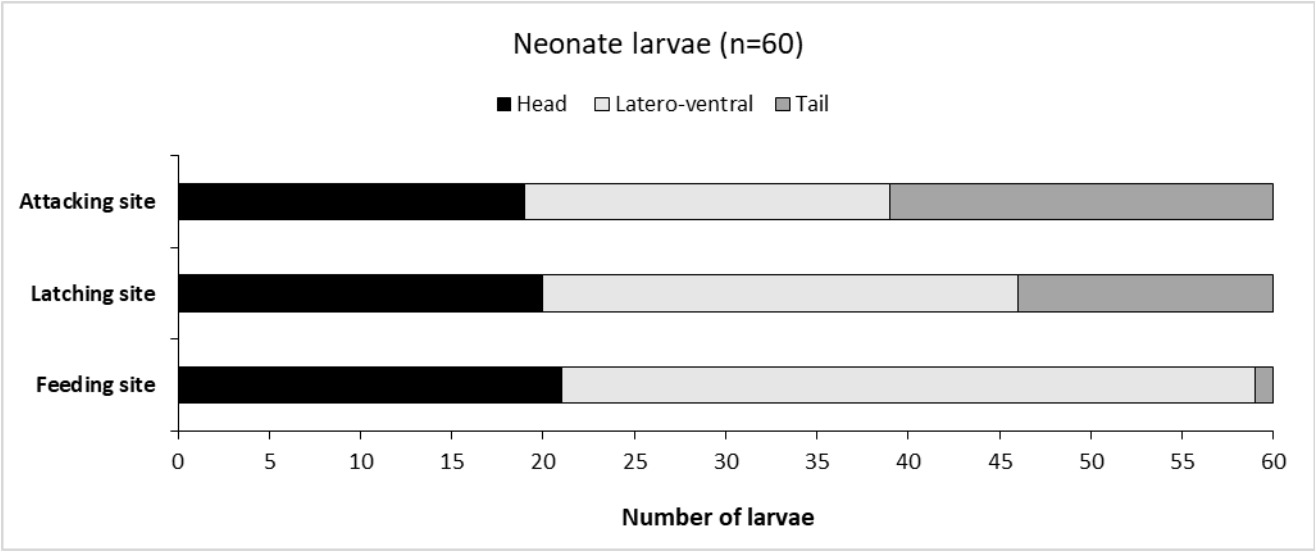


Figure 6 a

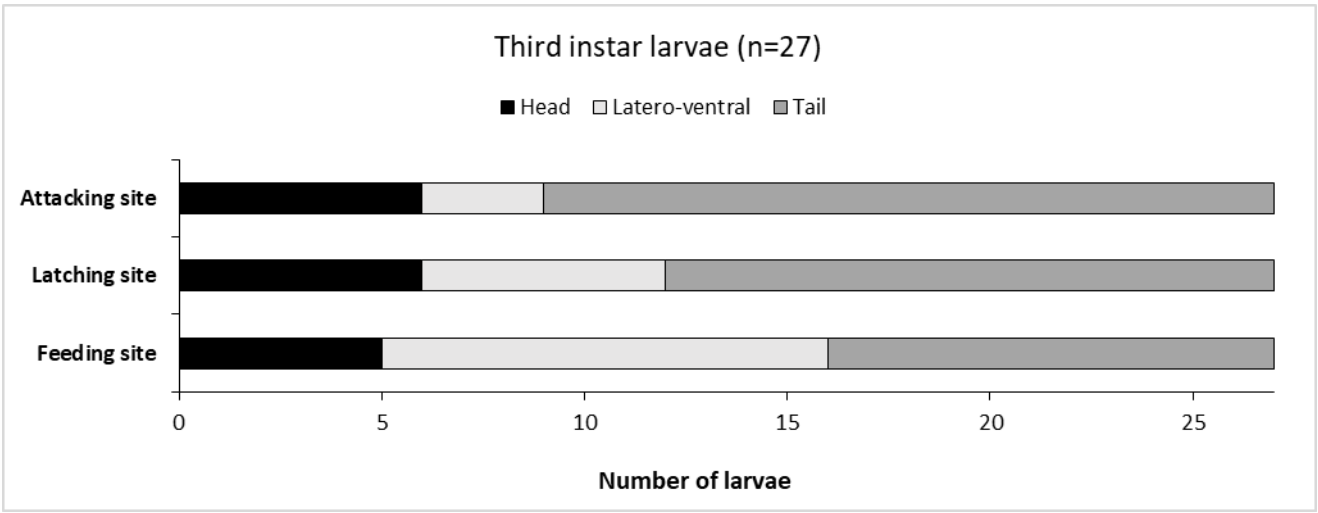
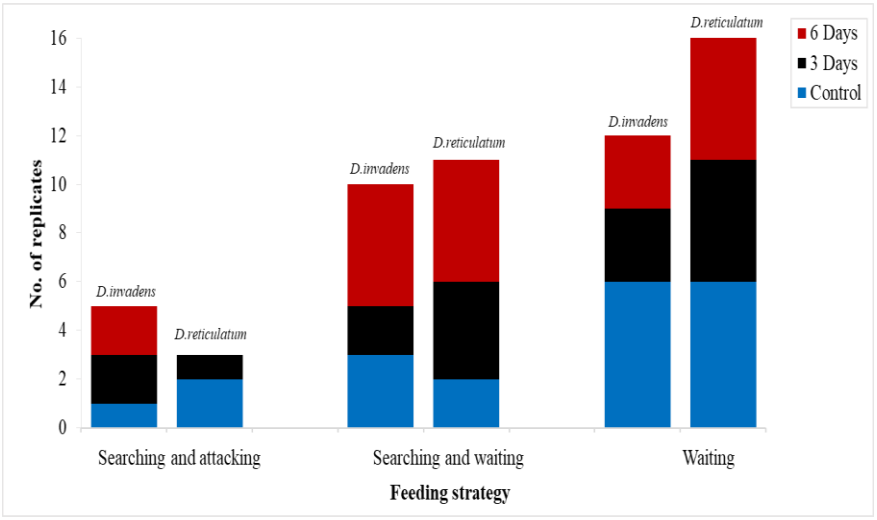
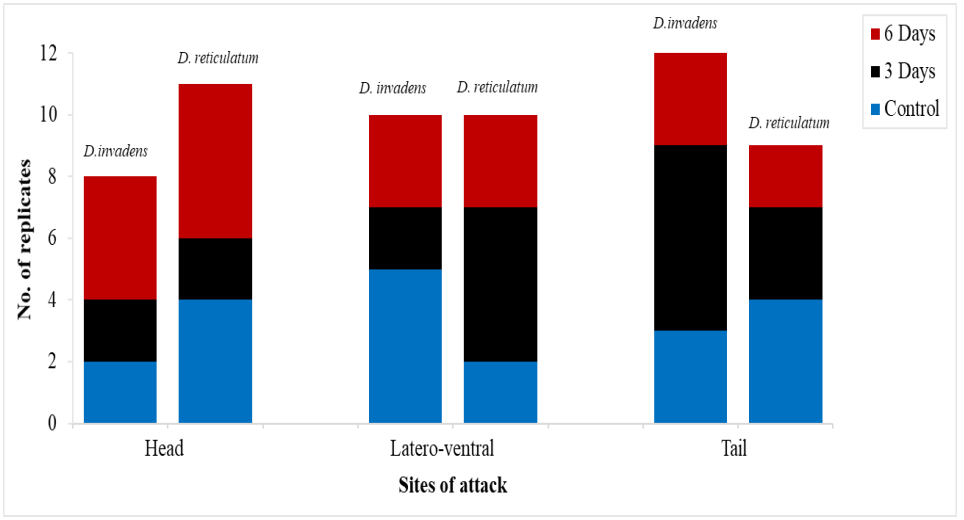


Figure 6 b

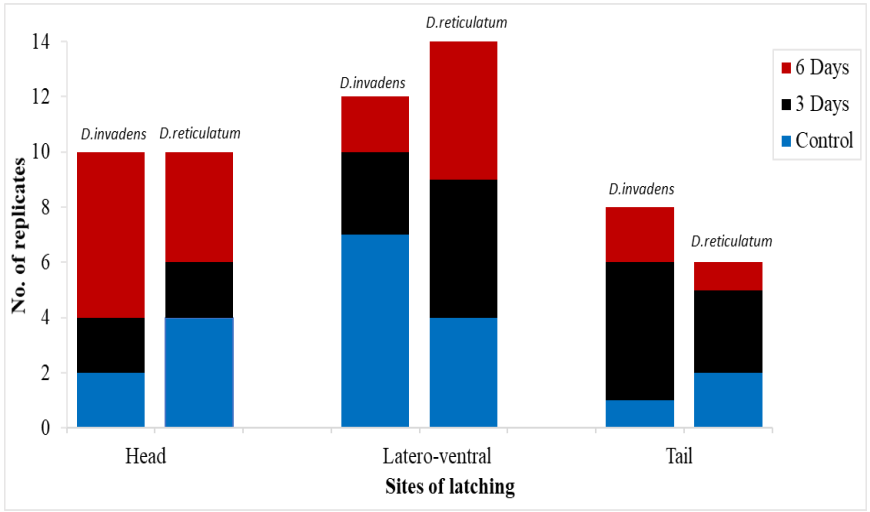
Appendix 1: Larval behavior-neonate larval behavior (a-d); third instar larval behavior (e-h).



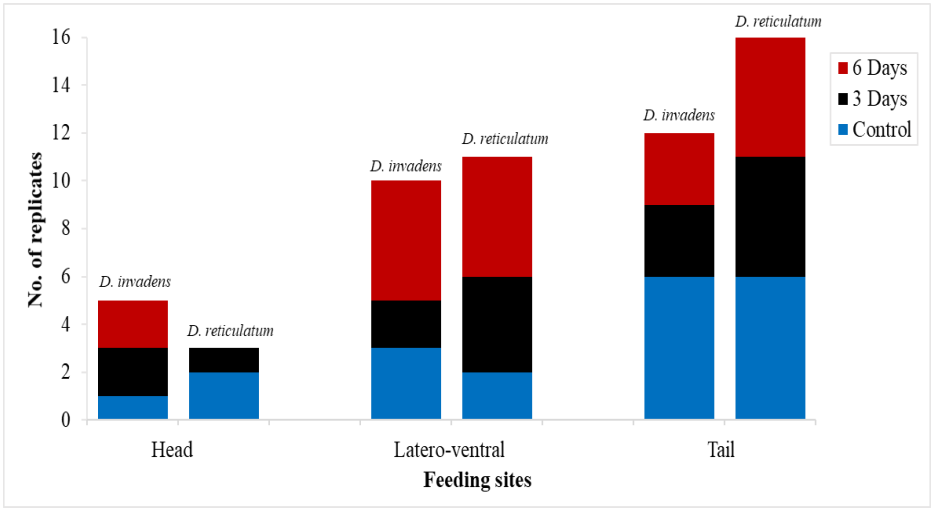
a- Feeding strategy of neonate *T. elata* larvae on both slug species



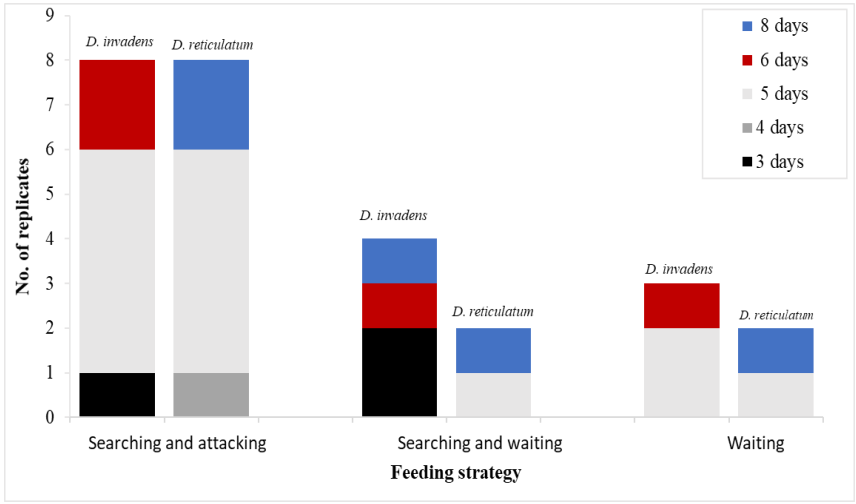
b- Sites of neonate *T. elata* larvae attack on both slug species



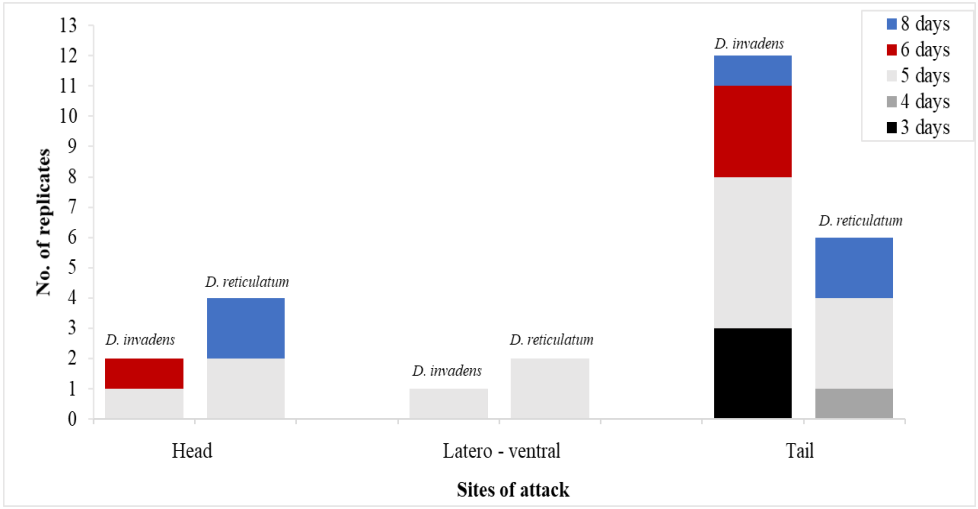
c- Latching sites on both slug species by neonate *T. elata* larvae



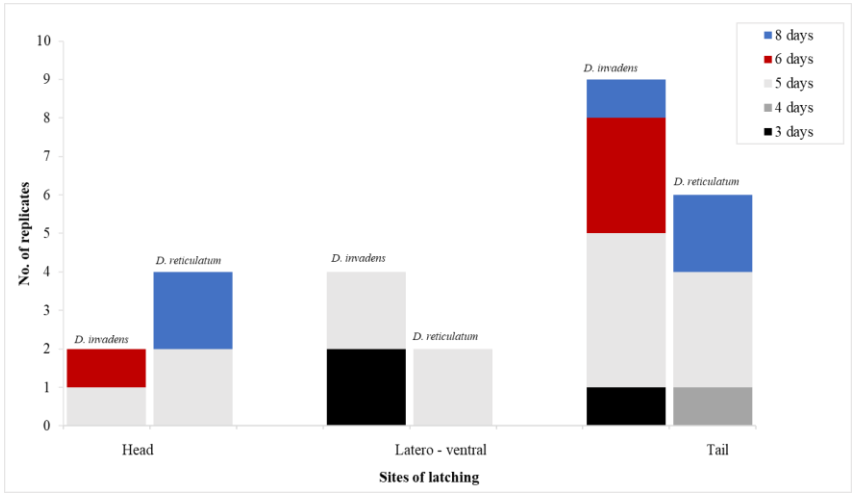
d- Feeding sites by neonate *T. elata* larvae on both slug species



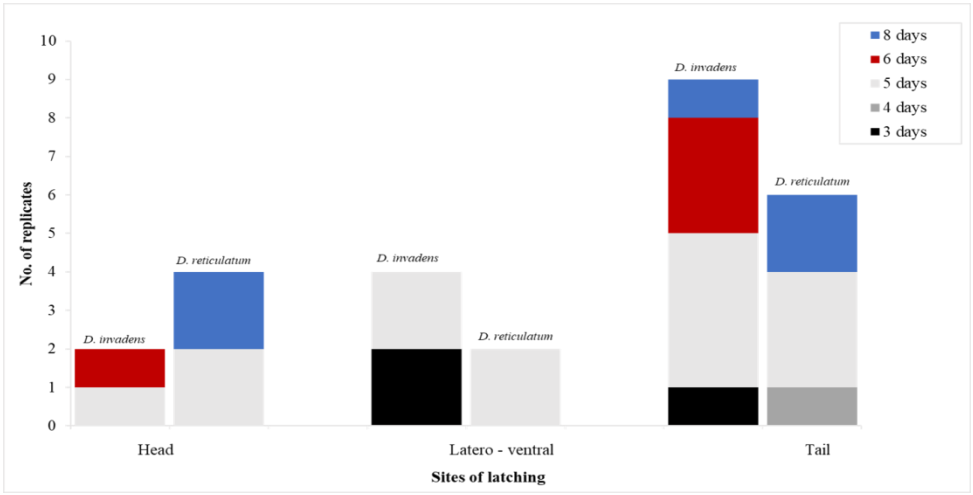
e- Feeding strategy of third instar *T. elata* on both slug species



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



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



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