Nutritional ecology of predaceous *Tetanocera elata* larvae and the physiological effects of alternative prey utilisation

Abstract

Tetanocera elata Fabricius (Diptera: Sciomyzidae) is an obligate mesoparasitoid of the pestiferous *Deroceras* spp. slugs in the first and second larval instars and then emerges to become a free-living predator of terrestrial slugs in the third instar. To determine the biological control potential of *T. elata*, naïve third-instar larvae were exposed to a range of prey slug species (*Deroceras reticulatum*, *Arion hortensis*, and *Tandonia budapestensis*) in no-choice, pairwise two-choice, and three-choice feeding assays. While larvae showed little prey preference, typically attacking the first individual with which they came into contact, *Arion hortensis* was significantly preferred over *T. budapestensis* in two-choice trials ($P = 0.0484$). Larvae were also more efficacious at predating *D. reticulatum*, in that significantly fewer larval attacks preceding feeding were required for *D. reticulatum* than for *A. hortensis* or *T. budapestensis* ($P = 0.0008$ and $P = 0.0059$, respectively). Larvae reared on *D. reticulatum* in culture following trials also experienced the highest survivorship to the start of pupariation. While these results suggest that *D. reticulatum* may remain the ideal prey for third instar *T. elata* larvae, they also demonstrate the ability of larvae to survive on alternative species. The implications of these findings in the context of using *T. elata* as a biocontrol agent are discussed.

KEY WORDS: biological control, prey preference, prey range, mollusc

1. Introduction

Terrestrial molluscs, in particular slugs (MacDonald 2009; Douglas and Tooker 2012; Howlett 2012), cause considerable amounts of damage to cereal and young vegetable crops (Hunter 1968; MacDonald 2009), and have been recorded as causing between £8 and £10 million (GBP) worth of damage to such crops in the UK (MacDonald 2009). Slug damage is due largely to the failure of crop seeds as a result of feeding damage to the seed or young seedlings. Additional damage can be caused by slug feeding on mature plant tissue and crop products (e.g., salad leaves or fruiting bodies), and there is evidence that slugs can act as vectors of plant diseases (Douglas and Tooker 2012).

Conventionally, slug populations are controlled using slug pellets containing methiocarb or metaldehyde as the active ingredient. However, due to concerns regarding non-target toxicity of methiocarbs and evidence that metaldehyde enters public waterways (Howlett 2012), use of methiocarbs has recently been restricted by the European Union (European Commission 2014; European Commission 2018) and metaldehyde has been banned from the UK from 2020 (Anonymous 2018). Even ferric phosphate, used in organic cultivation with variable success (Iglesias et al. 2001; Speiser and Kistler 2002; Rae et al. 2009), may incur negative effects on earthworms due to iron build-up, especially in the presence of chelating chemicals (Langan and Shaw 2006; Edwards et al. 2009). The only biocontrol option currently available for slug control is the soil-living nematode *Phasmarhabditis hermaphrodita* Schneider (Rhabditida: Rhabditidae) (Glen and Wilson 1997; Rae et al. 2007). Application of *P. hermaphrodita* has shown variable levels of slug control under field conditions (Howlett 2012; Rae et al. 2009; Kozłowski et al. 2014), and

47 does not guarantee a reduction of high-density slug populations below economic injury
48 levels. Coupled with this are the issues of expense and shelf life of the biological control
49 agent (MacDonald 2009; Glen and Wilson 1997; Grewal et al. 2005). In addition, while *P.*
50 *hermaphrodita* parasitises a range of slug species, they are not universally effective
51 (Dankowska 2006; Rae et al. 2007; Pieterse et al. 2017) with larger hosts often able to
52 withstand or recover from infection (Speiser et al. 2001).

53 With this in mind, there has been considerable and ongoing research conducted to
54 identify and evaluate other potential natural enemies that could be used as components of
55 integrated slug pest management programmes. Sciomyzidae (Diptera) have been the topic of
56 extensive research for the biological control of various terrestrial and semi-aquatic molluscs
57 (Berg 1953; Knutson et al. 1965; Gormally 1988; Vala et al. 2000; Knutson and Vala 2011;
58 Murphy et al. 2012; Hynes et al. 2014a). Numerous studies have suggested that the functional
59 responses exhibited by many species of Sciomyzidae may demonstrate effective biological
60 control of molluscs (Eckblad 1973; Haab 1984; Beaver 1989; Manguin and Vala 1989;
61 Knutson and Vala 2011). Some species within the genus *Tetanocera* (Diptera: Sciomyzidae)
62 have evolved as specialist predators of terrestrial slugs (Knutson et al. 1965; Berg and
63 Knutson 1978). Specifically of interest for agriculture is *Tetanocera elata* Fabricius, which
64 has been shown to feed on the prominent agricultural pest *Deroceras reticulatum* Müller
65 (Stylommatophora: Agriolimacidae) (Knutson et al. 1965). A multivoltine species producing
66 two to three generations per year, *T. elata* undergoes three larval instars before pupating and
67 becoming quiescent over winter. First and second instar larvae are obligate mesoparasitoids
68 of *D. reticulatum*, and occasionally on closely related species such as *Deroceras laeve* Müller
69 and *Deroceras invadens* Reise, Hutchinson, Schunack, & Schlitt (Knutson et al. 1965;
70 D'Ahmed et al. 2019). Neonates burrow into the host either under the mantle near the
71 pneumostome or (less frequently) through the optical tentacles, where they feed on mucous

and necrotising tissue of the host as they develop (Knutson et al. 1965). Upon maturing to late second instar, parasitoid larvae typically kill their neonate host through catastrophic tissue damage. Free-living late second instar larvae will continue to feed on the host carcass as they develop into the third and final larval instar. Third instar *T. elata* larvae are free-living and undergo a behavioural and ecological shift from parasitoid to predaceous (Knutson et al. 1965; Hynes et al. 2014a; D'Ahmed et al. 2019). These larvae are voracious and have the capacity to kill from six to twelve prey slugs before suspension of feeding in the pre-pupal window (Knutson et al. 1965; Hynes et al. 2014b; D'Ahmed et al. 2019).

Any species considered for biological control should ideally fulfil several basic requirements. Perhaps most importantly, biological control agents should be specific to the host or prey species they are intended to control (Murdoch et al. 1985). *Tetanocera* species are known to be oligophagous and while parasitoid *T. elata* have a very narrow potential host range, free-living predaceous larvae have been observed attacking and feeding on species other than *D. reticulatum* in laboratory trials (Knutson et al. 1965). It has been anecdotally considered that the larval shift from parasitoidism to predation is also associated with an ecological shift from specialism (e.g., host-specific parasitoids) to generalism (e.g., predators), however this has not been specifically examined or quantified. Likewise, although third instar *T. elata* larvae have the ability to kill alternative prey species (Knutson et al. 1965) and have been shown to discern between healthy and *P. hermaphrodita*-exposed *Deroceras* spp. (D'Ahmed et al. 2019), there has been no study of prey preference, nor an examination of any physiological effects that feeding on various prey species may incur.

The current study addressed these gaps in knowledge by exposing naïve predaceous third instar *T. elata* larvae to their known prey *D. reticulatum* as well as two additional potential prey species, *Arion hortensis* Férussac and *Tandonia budapestensis* Hazay (Stylommatophora: Milacidae). All three species are native across Europe and are pestiferous

97 species of economic importance (Douglas and Tooker 2012; Howlett 2012), commonly
98 occurring in arable agroecosystems (Hunter 1968). Additionally, these species have adopted a
99 global distribution associate with agricultural intensification, having been introduced into
100 regions including North and South America, Australia and New Zealand. Larvae were
101 presented with prey species in choice and no-choice assays, which were used to determine
102 prey preference. Additionally, the current study examined, for the first time, the physiological
103 effects of different prey species on developing *T. elata* larvae. Feeding efficiency,
104 survivorship, and developmental rates were considered together to gauge suitability of the
105 three potential prey species. The combination of prey suitability and preference provides
106 valuable insight into the potential and realised prey range of predaceous *T. elata* larvae,
107 which is an essential consideration to evaluate the potential for the use of *T. elata* as a
108 biological control agent of slugs in European horticulture.

109

110 ## 2. Materials and Methods

111

112 **2.1 Specimen Collection and Colony Maintenance.** *Tetanocera elata* colonies were
113 established using field-collected adults to ensure the availability of larval instars as required.
114 Adult *T. elata* were collected from dry grassland field sites in western Ireland (counties
115 Galway, Clare, and Mayo) (Supplementary Table 1) from July to August 2017 by passing a
116 heavy-duty sweep net (0.3 m long handle; 0.1 pore net; 0.5 m aperture) through tall
117 vegetation. Specimens were identified in the field using morphology as described by
118 Rozkoŝný (1984; 1987) and *T. elata* removed from sweep nets using acrylic barrel-style
119 pooters (Watkins & Doncaster, The Naturalists, Hawkhurst, Kent, England) for transport
120 back to the laboratory. Species identification and sex were confirmed using a dissecting

microscope (Olympus SZ40, X6.7 to X40 magnification) in the laboratory, and colonies were subsequently initiated by placing mixed-sex groups (approx. 1:1 M:F) of *T. elata* from the same collection location and date in vinyl and polyester mesh cages with a single 17 cm sleeve (24.5 x 24.5 x 24.5 cm; Bugdorm model 4222, MegaView Science, Taiwan). Cages were furnished with a honey-yeast diet (Hynes et al. 2014a), wet cotton wool to provide hydration, and wooden sticks for perching/oviposition. Colonies were maintained under laboratory ambient conditions (18-22°C, 42-70% RH), with photoperiod on an approximately 9:15 (L:D) cycle under incandescent room lighting supplemented by natural light from a large east-facing window on an approximately 16:8 (L:D) summer photoperiod. Cages were checked daily and any observed eggs were removed using a damp fine-hair paintbrush and transferred to Petri dishes for larval rearing (see Section 2.2).

Slug specimens collected for *T. elata* larval rearing and prey preference trials consisted of *D. reticulatum*, *A. hortensis*, and *T. budapestensis*. Individuals of all three species were collected by deploying de Sangosse slug traps (de Sangosse, France) on grassy areas on grounds of the National University of Ireland Galway. Collections were conducted by checking traps on a weekly basis and hand-collecting individuals of the appropriate species. Identifications were confirmed using morphological keys (Rowson 2014) and independent colonies were maintained for each species. Slugs were kept in cohorts of 10-12 individuals of similar size on damp tissue in ventilated 650 ml clear plastic boxes (17 x 11.5 x 4cm, L x W x H), and fed with dry porridge oats and organic carrot. Colonies were maintained at 16°C and ambient RH in darkness within an environmental chamber (LTE Qualicool, LTE Scientific Ltd., Greenfield, Oldham, UK).

2.2. Larval Rearing of *Tetanocera elata*. Eggs removed from *T. elata* adult cages were transferred into 5 cm Petri dishes lined with a damp cotton pad topped with filter paper (Grade 1 qualitative, 55 mm circles, GE Whatman, Marlborough, MA, USA) and sealed with

Parafilm M (Bemis NA, Neenah, WI, USA), with eggs being grouped by date of collection and parent collection site. Petri dishes were maintained under identical laboratory conditions as adult colony cages and were observed daily for larval hatching.

First instar larvae were transferred via paint brush from their natal Petri dishes onto a *D. reticulatum* host taken from slug colonies. Neonates were placed onto the mantle of the slug host near the pneumostome to enhance the likelihood of successful parasitism. Each neonate and its host were housed individually within 5.5 x 5.5 x 3 cm (L x W x H) ventilated plastic boxes lined with damp cotton pads topped with filter paper, as was done for egg dishes. A small portion of dry porridge oats was placed in each box to provide food for the host as parasitoids matured. Boxes were observed every 2-3 days to track maturation of *T. elata* larvae, which were observed by gently lifting the edges of the mantle of the host to view the protruding spiracles of the larvae. If the original host was killed before *T. elata* larvae reached third instar, a second host was provided for the larva from *D. reticulatum* colonies. Once *T. elata* larvae were confirmed to have matured to the predaceous third instar, the remains of the neonate host carcass were removed, and larvae were maintained without food until larval gut content was observed to be < 50% full at which stage the larvae were utilised for prey choice trials.

2.3. Setup and Recording of Prey Preference Assays. Prey preference was observed for third instar *T. elata* larvae by exposing naïve larvae to one, two, or three slug species concurrently in choice or no-choice arenas. Each individual (*T. elata* larva and slug prey) was used only once to ensure truly independent replicates, and all slugs used were of similar weight. No-choice treatments consisted of a larva being exposed to either *D. reticulatum* (n = 10), *A. hortensis* (n = 13), or *T. budapestensis* (n = 15). Two-choice treatments presented larvae with a pairwise choice of prey species: *D. reticulatum/A. hortensis* (n = 12), *D. reticulatum/T. budapestensis* (n = 11), or *A. hortensis/T. budapestensis* (n = 13). Arenas with

171 the three-choice treatment presented all three potential prey species simultaneously to a larva
172 (n = 14). Trial arenas consisted of a 9 cm Petri dish lined with damp filter paper (Grade 1
173 qualitative, 90 mm circles, GE Whatman, Marlborough, MA, USA). Slugs were transferred
174 into arenas first using a flat fine-haired paintbrush; in two- and three-choice trials, prey
175 individuals were placed at opposite ends of the arena, with the brush cleaned between slugs.
176 *Tetanocera elata* larvae were placed either on the opposite side of the arena from prey (no-
177 choice treatments) or in the centre of the arena equidistant from all prey (choice treatments)
178 using a separate paintbrush which had no contact with slug mucus.

179 Because larvae of Sciomyzidae are negatively phototactic (Mc Donnell et al. 2007),
180 all trials were run within wooden chambers (94 x 66 x 60 cm) which excluded light
181 contamination. Chambers were each lit with 2-3 infrared LED light sources (Abus
182 TVAC71200), and video recorded using an IR-capable digital camera (Colour Sony SUPER
183 HAD II CCD) mounted on the top of each chamber. Trials ran for 3 hours (after the
184 methodology of Hynes *et al.* 2014a). Videos of the feeding assays were recorded and
185 examined using EthoVision XT Version 10.1 (Noldus Information Technologies Inc.,
186 Wageningen, Netherlands) using a package for tracking the movement and behaviour of
187 multiple individuals. Counts of the number of attacks and feeding events made by *T. elata*
188 larvae per slug species were used as a measure of prey preference.

189 **2.4. Measurement of Prey Suitability.** Immediately after the conclusion of each
190 feeding trial, specimens were removed from experiment chambers and larvae were returned
191 to colony rearing boxes along with the prey individual on which they were feeding at the time
192 of trial end. Larvae continued to receive their chosen prey in laboratory cultures *ad libitum*
193 until the larva either died or began pupariation. Slugs provided for feeding were similar in
194 size/weight, as was confirmed by statistical comparisons of the mean biomass given to each
195 larva having no significant differences between prey species (P = 0.1468, permutation F

196 tests). If no feeding occurred during trials, larvae received *D. reticulatum* as the default prey
197 species. Rearing boxes were checked every 2-3 days to assess survivorship as well as to
198 perform enclosure maintenance and provide new prey as necessary. Development time of
199 third instar larvae to pupariation, survivorship to pupariation, and the total number and
200 biomass of prey provided to each larva was recorded for each individual to provide a measure
201 of prey suitability. Larvae undergoing pupariation were typically considered dead when
202 decomposition was observed. In a small number of instances, fully-formed pupae never
203 produced adults. These puparia were allowed to remain undisturbed for approx. 9 months
204 (into the subsequent summer season to account for the potential of the formation of an
205 overwintering pupa), then dissected. All dissected puparia were confirmed to have degraded.

206 **2.5. Statistical Analyses.** Prey species preference was determined by comparing the
207 number of trials where feeding occurred compared to those where feeding did not occur on
208 each prey species using a Fisher's Exact test and *post-hoc* Dunn tests. The number of attacks
209 preceding a successful feeding event (i.e., handling time) was evaluated using Kruskal-Wallis
210 tests with *post-hoc* Dunn tests where Kruskal-Wallis values were significant. Larval
211 survivorship to pupariation was compared between prey species using a 3x3 Chi-squared
212 table followed by a *post-hoc* Dunn test for pairwise comparisons, and development rates were
213 analysed using ANOVA or Welch's t-test according to normality and variances of the data
214 sets. Prey consumption (number of individuals and biomass) by *T. elata* larvae in colony
215 were compared using permutation F tests. Analyses were performed using R (R version 3.2.5,
216 R Core Team 2013, The R Foundation for Statistical Computing, Vienna, Austria) in R
217 Studio.

218

219 **3. Results**

220

221 **3.1. Prey Preference.** Prey preference was measured by comparing the number of
222 trials where feeding occurred with the number of trials where larvae did not feed for each
223 slug species. Across all choice levels (e.g., no-choice, two-choice, and three-choice) feeding
224 occurred on all potential prey species during the three hour observation period. Naïve *T. elata*
225 larvae attacked prey slugs at least once in 91% of all trials, with successful feeding occurring
226 in 74% of all trials. Statistical comparisons were only made between species at the same
227 choice level (i.e., feeding rates were compared in two-choice trials and a separate comparison
228 was made for three-choice trials); additionally, feeding rates were not compared statistically
229 between species in no-choice trials, as the experimental setup was not appropriate for this
230 type of comparison (i.e., no-choice trials generated a mix of dependent and independent
231 variables that would not allow for accurate comparison between and within species).

232 In no-choice trials, all *D. reticulatum* specimens (100%) exposed to *T. elata* were fed
233 on successfully by larvae in comparison to just 67% and 46% for *T. budapestensis* and *A.*
234 *hortensis*, respectively (Table 1). In two-choice trials when the data are combined for each
235 slug species tested (Fig. 1), *D. reticulatum*, with a 52% success predation rate, was again the
236 slug species most successfully preyed upon by *T. elata* larvae in comparison to *A. hortensis*
237 (44%) and *T. budapestensis* (25%), respectively. In addition, the number of successful
238 feeding events by *T. elata* larvae on *A. hortensis* was significantly greater ($P = 0.0484$) than
239 on *T. budapestensis* in the *A. hortensis* / *T. budapestensis* two-choice trial (Table 1). In
240 contrast, although no significant differences were detected in the three-choice trials, it is
241 interesting to note that when *T. elata* larvae had a choice between the three slug species, *D.*
242 *reticulatum* was predated upon least frequently (14%) in comparison to *A. hortensis* (36%)
243 and *T. budapestensis* (21%) (Table 1). In addition, as the treatments progressed from no-
244 choice to two-choice and three-choice trials, the percentage of successful feeding events on

D. reticulatum decreased from 100% to 52% to just 14%, and on *T. budapestensis* from 67% to 25% to 21%. However, for *A. hortensis*, there was little difference in the percentage of successful feeding events between no-choice (46%), two-choice (44%) and three-choice (36%) trials (Fig. 1).

3.2. Prey Suitability. Suitability of each prey species was determined by the number of preliminary attacks made by a larva before successful feeding commenced (i.e., handling time), larval survivorship to pupariation, and third instar development time (to pupariation).

3.2.1. Efficacy of attack and feeding. For the purposes of this study, an attack was defined as a larva extending its mouthparts into prey tissue in a brief contact which typically lasted approximately 1 second or less. This differed from larval feeding which was marked by prey being penetrated by the larva's mouthparts for an extended period of time coupled with subtle rippling contractions of the anterior body of the larva and the raising of the posterior spiracles (as described by Hynes *et al.* 2014a). When examined as a function of prey species or choice level, the number of attacks prior to a successful feeding event did not differ significantly according to Kruskal-Wallis tests (Supplementary Table 2) although larvae required a maximum of just three attacks before feeding successfully on *D. reticulatum*, compared with a maximum of five attacks being required in some cases for the other two slug species (Fig. 2). When all feeding events were pooled across choice levels, however, there were significant differences in the number of attacks required prior to feeding ($P = 0.00359$, $\chi^2 = 11.258$, $df = 2$) between the three potential prey species (Table 2). Larvae were able to begin feeding on *D. reticulatum* after significantly fewer attacks than on *A. hortensis* ($P = 0.0008$) and *T. budapestensis* ($P = 0.0059$), with no significant difference ($P = 0.3098$) between *A. hortensis* and *T. budapestensis* (Table 2).

3.2.2. Survivorship. Larval survivorship was comprised of two measures: (1) full formation of a puparium and (2) attempted or partial pupariation (where the larva died during pupariation and failed to complete a viable puparium). The two measures were combined to reflect overall larval survivorship to the beginning of pupariation, which was significantly affected by prey species ($P = 0.0435$, $\chi^2 = 9.8221$, $df = 4$) (Table 3). The rates of partial and full pupariation were also considered independently, with greater survivorship levels observed for larvae fed on *D. reticulatum* than for those reared on *T. budapestensis* when partial pupariation occurred ($P = 0.0348$) (Table 3). All other pairwise comparisons between prey species and pupariation success relevant to the study were non-significant (Supplementary Table 3).

One adult female and one adult male, reared as larvae on *D. reticulatum* and *T. budapestensis* respectively, successfully eclosed (Table 3), but no adults eclosed from *A. hortensis*-reared pupae. When comparing rates of full pupariation, larvae reared on *A. hortensis* showed slightly higher survivorship (25%) than *D. reticulatum* (16%), with *T. budapestensis* only forming a single puparium (6%). A greater percentage of larvae reared on *D. reticulatum* following feeding trials reached at least the partial puparium stage (64%) compared to those reared on *A. hortensis* (50%) or *T. budapestensis* (25%). It is worth noting that a considerable majority (84%) of pupariation attempts overall resulted in death before successful pupariation was accomplished for larvae reared on all prey species combined.

3.2.3. Larval development rate. Prey species did not significantly affect the overall developmental rates (e.g., combined development of fully and partially pupariating individuals) of *T. elata* larvae ($P = 0.4574$, $F = 0.9529$, $df = 5$) (Fig. 3). Of the larvae which successfully pupariated, those reared on *D. reticulatum* reached pupariation at a slightly faster rate (60.44 d ± 8.13) compared to those reared on *A. hortensis* (63.00 d ± 1.78, respectively) (Table 3), although the single larva to complete pupariation on *T. budapestensis* was faster

than the mean of both (45 d) (Table 3). There was no observed difference in development time to full puparia between larvae reared on *D. reticulatum* and *A. hortensis* (P = 0.7659, Welch's t-test) (Fig. 3). The two adult eclosions reflect a different trend than the mean development rates; puparial duration for the larva reared on *D. reticulatum* was considerably faster than for the larva reared on *T. budapestensis* (25 d and 45 d, respectively). Developmental rate to successful puparia could not be statistically compared for larvae reared on *T. budapestensis* because only a single puparium was formed.

Development rate to partial pupariation was slower for larvae fed on *D. reticulatum* (70.93 d ± 5.18) than for larvae reared on *A. hortensis* (57.50 d ± 10.84) and *T. budapestensis* (46.00 d ± 4.58). As with larvae which successfully completed pupariation, ANOVA analysis indicated that prey species had no significant effect on the development rate of larvae only achieving partial pupariation (P = 0.2192, F = 1.5946, df = 2) (Fig. 3).

4. Discussion

The preference for prey species, or lack thereof, demonstrated by predaceous *T. elata* larvae was complex and variable. Similar to observations by Knutson *et al.* (1965), larvae were observed feeding on a range of prey species. In the current trial, larvae attacked and fed on all potential prey species offered at all choice levels. The only observed significant difference in feeding rate, that of *A. hortensis* being predated significantly more frequently than *T. budapestensis* in paired two-choice trials, could indicate that *A. hortensis* is more palatable or easier to predate, which contradicts Knutson *et al.* (1965) who observed *T. elata* refusing to feed on *A. hortensis*. In other treatments, rather than exhibiting a clear preference between prey, larvae instead tended to attack and proceed to feed on whichever individual

317 they encountered first, regardless of species. Consequently, there must be consideration of the
318 probability that a number of these feeding events may have occurred somewhat randomly.
319 Hynes *et al.* (2014a) and D'Ahmed *et al.* (2019) observed that third instar *T. elata* larvae
320 regularly displayed a "search-and-wait" or "wait" behaviour (54% and 40% of trials,
321 respectively) whereby larvae largely remained stationary until a prey individual came into
322 contact with the larva as a result of the prey's movement. The nature of the feeding assays in
323 the current study (where all trials were run in 9 cm Petri dishes, regardless of the prey
324 density) inherently increased the probability that larvae would encounter a prey individual of
325 any species as the number of individuals within trial arenas increased. Alternatively, *T. elata*
326 larvae may exhibit variable functional responses based on prey density where higher prey
327 density could result in lower prey preference. Such responses have been observed for
328 *Tetanocera ferruginea* Fallén (Barker et al. 2004), and warrant further exploration for the
329 closely-related *T. elata*.

330 Feeding by larvae in no-choice trials demonstrated a clear affinity for *D. reticulatum*,
331 representing the only observed instance of 100% feeding rate in the trial. Likewise, in
332 pairwise trials where *D. reticulatum* was an option, it was fed on at higher (though non-
333 significant) frequencies than other prey options. The elevated rates of feeding on *D.*
334 *reticulatum* may be the result of a number of pre-existing conditions. First, *D. reticulatum* is
335 the optimal neonate host (Knutson et al. 1965; D'Ahmed et al. 2019), and the species on
336 which all larvae used in trials were reared in the parasitoid first and second instars. While the
337 third instar larvae used in trials were considered naïve, as they had not been given any slug
338 meal once they matured to the free-living predaceous stage, they did have some prior
339 association with *D. reticulatum* as they were allowed to continue feeding on the original
340 neonate host carcass for a short period after maturing to third instar. This may have
341 predisposed larvae toward feeding on a species with which they already had some (limited)

prior experience (Dillon et al. 2014). Alternatively, due to *D. reticulatum* being the neonate host, *T. elata* may be evolutionarily predisposed to predating on this species. While *D. reticulatum* does have considerable predator-avoidance defences in the form of exudation of a calcium-rich, viscous mucus (O'Hanlon et al. 2018), *T. elata* larvae have likely evolved coping strategies which allows them to parasitise and predate *D. reticulatum* more efficiently. Larvae were able to successfully feed on *D. reticulatum* after fewer attacks than either alternative species, supporting this potential of co-evolved strategies of predation of *T. elata* toward their parasitoid host. In contrast to handling time on *A. hortensis* and *T. budapestensis*, which increased as prey density increased, larvae began feeding on *D. reticulatum* most rapidly in three-choice trials, though there was no significant increase or decrease in handling time for *D. reticulatum* between choice levels.

Survivorship of larvae following trials was also greater on *D. reticulatum* than on alternative prey. Larval performance reflected a gradient of prey suitability, both for partial pupariation and full pupariation, with *D. reticulatum* being superior, *A. hortensis* being next favourable, and *T. budapestensis* least successful for survivorship. Across all species, larvae progressing into pupariation experienced high mortality, indicating this may be a particularly vulnerable point for *T. elata* larvae. Similar development times across prey species may support previous observations (ABE, unpublished data) which indicate that pupariation in *T. elata* could be related to consumption of a certain threshold amount of prey biomass. Though non-significant, the shorter development times witnessed for larvae reared on *A. hortensis* and *T. budapestensis*, combined with lower puparial weights, could suggest that these prey species are less suitable. It is worth noting that no adults successfully eclosed from puparia of larvae reared on *A. hortensis*. Larvae fed on *D. reticulatum* and *T. budapestensis* each produced one adult (female and male, respectively), though larvae pupariated at higher rates after being reared on *D. reticulatum*.

When taken together, the combination of feeding efficiency, survivorship, and developmental rates indicate that *D. reticulatum* may still be the superior prey species for *T. elata* larvae. Any differences in prey suitability may be due to several factors, from palatability (resulting in increased biomass consumption), the provision of essential nutrients, or ease of attack (Omkar 2005). Considering the ease with which larvae commenced feeding on *D. reticulatum* compared to other species, it seems likely that predating *D. reticulatum* poses a lower energetic cost to *T. elata* larvae. It is also reasonable to posit that *D. reticulatum* may provide nutritional components that align with the metabolomic needs of *T. elata* larvae entering the pupal phase more effectively than *A. hortensis* or *T. budapestensis*.

When all considerations are taken together, *T. elata* appears to be a viable option for safe and efficacious biological control for pestiferous slugs in European horticulture. While trials demonstrated the ability of larvae to utilise alternative prey, larvae experienced reduced performance and physiological trade-offs when their diets were restricted to particular slug species. It appears that *D. reticulatum* is a superior prey species and may provide nutritional components lacking in other prey species which *T. elata* larvae require to complete development. This, combined with superior location of *D. reticulatum* populations (Hunter 1966) and synchronicity with *T. elata* life history (Speight and Knutson 2012), makes any consequential prey shift unlikely to be realised under field conditions.

Although the outcomes of this study are optimistic, further research should be undertaken prior to any meaningful utilisation of *T. elata* in a biological control context. High mortality rates experienced by larvae should be examined in greater detail, and other studies may investigate additional aspects of larval fitness. If larval survivorship can be enhanced, an investigation of the impacts of alternative prey on adult longevity, reproductive capacity, and progeny fitness (*via* Aldrich 1986; Legaspi et al. 1996) would be highly enlightening and would complement the assessment of physiological suitability of prey species investigated

here. Further studies may also investigate choice of additional slug species *T. elata* larvae are likely to encounter in agroecosystems, as this study was not exhaustive. Additionally, feeding choice and physiological studies can be undertaken in more natural conditions. Trials described here were run in sterile, artificial arenas and larvae were reared under environmental conditions (e.g., temperature, relative humidity, photoperiod) which had been determined for optimal larval growth in laboratory cultures (Hynes et al. 2014b). A difference in prey choice and/or survivorship may be observed if larvae are maintained under more natural conditions (e.g., in boxes with soil, plant material, etc.) with access to a range of slug species rather than being restricted to one species for the duration of the predatory phase. This could also identify use of non-prey food items essential to larval development that are currently unknown. These topics will further enhance our practical knowledge of *T. elata* ecology and physiology, and contribute to enhancing the efficacy of an eventual conservation biological control programme.

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Table 1. Number and percentage of successful feeding events by *Tetanocera elata* larvae on *Deroceras reticulatum*, *Arion hortensis*, and *Tandonia budapestensis* at each choice level. All P-values are the result of Fisher's Exact Tests comparison of the number of trials where feeding occurred compared to trials where feeding did not occur. Comparisons were made per prey species within choice levels.

Treatment	No. of slugs exposed	No. of successful feeding events	% of successful feeding events	P-value
No-choice				†
D. reticulatum	10	10	100	-
A. hortensis	13	6	46	-
T. budapestensis	15	10	67	-
Two-choice				
D. reticulatum | *A. hortensis*	12	6 | 4	50 | 33	0.3401
D. reticulatum | *T. budapestensis*	11	6 | 4	55 | 36	0.3350
A. hortensis | *T. budapestensis*	13	7 | 2	54 | 15	**0.0484***
Three-choice	14	-	-	0.5437‡
D. reticulatum		2	14	-
A. hortensis		5	36	-
T. budapestensis		3	21	-

† No statistical comparisons were made of no-choice data. which generated a mix of dependent and independent variables that would not allow for accurate comparison between and within species

‡ Since results for three-choice prey preference in a 3x2 table were non-significant, pairwise comparisons were not made.

551

552 **Table 2.** Median and range (min – max) of the number of attacks preceding successful
553 feeding events undertaken by *T. elata* larvae for each prey species in no-choice, two-choice,
554 and three-choice treatments. Statistical comparisons were made using Kruskal-Wallis tests
555 with *post hoc* Dunn's tests.

	No-Choice	Two-Choice	Three-Choice	Experiment-Wide
	Median (range)	Median (range)	Median (range)	Median (range)
Deroceras reticulatum	1 (1 – 2) *n = 9*	1 (1 – 3) *n = 12*	1 (1) *n = 2*	1 (1 – 3)[a] *n = 23*
Arion hortensis	1.5 (1 – 3) *n = 6*	1 (1 – 5) *n = 11*	4 (1 – 5) *n = 5*	2 (1 – 5)[b] *n = 22*
Tandonia budapestensis	1 (1 – 3) *n = 10*	2 (1 – 4) *n = 6*	3 (2 – 5) *n = 3*	2 (1 – 5)[b] *n = 19*

556

557 Different superscript letters indicate significance differences (DR/AH P = 0.0008; DR/TB P =
558 0.0059) between species, following significant Kruskal-Wallis comparison (P = 0.00359, χ^2 =
559 11.258, df = 2).

560 **Table 3.** Development time in days (d) and survival rates of third instar *Tetanocera elata* larvae reared on *Deroceras reticulatum*, *Arion*
561 *hortensis*, or *Tandonia budapestensis*. Numbers of replicates for Mean developmental rates are the same *n* listed for corresponding Survivorship
562 categories.

Prey species	Total no. larvae	Mean no. prey consumed (± SE)	No. surviving larvae (%)			Mean developmental rate (d ± SE)		Adult longevity (d)
			Partial puparium	Full puparium	Adult eclosion	Partial puparium	Full puparium	
Deroceras reticulatum	56	3.26 ±0.31 *n = 114*	27* (48%)	9 (16%)	1 (2%)	70.93 ± 5.18	60.44 ± 8.13	3
Arion hortensis	16	2.13 ±0.58 *n = 17*	4 (25%)	4 (25%)	0 (0%)	57.50 ± 10.84	63.00 ± 1.78	-
Tandonia budapestensis	16	2.00 ±0.58 *n = 8*	3* (19%)	1 (6%)	1 (6%)	46.00 ± 4.58	45.00	3

563

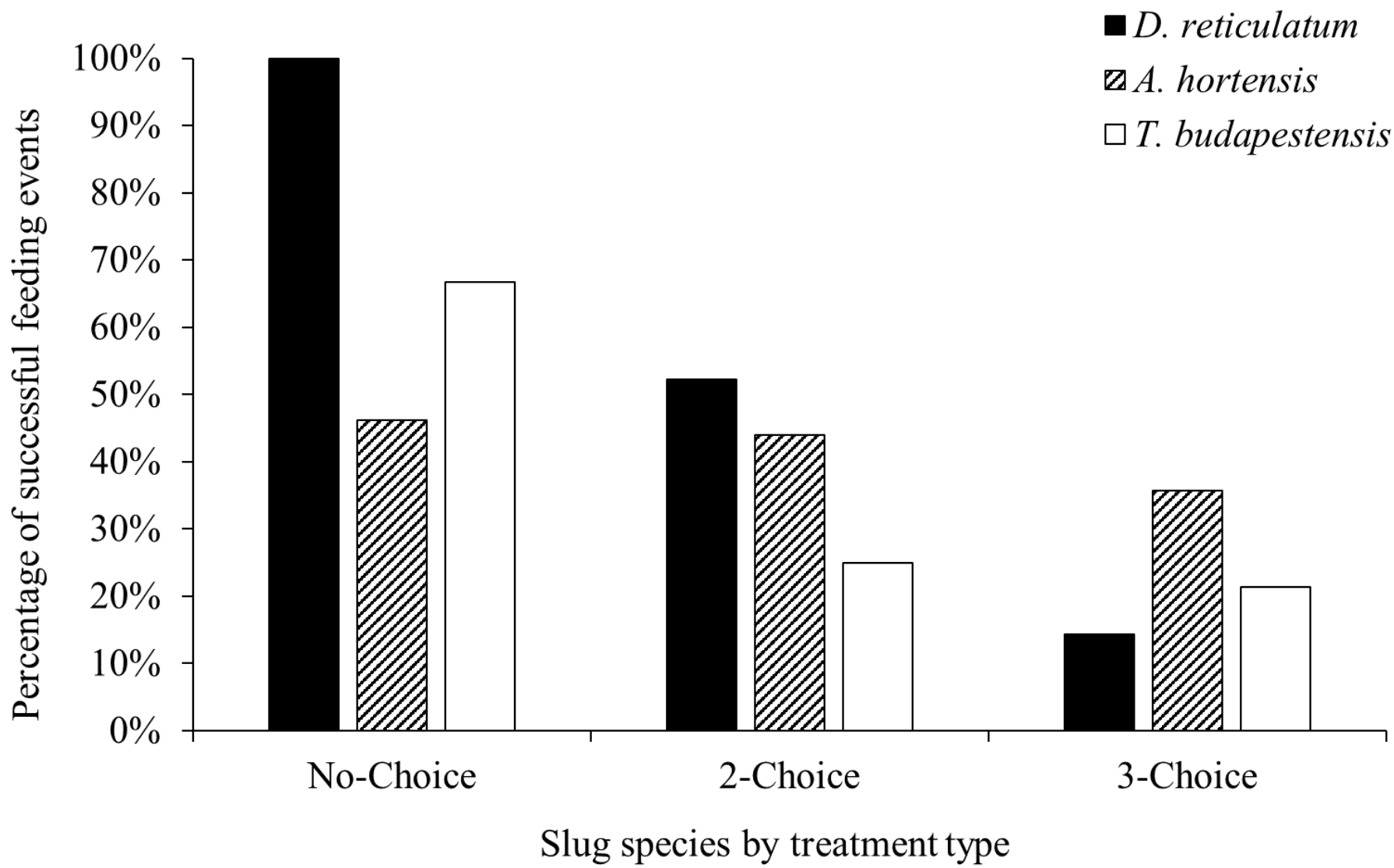
564 Asterisks indicate statistically significant differences in survivorship (P = 0.0348) between individuals completing partial pupariation reared on
565 *D. reticulatum* compared to on *T. budapestensis*. Comparisons were made using a Chi-square test (P = 0.0435, χ^2 = 9.8221, df = 4) followed by a
566 *post-hoc* Dunn's test.

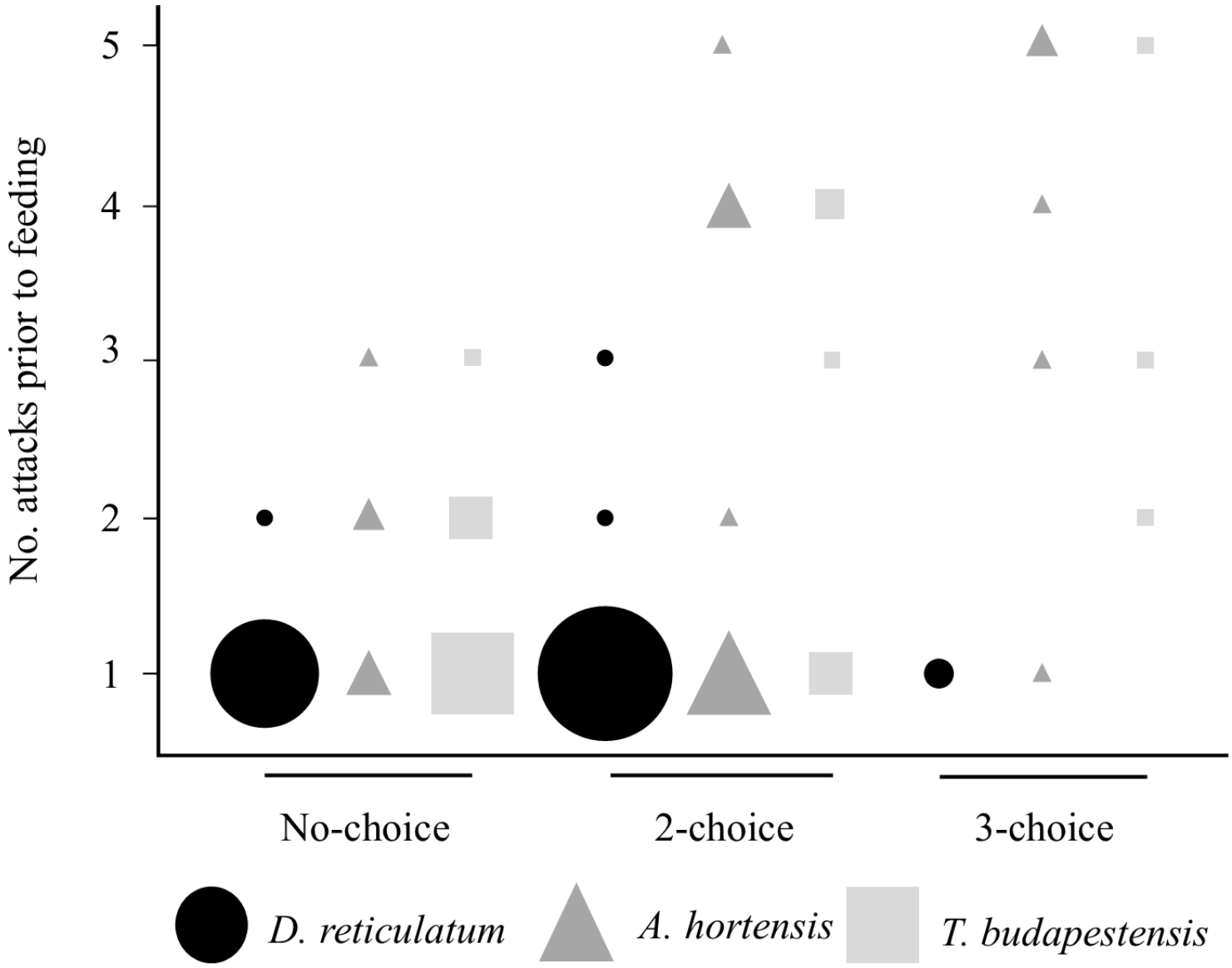
Figure 1. Percentage of successful feeding events by third instar *Tetanocera elata* larvae on each of three prey species in no-choice, two-choice, and three-choice feeding trials. Data for pairwise two-choice trials are pooled to illustrate percentage successful feeding events on each slug species overall.

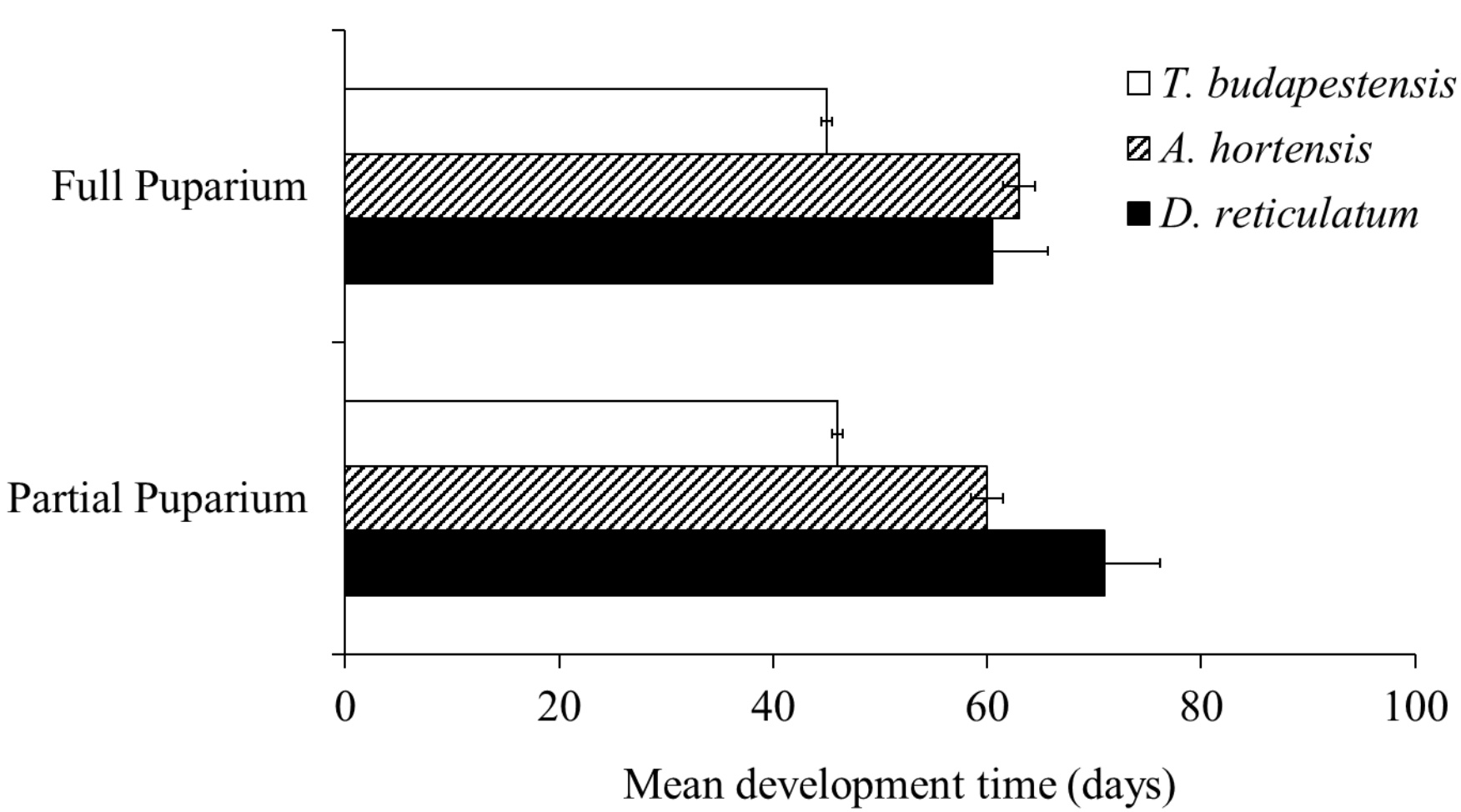
Figure 2. Number of attacks (i.e., handling time) of *Tetanocera elata* larvae on each prey species across choice levels. Markers are scaled to reflect the number of observations.

Figure 3. Mean developmental rates (± SE) of third instar *Tetanocera elata* larvae reared on *Deroceras reticulatum, Arion hortensis*, or *Tandonia budapestensis*. Larvae are separated by survivorship types: partial puparium (e.g., those that died while pupariating) and full puparium (e.g., those that successfully completed formation of a puparium).

579


D. reticulatum
A. hortensis
T. budapestensis
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of successful feeding events
No-Choice
2-Choice
3-Choice
Slug species by treatment type


No. attacks prior to feeding
5
4
3
2
1
No-choice
2-choice
3-choice
D. reticulatum
A. hortensis
T. budapestensis


Full Puparium
Partial Puparium
T. budapestensis
A. hortensis
D. reticulatum
0
20
40
60
80
100
Mean development time (days)

609

610 **Supplemental Table 1.** Locations and description of field sites where *Tetanocera elata*

611 adults were collected, June through August 2017.

Site name	County	GPS	Habitat description	No. specimens collected
Cow Park	Galway	53°13'47.7"N 8°52'20.0"W	Dry grassland meadow with some wet grassland mosaic; unmanaged public amenity area; former village grazing field.	6♂ 11♀
Burren	Clare	53°00'53.4"N 9°04'30.1"W	Dry grassland meadow; seminatural grassland surrounded by hazel scrub; occasionally grazed.	2♂ 2♀
Mulranny	Mayo	53°54'21.9"N 9°45'22.4"W	Patchy dry and wet grassland; small plot adjacent to carpark and visitor centre; traditionally grazed but currently unmanaged.	2♂ 5♀

612

Supplemental Table 2. P and χ^2 values (df = 2 for all) of Kruskal-Wallis tests using a χ^2 distribution for larval feeding efficiency as a function of prey species and choice level.

Factor	Level	Treatments compared	P-value	χ^2
Choice type	No-choice	DR x AH x TB	0.2156	3.0683
	2-choice	DR x AH x TB	0.1518	3.7710
	3-choice	DR x AH x TB	0.1688	3.5577
Prey species	*D. reticulatum*	No-choice x 2-choice x 3-choice	0.7828	0.48986
	A. hortensis	No-choice x 2-choice x 3-choice	0.1669	3.5803
	T. budapestensis	No-choice x 2-choice x 3-choice	0.1042	4.5233

Supplemental Table 3. Pairwise P-values of *post-hoc* Dunn's tests for number of larvae within each survivorship category following a significant Chi-square test (P = 0.0435, χ^2 = 9.8221, df = 4).

		No pupariation			Partial pupariation			Full pupariation	
		DR	AH	TB	DR	AH	TB	DR	AH
No pupariation	*A. hortensis*	0.2183							
	T. budapestensis	0.3977	0.3020						
Partial pupariation	*D. reticulatum*	0.3977	0.1489	0.3020					
	A. hortensis	0.1217	0.3487	0.1821	0.0769				
	T. budapestensis	0.0599	0.2183	0.0974	**0.0348***	0.3487			
Full pupariation	*D. reticulatum*	0.3020	0.3977	0.3977	0.2183	0.2584	0.1498		
	A. hortensis	0.1217	0.3487	0.1821	0.0769	0.5000	0.3487	0.2584	
	T. budapestensis	0.0348[1]	0.1498	0.0599	0.0190[1]	0.2584	0.3977	0.0974	0.2584

[1]While these results are significant, the groups compared were not relevant to the study and are therefore not discussed.