Integrative evaluation of *Tetanocera elata* (Diptera: Sciomyzidae) for slug pest management in agriculture

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DECLARATION

I, Allison J. Bistline-East, hereby verify that this thesis is all my own work and that I have not obtained a degree at this university or elsewhere on the basis of this work.

ABSTRACT OF THE THESIS

Terrestrial gastropods are pervasive pests, especially of agriculture in temperate regions. While current management options exist, they primarily consist of baited pellets with active ingredients such as methiocarb or metaldehyde. These pesticides are highly effective at controlling slug populations and feeding damage incurred on crops, but they are also now widely accepted to have devastating non-target effects on other macroinvertebrates, mammals, birds, and invertebrate communities in nearby soil and waterways. With the use of methiocarb currently being suspended by the European Union and metaldehyde recently disallowed in the United Kingdom, and other available methods (e.g., ferric phosphate pellets, inundative biological control using the parasitic nematode *Phasmarhabditis hermaphrodita*) demonstrating inconsistent efficacy, there is a clear and pressing need for the development of additional, sustainable control strategies for pestiferous slugs.

Knowledge of marsh flies (Diptera: Sciomyzidae) being natural enemies of molluscs has garnered growing interest since the first early observations of the family in the 1950s. This diverse, globally distributed family is composed of a multitude of species which have evolved to be associated with one or a small suite of mollusc species, either as predators or parasitoids. Many of these are associated with semi-aquatic snails and have been the focus of biological control aimed at reducing the intermediate vectors of filarial diseases. A small subset of the Sciomyzidae, however, have evolved to target terrestrial slugs. *Tetanocera elata* is a species possessing a wide Holarctic distribution (including Ireland), and is an obligate parasitoid and predator of *Deroceras reticulatum* (Stylommatomorpha: Agriolimacidae) in the larval stage; both of which make this species a prime candidate for use in slug control.

Previous research has addressed essential questions of *T. elata* biology and behaviour, such as the determination of optimal rearing conditions of laboratory cultures and describing predating behaviour of larvae on *D. reticulatum*. However, before *T. elata* can be realistically considered as a biological control agent, the existing knowledge base requires expansion in several key areas. The research presented in this thesis addresses many of these areas.

Of primary importance is an understanding of the biological requirements of *T. elata* at each life stage. While previous investigations are predominantly focused on larval requirements (as this is the life stage which affects pest slugs), almost nothing to date on adult requirements has been investigated. Such requisites were examined to determine dietary components utilised by *T. elata* (and other marsh fly species) in nature. Studies presented here discovered that adult marsh flies feed on hemipteran honeydew. Another important factor for the evaluation of *T. elata* as a biological control agent is a determination of its prey range and preference, in order to avoid potential non-target effects. Choice and no-choice behavioural assays were conducted to determine the realised prey range of predaceous larvae, with additional investigation into the physiological consequences of the utilisation of alternative prey species on larval survivorship. Larvae showed no clear preference for prey species, however attacks were more efficacious on *D. reticulatum*. Finally, in an effort to optimise the establishment of *T. elata* populations within agroecosystems, an evaluation of habitat requirements was undertaken. This study addressed potential ecological associations of *T. elata* with plant community as well as structural features such as plant growth forms and field boundaries, finding that *T. elata* presence were correlated with hedgerow proximity and height and percentage cover of dead vegetation. These results will form the basis for any future ecological management recommendations.

Overall, the findings presented here contribute to the progression of realising the full ecological potential of *T. elata* for pest control and propose how this research may be applied in an ecological management and conservation biological control context.

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Chapter 1 General Introduction

Food production and security is perhaps the single most important industry across the globe. Latest estimates value total worldwide agricultural revenues at over \$7 x 10^{15} USD each year (FAOSTAT 2016), with agricultural labour comprising 40% of global employment (United Nations 2019). Even with such large-scale output, the UN (2019) estimated that over 800 million people are undernourished. With approximately 15% of the yield of staple crops (e.g., maize, rice, wheat, soybeans, potato) lost to animal pest damage each year (Oerke 2006) and expected to be further augmented by progressing climate change (Gregory et al. 2009), it is clear that food security is a priority area to be advanced to sustainably meet the needs of the growing world population.

1.1. Agriculture in Ireland

Agriculture is a critical component of the Irish economy, with over 137,000 farms across the country (Dillon et al. 2017). It contributes a Gross Agricultural Output (GAO) of €6.9 billion per annum (DAFM 2016) and accounts for nearly 175,000 jobs, translating to 8% of all employment nationwide (DAFM 2018). While a majority of primary agriculture produces silage, hay, and grazing for sheep and cattle, approximately 8% of agricultural land area in Ireland is arable (e.g., cereals, vegetables, fruits), and generates an estimated €1.4 billion per year (An Bord Bía 2017). Cereals, including wheat, oat, and barley, comprise the majority of arable farming land (86%), with the remaining area consisting of mixed fruit and vegetable agriculture and general nursery plant horticulture (Table 1.1) (CSO 2016).

Horticultural revenues have increased since 2016, reflecting overall trends of increased yields and lowered production costs, with an average output valuation of €6,693 per farm (Dillon et al. 2017; DAFM 2018). Additionally, the value of cereals, fruits, and vegetables exported from Ireland are steadily increasing (Table 1.2) (CSO 2017). There has also been a rise in the amount and value of these commodities imported (Table 1.2), indicating a market where consumer demand currently outpaces supply. With this in mind, it is evident that conservation of current yields is highly important.

Table 1.1. Total arable crop representation in Irish agriculture. Land area (hectares) and farm numbers are given in thousands. Data taken from the Central Statistics Office Farm Structure Survey (CSO 2016).

Crop	Area (ha)	No. farms
Cereals		
Wheat (winter)	60.3	2.7
Wheat (spring)	7.5	0.8
Oat (winter)	13.3	1.1
Oat (spring)	10	1.4
Barley (winter)	74.7	8.2
Barley (spring)	114.6	8.2
Other crops		
Beans/peas	12.5	1.1
Oilseed rape	9.9	0.6
Potato	9	1.1
Consumer vegetables	3.7	0.4
Consumer fruit	0.7	1.2
Additional crops	7.6	2.1
Nursery/general horticulture	0.5	0.1
Total Cereals	280.4	22.4
Total Other	43.9	6.6
Grand Total	324.3	29

Ireland, especially in the west, is both temperate and humid, with a 30-year average rainfall of 105 mm and temperature ranging from 5°C (winter) to 16°C (summer) (Met Eireann 2018a, b). While this is favourable for numerous crops, it is also favourable for pests. It is therefore unsurprising that Ireland is among the countries (along with the UK, France, and Netherlands) whose agriculture is most severely impacted by terrestrial slug damage (Speiser et al. 2001).

1.2. Terrestrial Slugs

Terrestrial slugs (Gastropoda) contribute considerably to food crop loss due to pests in Ireland and other temperate regions. While no explicit measure is currently available, it has been estimated that economic losses incurred by slugs in UK vegetable farming could value between £8 and £10 million GBP annually (MacDonald 2009); another figure estimates that losses of up to £15 million in potatoes alone can be attributed to slug damage (Twinning et al. 2009). Further reports from the UK speculate that potential damage to cereal crops could range up to £43 million in the absence of any slug control measures (Nicholls 2014). Due to the geographic and climactic proximity of Ireland to the UK, it is reasonable that similar impacts may be assumed for Irish horticulture.

Such slugs inflict damage in two ways: (1) causing failure of establishment of newly sown seedlings and (2) direct tissue damage of mature and/or fruiting plants. Seedling failure is caused as a direct result of slugs grazing on seeds and young sprouts in the soil, as they consume the young and vulnerable germinating tissue. Other damage is caused to mature plants when slugs feed on stem and leaf tissue, affecting the health and viability of the crop plant, as well as on the portion of the crop marketed as food product (e.g., salad leaves or the fruiting bodies of vegetable and fruit crops). Additionally, slugs have the potential to serve as vectors of fungal and bacterial plant pathogens (Dawkins et al. 1986; South 1992; Hoffman & Rao 2013). In addition to this explicit damage, food crops are rendered less marketable through contamination of mucus and faeces left behind as slugs crawl over the food.

Table 1.2. Valuation of arable agriculture exports from and imports into Ireland. Valuation is in thousands of euros. Change percentages given in italics indicate negative values. Data taken from the Central Statistics Office Trade Statistics Report (CSO 2017).

Commodity	2016		2017		Change (%)	
	Value (€)	Tonnes	Value (€)	Tonnes	Value	Tonnes
Exports						
Cereals†	381,413	367,317	417,086	419,322	9.4%	14.2%
Fruit/vegetable	278,390	179,161	299,215	172,444	7.5%	*3.7%*
Oilseed rape and similar	13,763	16,154	8,099	9,237	*41.2%*	*42.8%*
Imports						
Cereals‡	1,049,082	1,978,977	1,098,551	2,317,964	4.7%	17.1%
Fruit/vegetable	1,211,418	1,033,912	1,252,493	1,023,416	3.4%	*1.0%*
Oilseed rape and similar	28,289	75,404	33,124	87,089	17.1%	15.5%

† Includes prepared goods made of cereals (e.g., breads, baking products); does not include beverages.

‡ Primarily consists of maize imported for livestock fodder.

Deroceras reticulatum (Müller) is one of the most serious pest molluscs in agriculture throughout the world (Howlett 2012). Other species of import in agriculture consist of agriolimacid and arionid species, including *Arion hortensis* (Férussac), *A. distinctus* Mabille, *A. vulgaris* Moquin-Tandon, and *Tandonia budapestensis* (Hazay) (Hunter 1966; Port & Port 1986; South 1992; Howlett 2012). These species, unlike *Deroceras* spp., are largely restricted to cropping fields as they rely on the soil disturbance caused by tillage but have difficulty colonising natural or seminatural habitats due to their difficulty or inability to penetrate non-disturbed soil (Hunter 1966).

Three dominant pestiferous species, *D. reticulatum*, *A. hortensis*, and *T. budapestensis*, are widely spread and form dense aggregates in agriculture, and as such are of primary interest to this thesis. Their population ecologies are offset from one another and there seems little direct competition for resources where the three occur sympatrically. *Deroceras reticulatum* overwinters primarily in the egg stage, with the densest populations forming over favourable spring and summer months. Conversely, *A. hortensis* populations are lowest in summer and increase in winter months, resulting in *A. hortensis* being the primary pest of winter crops. While *D. reticulatum* and *A. hortensis* both mature and reproduce on similar time scales, *T. budapestensis* is a slower-developing species, requiring approximately 18 months to complete one generation. As a result of this, *T. budapestensis* populations rarely reach the densities observed for *D. reticulatum* and *A. hortensis*, rather existing as stable moderate-density populations with no marked seasonal expansion or decline. If and when these species co-occur, the result is a constant pressure of pest damage to any crop at any time.

1.2.1. Control options for slugs in agriculture

Options for the control of any pest are highly varied. The primary aim of any pest control programme is to reduce target pest population density to below a defined level, above which economic losses will be incurred due to crop damage or yield reduction; this is known as the economic injury level (EIL) and varies based on the crop being grown and the target pest (Stern et al. 1959). Ideally, this is accomplished through regular monitoring of the pest in question, only taking prescriptive action (e.g., pesticide application) once pest densities reach or exceed the economic threshold. This threshold indicates the levels of pest populations at which action needs to be taken to prevent reaching EIL, and incorporates the time such action requires to suppress population densities (Stern et al. 1959). For sustainable agricultural practices, integrated control should be the aspiration, employing natural and conservation

biological control as the default, and only turning to chemical pesticide use when economic thresholds are exceeded. There are numerous difficulties, however, when considering such approaches for pestiferous slugs, many of which can be attributed to the pests' biology and ecology (see Section 1.2). Currently accepted methods for terrestrial slug control are discussed in detail below.

1.2.1.1. Chemical slug control

Slugs in Irish and UK agriculture are conventionally combatted using liberal application of chemical molluscicide. Use of chemical molluscicide in the UK alone has been estimated at 10 million kg per year (Port & Ester 2002). The two most widely available chemical pesticides in the EU are the carbamate ester methiocarb ($C_{11}H_{15}NO_2S$) (= mercaptodimethur) (NCBI 2019a) and the acetal metaldehyde ($C_8H_{16}O_4$) (= metacetaldehyde) (NCBI 2019b). The mode of action for methiocarb is as an acetylcholine esterase inhibitor, resulting in paralysis and death through starvation (Ester & Nijenstein 1995). Metaldehyde, in contrast, functions primarily by causing excess secretion and irreparable damage to mucus cells in gastropods (Bieri 2003; MacDonald 2009), causing death through dehydration. Both compounds are typically administered in dry pellet form, spread evenly across the topsoil within and between crop rows using a calibrated spreader (MacDonald 2009).

Chemical molluscicides, especially methiocarb, cause significant reductions in pest slug populations and slug-incurred crop plant damage (Ester & Nijenstein 1995; Bailey 2002; Douglas & Tooker 2012; Howlett 2012). However, there is now ample evidence that these pesticides also incur significant non-target and environmental damage as well. Methiocarb, with a broadly targeting mode of action, cause considerable damage to invertebrate populations in neighbouring soil, as well as to aquatic invertebrates as runoff introduces these compounds into waterways (Cloyd 2012; Sanchez-Bayo & Goka 2014), with the potential to have acute and long-lasting effects (European Commission 2013). While metaldehyde is considered a safer alternative to methiocarbs, with a lifespan of only approximately 12 days (DT_{50}) and evidence of non-persistence in the environment (Bieri 2003), a single slug pellet with 5% active ingredient can contaminate 10,000 litres of water at levels lethal to invertebrates (> 0.1µg/L) (MacDonald 2009). Metaldehyde also incurs toxic effects on mammals and birds (South 1992; Rumbeiha 2014).

The pervasive use of methiocarb pellets was reviewed by the European Commission initially in February 2014, and subsequently found to be so detrimental that its usage was

restricted to protective seed coatings (European Commission 2014). The timeline to phase out methiocarb molluscicide topical and pellet application was initially 30 September 2014 (European Commission 2014). This deadline has been extended almost annually as more evidence is gathered regarding the toxicity and non-target impacts of methiocarbs, and the current deadline for end-of-use stands at 31 July 2020 (European Commission 2015, 2018, 2019). Metaldehyde is currently approved for use as a molluscicide with certain application restrictions in the EU (European Commission 2011), but its usage authorisation in the UK has recently been withdrawn, effective 30 June 2020 (HSE 2018). Despite the recent sequence of extensions, it seems likely that the use of these substances will eventually be phased out; when this happens, conventional tillage farmers will be left at a loss for effective slug control. Additionally, organic farmers regularly face slug damage to their crops and are unable to use either methiocarb or metaldehyde for pest control.

Aside from these two prominent chemical pesticides, there are emerging options for slug control that are less environmentally damaging and approved for use in organic agriculture. Perhaps the best known of these is ferric phosphate ($FePO_4$), also applied to soil surfaces in ingestible pellet form. This substance is approved for use throughout the EU until 2030 as a molluscicide (European Commission 2011, 2015) and is also authorised in organic farming (IOFGA 2012). Ferric phosphate works as a calcium metabolism disruptor in molluscs which inhibits feeding, and has the advantage of degrading into phosphorus and iron in soil (MacDonald 2009; Howlett 2012). While it is generally considered less toxic than either methiocarb or metaldehyde, recent studies have suggested that a build-up of excess iron can negatively affect soil community and earthworm health (Langan & Shaw 2006; Edwards et al. 2009), and runoff into nearby waterways can contribute to phosphorus eutrophication (Schindler et al. 2008). Cropping fields treated with ferric phosphate have also shown high variability of success with regard to slug pest control (Iglesias et al. 2001; Speiser & Kistler 2002; Rae et al. 2009).

1.2.1.2. Cultural and physical control of slugs

Cultural control generally refers to management practices which can be altered to the direct output of reducing the population of a target or general suite of pest species (Van Driesche & Bellows 2001). For pestiferous slugs, there are three primary approaches. First, and perhaps easiest, is to maintain the local landscape in a way that will not foster pest population build-up such as removing weed growth within or surrounding crop fields and eliminating excess post-harvest crop residue (MacDonald 2009; AHDB 2016). Such plant material offers

shelter and food sources for slugs, and allow populations to grow even outside of cropping periods.

Seed bed establishment and mechanical manipulation are a second option for cultural control. Compacted seed beds with compressed soil make it difficult for slugs to access germinating plants (MacDonald 2009) and drilling of seeds to depths > 4 cm will prevent slug damage to seedlings in even open, cloddy soil (AHDB 2016). Repeated mechanical manipulation (i.e., tilling) disrupts slug aggregations and can expose individuals and eggs to unfavourable conditions (e.g., hot and dry in summer or exposure to frost/freezing temperatures in winter) (MacDonald 2009). Tilling can also help to compress soil between plantings, restricting the dispersal of slug populations (Howlett 2012). It should be noted, however, that such intensive cultivation is often at odds with modern sustainable agricultural frameworks in Ireland, and may even disqualify farmers from certain subsidizations through agri-environment schemes such as the current Green, Low-Carbon Agri-Environmental Scheme (GLAS) because of the associated fuel consumption/carbon emission of running farm machinery and negative effects on soil health and erosion (Howlett 2012; DAFM 2016).

Finally, strategic management practices can be highly valuable for limiting the impacts of many pest species, including slugs. Herbaceous and leafy crops provide an ideal environment and food source for slugs, therefore such high-risk crop rotations (e.g., oilseed rape followed directly by winter wheat) should be avoided (Howlett 2012; AHDB 2016). More beneficial rotations for reducing slug damage may be to follow leafy crops with others with shorter germination or growing periods (e.g., winter wheat followed by potatoes) (South 1992) or replacing susceptible varieties with more resistant ones (e.g., in potatoes) (Winfield et al. 1967). Additionally, the establishment of beetle banks and buffer strips can increase the abundance and diversity of natural enemies, resulting in reduced slug population densities via predation between June and September (MacDonald 2009; AHDB 2016). Such management practices can be easy to implement but require a certain amount of pre-planning and expert knowledge of the target pest to be most effective.

1.2.1.3. Biological control of slugs

Currently the only available biological control agent specifically targeting terrestrial slugs is the soil-dwelling nematode *Phasmarhabditis hermaphrodita* Schneider (Rhabditida: Rhabditidae) (Glen & Wilson 1997; Rae et al. 2007). Marketed in Europe as Nemaslug (BASF, Ludwigshafen, Germany), this endemic nematode is reared in mass cultures

and applied to cropping systems in the infective juvenile (IJ) form which migrate into the bodies of host slugs where they mature and reproduce (Wilson et al. 1993). The bacterium *Moraxella osloensis* Jebasingh, Lakshmikandan, Rajesh, & Raja (Pseudomonadales: Moraxellaceae), thought to be opportunistically associated with *P. hermaphrodita* in biological control (Rae et al. 2010), causes mortality in the slug host while *P. hermaphrodita* consume the host carcass (Rae et al. 2007).

These nematodes are used as inundative biological control – where a large volume of natural enemy is released cyclically to eradicate pests – and therefore require high investment of both labour and cost as they do not persist in crop fields, with multiple applications required in a single growing season for effective reduction of slug damage (Rae et al. 2007; Pieterse et al. 2017; Michaud 2018). The ability to infect multiple slug species makes *P. hermaphrodita* an attractive option in agriculture, however there is evidence that this biological control agent may be less efficacious against species other than *D. reticulatum*, with larger *Arion* spp. often immune to or able to recover from infection (Speiser et al. 2001; Dankowska 2006; Pieterse et al. 2017). Additionally, *P. hermaphrodita* have a short shelf-life because it is sold as a live culture (Howlett 2012) and have demonstrated unreliable rates of slug control (Glen & Wilson 1997; Speiser et al. 2001; Rae et al. 2007). Protracted mass-rearing of these nematodes in laboratory media could also cause reduced virulence, further reducing their usefulness. While *P. hermaphrodita* is a useful tool for controlling slug populations, additional options will need to be explored to fully replace reliance on chemical molluscicides.

Marsh flies (Diptera: Sciomyzidae), a globally distributed family, have been known natural enemies of terrestrial and semiaquatic molluscs since Berg (1953). Member species of this family have been the subject of extensive and ongoing research for use against a variety of molluscs, including semiaquatic snails which are intermediate hosts for filarial and trematode diseases (Berg 1953; Gormally 1988; Vala et al. 2000; McDonnell et al. 2005; Knutson & Vala 2011), and studies have indicated that Sciomyzidae demonstrate functional responses appropriate to species used as biological control agents (Eckblad 1973; Haab 1984; Beaver 1989; Manguin & Vala 1989; Knutson & Vala 2011). Nine species, four of which are within the genus *Tetanocera*, have evolved to feed specifically on terrestrial slugs (Table 1.3) (Knutson & Vala 2011; Murphy et al. 2012). Of these, *Tetanocera elata* (Fabricius) is the only species with a Palaearctic distribution; an analogous species, *Tetanocera plebeja* (Loew), fills the same niche in North America (Knutson & Vala 2011). The host-specific association *of T.*

elata with pestiferous terrestrial slug species, along with its native distribution, makes it a species of interest for use in biological control.

1.3. *Tetanocera elata*

Since the first observations of Knutson *et al.* (1965), *T. elata* has been recognised as a natural enemy of terrestrial slugs. These initial studies described the basic biology and ecology of *T. elata* and made preliminary identification of species within its host range (Knutson et al. 1965). This knowledge was employed primarily in an ecological context (e.g., for informing species lists and biodiversity surveys) for decades (Rozkoŝný 1984, 1987; Speight 2004a, b; Williams et al. 2009; Speight & Knutson 2012). But as sustainable agriculture and natural pest control have gained wider traction, interest the species has undergone a resurgence in the past few years as the subject of applied biological control of pestiferous slugs. Hynes and colleagues are responsible for many of the modern studies of *T. elata*, on which this thesis builds. Such modern works have determined optimal rearing conditions for laboratory cultures and temperature effects on egg hatch and adult longevity, as well as describing predatory behaviours of larvae (Hynes et al. 2014a, b, c). Most recently, D'Ahmed *et al.* (2019) described the interaction of *P. hermaphrodita* and *T. elata* larvae and speculated how such interactions could affect slug control in an integrated management scheme in which both natural enemies were employed.

1.3.1. Biology and development

The life cycle of *T. elata* (Fig. 1.1) consists of egg, three larval instars, pupa, and adult stages (Fig. 1.2A). Timing of each life stage can fluctuate based on environmental conditions, but maturation from egg to adult typically takes between 53 and 77 days in nature during the active flight period with favourable conditions (see Section 1.3.3, below) (Knutson et al. 1965; Hynes et al. 2014c). Under laboratory rearing conditions, eggs demonstrated the highest success rate of hatch (52%) when reared at 14°C (Hynes et al. 2014b), while larvae (Fig. 1.2B) develop best at 20°C (Hynes et al. 2014c). Females lay an average of 291 eggs, with a maximum range observed between 196 to 487 for a single individual, and do not seem to require repeated copulation events (Knutson et al. 1965; Beaver 1973; Hynes et al. 2014b; D'Ahmed et al. 2019).

Table 1.3. Summary of slug-killing Sciomyzidae species with additional documentation of distribution and feeding behaviour.

Species	Distribution	Exclusively Slug Feeders?
Euthycera arcuata (Fabricius)[1]	Nearctic	Unknown
Euthycera chaerophylli (Fabricius)[2]	Palaearctic, mesic woodland	Yes
Euthycera cribrata (Rondani)[2]	Mediterranean	No
Euthycera stichospila (Czerny)[2]	Mediterranean	No
Limnia unguicornis (Scopoli)[3]	Palaearctic	Unknown
Tetanocera clara Loew[2]	Nearctic, mesic woodland	No
Tetanocera elata (Fabricius)[2]	Palaearctic, widely distributed	Yes
Tetanocera plebeja Loew[2]	Nearctic, widely distributed	No
Tetanocera valida Loew[2]	Nearctic, mesic woodland	Yes

[1]GBIF Secretariat (2017a)

[2]Knutson & Vala (2011)

[3]GBIF Secretariat (2017b)

Tetanocera elata is a natural enemy of terrestrial slugs in its larval stage. Eggs are likely laid on vegetation near soil near aggregates of *D. reticulatum* (Knutson et al. 1965). In the first and second larval instars it is an obligate mesoparasitoid, with high host specificity to *D. reticulatum* and closely related species (*Deroceras laeve* Müller, *Deroceras invadens* Reise, Hutchinson, Schunack, & Schlitt) at lower frequency (Knutson et al. 1965; D'Ahmed et al. 2019). After hatching, neonates have been demonstrated to live up to a maximum of 13 days before starvation (D'Ahmed et al. 2019). Parasitoid infection occurs as neonate larvae burrow into the host tissue near the head, typically targeting the mantle near the pneumostome (Fig. 1.2C), or through an optical tentacle (Knutson et al. 1965). The parasitoid will occupy a singular host until the end of the second instar, feeding on mucus and decaying tissue. Upon the death of their neonate host (via massive tissue damage as the larva grows), larvae undergo a behavioural shift to become predaceous (Fig. 1.2D).

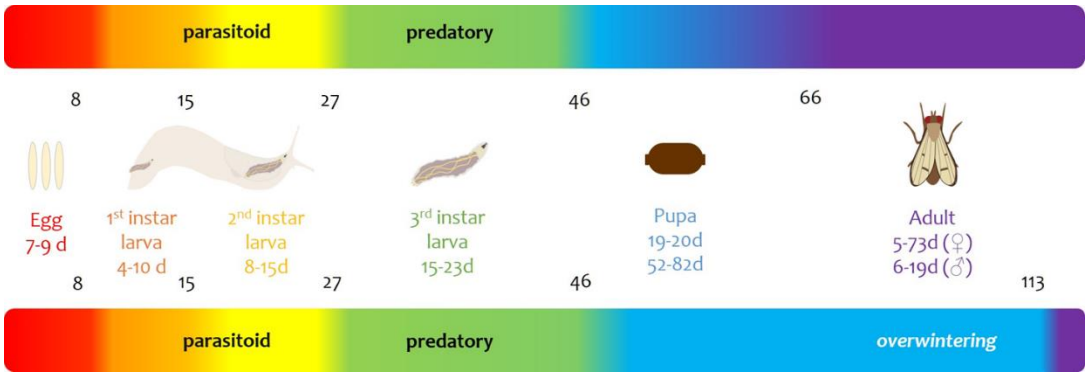

Figure 1.1. Life cycle and developmental timeline of *Tetanocera elata* under field typical conditions in Ireland. Top timeline reflects developmental rates in a summer/flight period generation, while the timeline on the bottom (and the corresponding bottom set of numbers for Pupa stage) reflect overwintering (quiescent) time periods. Generated from data in Knutson et al. (1965) and Hynes et al. (2014c).

Free-living third instar larvae also experience an expansion of their diet range, and have been documented feeding on *Tandonia budapestensis* (Hazay), *Tandonia sowerbyi* (Férussac), *Arion fasciatus* (Nilsson), *Arion intermedius* Normand, *Limacus flavus* (Linnaeus), *Malacolimax tenellus* (Müller), and *Geomalacus maculosus* Allman, in addition to host species *D. reticulatum* and *D. laeve* (Knutson et al. 1965; Giordani et al. 2014; Hynes et al. 2014a; Knutson & Vala 2011). Larval predating

behaviours have been classified into three strategies: search and attack (SA), search and wait (SW), or wait (W) (Hynes et al. 2014a). Searching behaviour is identified by larvae actively raising and moving the head in a side-to-side manner, sometimes paired with forward movement. If this sequence is coupled with attacking (SA), the larva reaches a prey individual and injects its mouthparts into its prey, immobilising it by injection of a neurotoxin (Knutson & Vala 2011) and eventually commences feeding. Searching may alternatively be followed by the larva ceasing activity (SW). Individuals displaying only a waiting behaviour (W) do not make any attempts at prey-finding. Both SW and W only result in attack on a prey individual if the slug incidentally contacted the stationary larva (Hynes et al. 2014a; D'Ahmed et al. 2019). Although this head movement while searching may suggest the utilisation of some chemosensory cues by larvae, subsequent investigation (Colton 2016) has indicated that *T. elata* larvae do not follow mucus trails as other *Tetanocera* spp. do (Barker et al. 2004).

While larval parasitoid host range has been examined extensively, little is known regarding the diet of adult *T. elata*, with the exception of the suitability of an artificial medium composed of honey and brewer's yeast, occasionally supplemented with milk powder or crushed snail tissue, for laboratory rearing (Knutson et al. 1965). Some anecdotal evidence has documented *T. elata* adults perching on flowering plants or potentially feeding on dead insect tissue/eggs or slug mucus (Berg & Knutson 1978; Knutson & Vala 2011), however this has not been explicitly examined or quantified.

1.3.2. Ecology

To date, not much is known regarding the specific ecology of *T. elata*. Aside from host/prey association, few other community interactions have been documented. Recently, D'Ahmed *et al.* (2019) demonstrated that third instar larvae more frequently attacked *P. hermaphrodita*-infected (and potentially immunocompromised) slugs than unexposed individuals. A trade-off in fitness was observed, however, with both parasitoid and predaceous larvae reared on nematode-infected slugs showing lower survivorship.

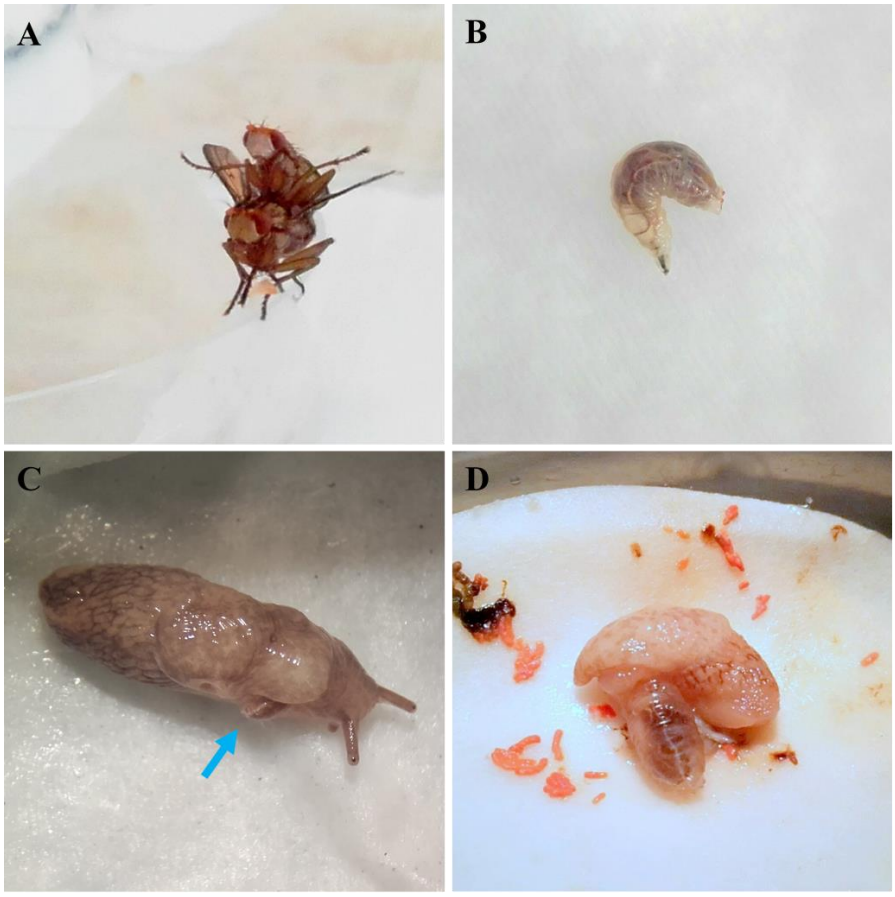

Figure 1.2. *Tetanocera elata* adult male and female mating (**A**), juvenile (**B**), second instar parasitoid spiracles protruding from under host (*Deroceras reticulatum*) mantle (**C**), and third instar predaceous larva attacking prey (*D. reticulatum*) (**D**).

Tetanocera elata is a wide-spread Palearctic species. Adult collection records range from the western boundaries of Ireland and the UK east to Japan, including records from France, Italy, the Netherlands, Germany, Denmark, Norway, Sweden, Finland, Czechia, Slovakia, Turkey, Kazakhstan, Russia, Mongolia, China, and Korea (Knutson & Berg 1971; Rozkoŝný 1984; Leclercq & Schacht 1986; Roller 1995; Rozkoŝný et al. 2010; Knutson & Vala 2011; EOL 2019). Surveys across this range have recovered *T. elata* adults from a disparate array of habitats, including wet and dry grasslands, abandoned horticultural land, on the banks of lakes and turloughs, and in bogs, fens, and woodlands (Knutson et al. 1965; Speight 2001; Speight 2004a, b; Williams et al. 2009; Knutson & Vala 2011; Speight & Knutson 2012; Maher et al. 2014; Carey et al. 2017). There is no known association between *T. elata* and any plant species, however Williams *et al.* (2009) have indicated that vegetation structure may be an important factor. Although commonly-occurring, the species is seldom

recovered at high densities when sampling, more frequently occurring in both active and passive sampling catches as singletons or doubletons (Chandler 1972; Speight 2004a, b; Williams et al. 2007; Carey et al. 2015, 2017). This trend may indicate that either surveys are being conducted in sub-optimal habitats, and as such that *T. elata* are able to survive when necessary across such an expanded range, but flourish at higher densities within more suitable habitats, or that the species simply does not form dense populations.

The active flight period of *T. elata* occurs in Ireland between late June and early October (Speight & Knutson 2012), and the species is believed to be either bi- or multivoltine. This flight period coincides with the population dynamics of *D. reticulatum* (see Section 1.2.1, above) which is ideal for the feasibility of *T. elata* as a biological control agent of *D. reticulatum*. Overwintering quiescence occurs in the pupal stage within the topsoil (Knutson et al. 1965; Speight & Knutson 2012) when *D. reticulatum* shelter within soil from winter freezing temperatures (Hunter 1966).

1.4. Conservation Biological Control

Biological control, the suppression of the population of one organism (i.e., the "pest") through the use of another (i.e., the "natural enemy"), is a widely used approach to pest control throughout agricultural and natural systems across the globe (Van Driesche & Bellows 2001). The most well-known of these is likely either classical biological control, which utilises natural enemies imported from the same home range as an invasive pest, or inundative (augmentative) biological control (as described for the case of *P. hermaphrodita*, Section 1.2.2.3); however additional biological control approaches exist to meet other pest control needs. Conservation biological control is one such approach, and has been gaining in favour and attention in the past two decades as a shift toward agricultural sustainability has occurred, as evidenced by numerous current studies reviewed by Crowder & Jabbour (2014) and Begg *et al.* (2017). Throughout the EU, there are even provisions through various agri-environmental schemes to support farmers wishing to employ conservation biological control mechanics (Rusch et al. 2010; Holland et al. 2016; Begg et al. 2017).

At its most basic, conservation biological control aims to improve control of pest species by enhancing the local environment to better support populations of natural

enemies. This can be accomplished in two main ways: (1) by improving the quality of the habitat and increasing available resources; and (2) by restricting pesticide usage in situations where it may negatively impact natural enemies (Holland et al. 2016). The most widely adopted approach is the enhancement of quality and area of semi-natural habitat on farms, including such features as hedgerows, beetle banks, flower or grass margins, and fallow land (Van Driesche & Bellows 2001; Nicholls & Altieri 2007; Begg et al. 2017). These features, though mechanistically different, all have similar outcomes of increasing invertebrate biodiversity. Semi-natural habitats provide numerous benefits for natural enemies, including alternative food sources (e.g., floral and extrafloral nectar), refuges (e.g., protection from environmental changes or areas away from where pesticide may be applied), sheltering and overwintering sites removed from areas of disturbance (e.g., crop harvest and tillage), and corridors for movement and dispersal (Van Driesche & Bellows 2001; Nicholls & Altieri 2007). It is commonly accepted that agricultural intensification and landscape simplification reduces biodiversity and can increase pest abundance and damage (Tscharntke et al. 2005; Nicholls & Altieri 2007; Jonsson et al. 2016); conversely, there is mounting evidence that improving landscape complexity and biodiversity within agroecosystems improves pest control and crop yield (Chaplin-Kramer et al. 2011; Tscharntke et al. 2012; Pywell et al. 2015). As a result, the success of conservation biological control is now well evidenced (Thies et al. 2011; Ramsden et al. 2015; Jonsson et al. 2016).

Because the focus of conservation biological control is manipulation of the environment and not the biological control agents themselves, this approach largely relies on the existence of natural enemies within the agroecosystem (or surrounding local landscape) and their ability to effectively control pest populations if adequately supported. In this context, this type of biological control is most useful for controlling indigenous pests (Van Driesche & Bellows 2001). While *T. elata* may not necessarily exist widely within agroecosystems, it is a native species and therefore remains a good candidate. A purely conservation biological control approach would likely not be effective, rather a combination of ecological engineering to provide a suitable habitat with requisite resources provided (conservation biological control) coupled with the intentional introduction of individuals (augmentative biological control) to initiate populations.

It has been suggested that it is possible to maximise the effects of biological control for a narrow range of pests by tailoring the approach to suit a single natural enemy of interest, however for such a programme to be successful explicit knowledge of the target organism is required to ensure all needs of the species are met (Holland et al. 2016). Specifically, factors such as dietary requirements for all life stages, association with host or shelter plants, overwintering period and location, and life cycle and phenology of the natural enemy should be identified in detail.

1.5. Overview of the Thesis

1.5.1. Aims and objectives

This thesis was designed to address specific gaps in the existing knowledge base relating to *T. elata*, with the ultimate goal of assessing the feasibility and advancing the potential of a biological control programme of pestiferous slugs in Irish agriculture using *T. elata*. Specifically, the desire is to design a conservation biological control programme, through which introduced populations of *T. elata* will establish within agroecosystems and offer self-sustaining, long-lasting control of slug populations with minimal continued input. As discussed in Section 1.4 (above), extensive knowledge of the biology and ecology of the target natural enemy must be established before such an undertaking may be considered viable.

The primary objectives of the thesis can, therefore, be summarised as follows:

1. To determine the biological requirements of *Tetanocera elata* adults, building on existing knowledge of larval requirements, for the establishment of a functional biological control scheme.
2. To evaluate the potential and realised prey range of this proposed biological control agent, and to anticipate its safety for use in agroecosystems.
3. To assess the physiological impacts of alternative prey utilisation on larvae.

4. To describe the ecological interactions of *T. elata* adults, with the aim of translation of these findings into agroecological management recommendations.

5. To identify the phenology and population dynamics of *T. elata* adults in nature, and to determine the voltinism of *T. elata* in Ireland.

1.5.2. Structure of the thesis

The thesis is submitted in article format, with Chapters 2 – 4 each representing an independent original research manuscript that has either been published or submitted for publication in relevant entomological journals. The preceding introduction (Chapter 1) gives context to the research and sets out the scope and objectives of the thesis project.

Chapter 2 discusses research undertaken to identify sources of adult diet in natural habitats. This laboratory study was conducted at family level, using many species of Sciomyzidae; however, because *T. elata* is the focal species of this thesis, a subset of individuals in this study consisting only of *T. elata* was evaluated independently. Results given here describe a new community ecological association not previously documented for any species of Sciomyzidae. This chapter was published in the Journal of Environmental Entomology.

Host range and alternative prey effects are reported in Chapter 3. Specifically, third instar larvae were used for this laboratory study, as existing research (Hynes et al. 2014a, b; D'Ahmed et al. 2019) has already established behaviours and host range of parasitoid larvae. Three of the most important pest slug species (*D. reticulatum, A. hortensis*, and *T. budapestensis*) comprise the prey choice options. Following choice trials to determine prey preference, this study also examined the prolonged physiological effects of different prey species on larval development and survivorship. This study has been submitted to BioControl and is currently under review.

The final study reported in the thesis (Chapter 4) is a series of field observations made with the goal of describing the important characteristics of local habitats where *T. elata* populations occur. This study was conducted over 12 months at a single site where *T. elata* have regularly been recovered, and serves as a case study with recommendations for future application. Also included in this chapter is an analysis of

T. elata population dynamics as reflected in adult collection numbers, with the perspective of determining both voltinism of the species in Ireland and confirming the synchronicity of *T. elata* with the target pests. It has been submitted to Agricultural and Forest Entomology.

Chapter 5 is a general discussion of the thesis findings, and serves to synthesise the results of these independent studies into a cohesive output. This chapter discusses the potential for applying the thesis findings to the development of a conservation biological control programme, including potential limitations, and concludes with a summary of key conclusions and recommendations for further research development. Appendices (I – III) include supplemental data and/or figures as indicated for each chapter.

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Chapter 2 Catching flies with honey(dew): Adult marsh flies (Diptera: Sciomyzidae) utilize sugary secretions for high-carbohydrate diets

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2.1. Abstract

Marsh flies (Diptera: Sciomyzidae) are a diverse family which provide valuable ecosystem services, including the biological control of mollusks which are agricultural pests and vectors of animal and human parasitic diseases. Additionally, some species may serve as important ecological bioindicators. Despite the extensive research on this family, most has centered on larval diet and behavior, as this is the life stage primarily used for biological control; virtually nothing is known about the natural dietary components of adult marsh flies. Our study aimed to close this knowledge gap by examining the dietary range and preference of adult marsh fly species. Individual flies were provided with five food choices in cafeteria-style food choice trials, consisting of crushed snail, freshly-killed slug, glucose solution, honey-yeast mixture (the standard laboratory rearing diet), or water. Sciomyzidae at family level displayed significant differences in food selection ($P = 0.0212$), with carbohydrates (honey-yeast and glucose solution) significantly preferred over protein options (mollusk tissue) or the water control ($P < 0.001$). This suggests that marsh flies may naturally maintain a carbohydrate-rich diet. Since many plants typical at field sites produce little or no nectar, a second experiment aimed to determine the source of these carbohydrates in nature. When presented with honeydew harvested from aphids (Hemiptera: Auchenorrhycha), *Tetanocera elata* individuals were observed to feed on dry honeydew and honeydew solution significantly more frequently than the water control ($P < 0.001$ and $P = 0.01969$, respectively) suggesting that honeydew may play an important role in adult marsh fly diet.

KEY WORDS: bioindicator, biological control, honeydew, mollusk, Sciomyzidae

2.2. Introduction

Marsh flies (Diptera: Sciomyzidae) are a cosmopolitan family with a global distribution with the exception of Antarctica. Across their range, they provide important ecosystem services, including biological control of aquatic and semiaquatic mollusks which are obligate intermediary hosts of medical and veterinary parasites (Gormally 1988), as well as terrestrial mollusks which are prominent pests of arable farming and horticulture (Knutson & Vala 2011). They may also serve as reliable bioindicators within wet grassland habitats (Carey et al. 2017a). All of these ecosystem services are highly relevant to ecologically sensitive and sustainable agriculture, especially in wet temperate zones such as Ireland, where many of these pestiferous mollusks thrive and where grasslands dominate the landscape.

Agricultural revenues comprised $7.1 quadrillion ($7.1x10^{15}) USD globally in 2016 (FAO 2016), and amongst staple crops (e.g., wheat, maize, rice, soybeans, potato) losses due to animal pest damage have been estimated to range from 7-20% depending on region and crop, with a global average of approximately 15% (Oerke 2006). In Ireland, arable fields, vegetable, and cereal crops account for approximately €1.4 billion per annum (An Bord Bia 2017). In the UK, which has similar agricultural figures to Ireland, approximately 15% of agricultural production is lost to pest damage. Responsible pests are comprised in large portion by numerous species of terrestrial slugs (e.g., *Deroceras reticulatum* Müller, *D. laeve* Müller, *Tandonia budapestensis* Hazay, *Arion hortensis* Férussac, *A. distinctus* Mabille, *A. vulgaris* Moquin-Tandon, and others) (MacDonald 2009; Howlett 2012), and in the UK, various slug species have been recorded to inflict £8 million in damages to crops annually (MacDonald 2009). Damage of this type is generally caused by a failure of seeds to complete germination, with slugs heavily damaging seeds and young sprouts. Additionally, residual mucus and feces can reduce marketability of mature crops, and some slug species have been shown to vector diseases between host plants (MacDonald 2009; Douglas & Tooker 2012). In addition to crop pests, many aquatic and semiaquatic mollusks are intermediate hosts of medical and veterinary diseases, providing an intermediate host for filarial diseases such as fascioliasis and schistosomiasis. *Galba truncatula* Müller (Gastropoda: Lymnaeidae), a common semiaquatic snail species found in Irish wet grasslands subject to periodic inundation, is an intermediate host of

Fasciola hepatica L. (Echinostomida: Fascioliclae), the cause of fascioliasis. Nearly 600 million livestock animals such as sheep and cattle are infected with *F. hepatica* worldwide each year, costing approximately $200 million in treatments (Boray 1985; Hillyer & Apt 1997; Williams et al. 2010).

Traditionally, pestiferous terrestrial mollusks have been chemically controlled using pesticides such as methiocarb and metaldehyde pellets. Though the most successful option for many years, such pesticides are often unreliable, with problems ranging from only affecting surface-active mollusks, to not being distributed to the correct areas of pest aggregation in sufficient amounts, to the pellets being distasteful to pests to ingest, to pests frequently "recovering" from poisoning (Howlett 2012). While ferric phosphate pellets have emerged recently as a more environmentally-friendly option, they suffer similar problems with efficacy as traditional pellets. Because of the demonstrated non-target risks associated with them, the use of methiocarb baits was banned by the European Union in 2013 (European Commission 2014; Nicholls 2014). This ban was extended with an order for further review until August 2019 (European Commission 2015, 2018), giving growers time to formulate alternative pest control approaches. While snail-borne diseases are commonly controlled by treating domestic animals with anthelmintic drugs, resistance to drugs is becoming a persistent problem in many parts of the world (Learmount et al. 2018). A promising alternative to chemical control may present in the form of native natural enemies for the targeted biological control of pestiferous or intermediate host mollusks by the augmentation or introduction of marsh flies, the larvae of some species being highly host-specific as mesoparasitoids, and others generalist predators, in early instars (Knutson & Vala 2011; Hynes et al. 2014a; Speight & Knutson 2012; Murphy et al. 2012). Berg (1953) first examined the potential for using marsh flies as biological control agents against agriculturally or medically pestiferous gastropods. Since this initial research, the larval stages of numerous species have generated significant interest due to their mollusk-killing abilities as possible biological control agents for mollusk-spread trematode diseases (e.g., *Ilione albiseta* Scopoli targeting *G. truncatula*) and agricultural pests (e.g., *Tetanocera elata* [Fabricius] controlling *D. reticulatum*) (Berg & Knutson 1978; Gormally 1988; Murphy et al. 2012; Hynes et al. 2014a).

Sciomyzidae have also been proposed as a potential wetland bioindicator due to their preference for such habitats (Speight 1986). Across Europe, agricultural intensification frequently threatens biodiversity in wet grasslands. This can be mitigated with the use of high-nature value (HNV) farming approaches and agricultural incentive schemes, and recent research on wet grassland biodiversity has found that marsh flies may be used as surrogates for the diversity of the wider dipteran population at trap-scale (Desjeux et al. 2014; Carey et al. 2017a). Adult marsh flies also demonstrate high fidelity to larval habitats (Williams et al. 2009). The characteristic host-specific mollusk association of several species suggests they may also prove valuable bioindicators for the mollusk community of a wetland, because the presence of adult marsh fly species generally indicates the presence of associated mollusks exclusive to their respective larval stages (Speight & Knutson 2012).

In a biological control context, there has been much research into marsh fly larval feeding behavior and host range, as this is the life stage which targets mollusks (Knutson & Vala 2011; Murphy et al. 2012). However, while the feeding habits of marsh fly larvae have been extensively studied, there remains very little knowledge of what adults feed on in nature (Knutson & Vala 2011). In order to successfully utilize marsh flies as either biological control agents or bioindicators, knowledge of their biology at every life stage must be improved. For biological control species, this could improve the design of mass-rearing programs (by providing a more natural diet for colonies, thus lowering stock mortality rate) and ensure the ecological requirements of field-released individuals are met to sustain introduced or augmented populations (McGeoch 1998;, Knutson & Vala 2011). Any species potentially used as bioindicators must have a knowledge base of the biology, phenology, feeding habits, and life cycle. While several of these criteria are already met for marsh flies, further elucidation of their adult feeding habits and behaviors could enhance their viability as bioindicators of dipteran diversity (McGeoch 1998; Knutson & Vala 2011).

To successfully survive and reproduce, insects require access to a diet consisting of carbohydrates and amino acids (Sabri et al. 2013). Superficial observations have been made concerning adult feeding behavior by Berg and Knutson (1978) and, although the observations are simply anecdotal, they suggest that adult marsh fly diet may include dead or dying insects, eggs, snail mucus, and a variety of wild plants, although the extent of these feedings have yet to be fully examined. Berg and Valley

(1985a, b) observed *Sepedon* spp. females feeding on nuptial gifts secreted by males, although their content remains unknown. Marsh flies have been observed on *Caltha palustris* L. by Judd (1964), a common wet grassland plant, and microscopic observations by Stoffolano *et al.* (2013) have revealed clusters of pollen on the antennae of *Sepedon fuscipennis* Loew. There are also images from various sources (e.g., online photo-sharing and insect identification forums) of adult marsh flies of various species perching on flowers (Knutson & Vala 2011). However, like most acalyptrates, marsh flies have a spongey proboscis in the adult life stage, which largely restricts them to consuming liquid or semi-liquid food sources (Coronado-Gonzalez et al. 2008). Unlike the larval stages, which have hooked mouthparts, the adult proboscis does not allow for an attack on live mollusks or insects or feeding on flower pollen.

In laboratory colonies, the most commonly-used diet for adult marsh flies is a combination of honey and brewer's yeast (Knutson & Vala 2011; Hynes et al. 2014b). The ability and success of raising adults on a honey-yeast paste indicates that marsh flies, like many other insects, require a high-carbohydrate diet in the adult life stage (as there is a high level of energy investment required for mate-finding or selecting oviposition sites). In nature, one of the most readily-available sources of a high carbohydrate diet is plant nectar, either from flowers or extrafloral nectaries; however, representative plant species within typical marsh fly habitats (e.g., wet or terrestrial grasslands) frequently lack either flowers with prolific nectar or extrafloral nectaries. These sites are generally dominated with grasses (Poaceae), rushes (*Juncus* spp.), or sedges (*Carex* spp.), interspersed with seasonal flowering plants and may be bordered by hedgerows (Appendix I.1). While some of the characteristic flowering plants present offer sugar-rich nectar (Pellmyr 2002; Hicks et al. 2016), such flowers comprise only a small percentage of the overall vegetation (Appendix I.1).

Honeydew-producing insects are known to provide a source of carbohydrates and amino acids for many insect species and microbial communities, and high abundances of honeydew producers have been shown to enhance insect activity and abundance (Gaigher et al. 2011; Eatough Jones et al. 2012). Aphids (Hemiptera: Sternorrhyncha: Aphididae) are the most common and well-studied of all honeydew producers (Pyati et al. 2011; Sabri et al. 2013), and have been observed in abundance at marsh fly study sites (ABE & JGJC *pers. obs*.).

Whether in the context of biological control agents or bioindicators, there is an extensive understanding of the feeding behavior of larval marsh flies (Berg & Knutson 1978; Knutson & Vala 2011), but there is little quantitative data regarding the feeding behavior and dietary preferences of adult marsh flies. Our study aimed to elucidate a consensus diet by: (1) ascertaining the major components of the preferred adult marsh fly diet across species; and (2) testing adult feeding alacrity on a diet source readily available to them in typically-surveyed marsh fly habitats. Because the diet of adult marsh flies in the wild is currently unknown, identifying what adults consume in nature could prove important to the success of future biological control implementation and in their use as effective bioindicators.

2.3. Materials and Methods

2.3.1. Acquisition and maintenance of experimental colonies

Flies (*Ilione albiseta*, *I. lineata*, *Pherbina coryleti*, *Tetanocera arrogans*, and *Tetanocera elata*) which were the subject of experiments were collected using a sweep net (0.3 m handle; 0.5 m diameter; 0.1 pore net) on tall vegetation in abandoned or unmanaged fields in Co. Galway, Ireland (Fossitt 2000; Appendix I.2). All species of Sciomyzidae were removed from nets using barrel-style pooters (Watkins & Doncaster, The Naturalists, Hawkhurst, Kent, England) and transported therein to the National University of Ireland Galway, where they were maintained in colony until they could be used for experiments. Marsh flies returned from field collections were identified based on morphology (Rozkoŝný 1984, 1987) and maintained in groups according to species. Flies were held in 24.5 x 24.5 x 24.5 cm vinyl and polyester mesh cages with a 17 cm sleeve (Bugdorm model 4222, MegaView Science, Taiwan) and provided with a laboratory diet of 3:1 honey-yeast mixture (Knutson et al. 1965; Hynes et al. 2014b) and cotton pads soaked with water. Wooden sticks were also placed within cages to provide additional resting perches. Flies were sexed before use in each trial, and sex and species were confirmed after each individual died. All cages were maintained at laboratory ambient temperature and relative humidity (18-22°C, 42-70% RH). Photoperiod was largely kept to laboratory ambient conditions under incandescent room lighting, but was supplemented by natural light from a large northeast-facing window providing an approximate 16:8 (L:D) summer photoperiod.

The two mollusk species used as diet choices in feeding trials were selected based on their being commonly predated on by numerous species of marsh fly (Berg & Knutson 1978; Knutson et al. 1965). Slugs (*D. reticulatum*) were obtained by placing de Sangosse slug traps (de Sangosse, France) on amenity lawns or grassy fields on college grounds. Traps were checked weekly and *D. reticulatum* collected and housed in controlled laboratory colonies. Slugs were maintained on damp tissue in 650ml clear plastic boxes (17 x 11.5 x 4cm, L x W x H) with small holes in the lids to provide ventilation, and fed with dry porridge oats. Occasionally eggs were discovered in boxes and were removed (to prevent cannibalism) and reared in 5 cm petri dishes until hatching, at which point juvenile *D. reticulatum* were transferred to standard rearing boxes. All *D. reticulatum* were maintained within an environmental chamber (LTE Qualicool, LTE Scientific Ltd., Greenfield, Oldham, UK) at 16°C. Snails (*Lymnaea stagnalis* L.) were acquired by dragging a 30cm pond net (EFE & GB Nets, Totnes, Devon, UK) at Rostaff turlough (53.485479, -9.1314339). Sediment was washed through fine metal sieves and snails extracted as needed for feeding trials.

Honeydew used in trials was obtained from mixed-species aphid (Hemiptera: Aphididae) colonies housed at NUI Galway to ensure that it was free of any possible contaminants. Colonies were initiated by collecting aphid-infested cuttings or whole plants (*Eruca sativa* Mill.) from Site 2 (Appendix I.2) and laying them adjacent to potted bell pepper plants (*Capsicum annuum* L. var. *annuum*) purchased as seedlings from a consumer garden center. Aphids were allowed to migrate naturally onto colony plants to avoid excess handling. Host plants were maintained within 76 x 62 x 62 cm plastic propagation tents (Ready Steady Grow PVC Propagation Dome) at laboratory ambient conditions (18-22°C, 42-70% RH) and natural light in an approximate 16:8 LD photoperiod provided by a southwest-facing window. Planthoppers used in marsh fly-hempiteran feeding interaction trials (mixed species within the family Cicadellidae [Wilson et al. 2015]) were collected using sweep nets at marsh fly collection sites. They were harvested concurrently with marsh fly collection using the same methodology described above.

2.3.2. General cafeteria trials

Cafeteria experiments were performed by releasing a single adult marsh fly into a testing cage identical to those used to maintain colonies (Bugdorm model 4222, MegaView Science, Taiwan) furnished with five different food choices simultaneously. These consisted of: slug (*D. reticulatum*) tissue (0.25g); snail (*L. stagnalis*, 0.25g); standard laboratory diet (3:1 honey-yeast mixture, 0.5g); 10% glucose solution (1ml); and water (1ml) as a control. Mollusks were killed immediately prior to commencing trials to prevent prolonged decomposition. *Lymnaea stagnalis* were crushed, while *D. reticulatum* were killed by freezing for one hour and subsequently crushed, both to kill the individual and expose internal tissue for improved access for feeding. The concentration of glucose solution used was determined by experimentally testing the preference of concentration of multiple marsh fly species in an earlier cohort (Appendix I.3). Each food type was placed individually within an open-topped 5 cm clear plastic Petri dish, and all Petri dishes were arrayed in randomized order in a circular configuration at the bottom of the testing cage. Trials ran for 5 hours, with observations of individuals occurring at 10 min intervals (n = 30 observations per trial), and each individual was used only once to obtain independent data. Trials were run under laboratory ambient conditions in a room with large southwest-facing windows with blinds drawn down to avoid phototactic responses. Light intensity (lumens) and temperature/relative humidity were measured at each observation using a LUX meter (HANNAHi 97500, Johannesburg, S. Africa) and Hygropalm thermometer (Protronic, Series 21, USA), respectively.

2.3.3. Honeydew cafeteria trials

Honeydew was collected from aphid colonies (see Section 2.3.1, above) by placing glass mirrors and Petri dishes beneath colony plants. Only passive collection techniques were utilized to ensure that aphids were not disturbed, thus reducing the likelihood of pheromones (e.g. alarm pheromone) in the honeydew as a result of excessive handling (Nault et al. 1973). Honeydew was harvested from collection surfaces weekly and stored in sealed Petri dishes at -21°C. Stored honeydew was removed from the freezer at least 1 hour before commencing feeding trials and thawed. Marsh flies used in trials (*Ilione albiseta*, *Tetanocera elata*) were

held in the laboratory with access to a honey-yeast diet and water for at least 48 hours after field collection to acclimate to laboratory conditions. Flies were not starved as previous studies showed no difference in feeding behavior between starved and fed flies (Naughton 2016).

Honeydew feeding trials were run as described for the general cafeteria trials, but with a difference in food choice. In these experiments, individuals were offered a choice of three food options simultaneously: dry honeydew (2g), honeydew solution (1ml), or water (1ml). Honeydew solution was created by dissolving dry honeydew on 6 cm^2 areas of the collection surface in 2ml water. Options were presented in individual open-topped 5 cm clear plastic Petri dishes arrayed at the bottom of a testing cage, and observations were recorded every 10 min for 5 hours (n = 30 observations per trial). Trials were run under laboratory ambient conditions, with temperature, relative humidity, and light intensity being recorded every hour.

2.3.4. Community level interactions

Following cafeteria experiments, additional adult Sciomyzidae (*Pherbina coryleti*, *Pherbellia schoenherri*, *Psacadina zerenyi*, *I. albiseta*, *I. lineata*, *Hydromya dorsalis*, *Sepedon spinipes*, *Tetanocera ferruginea*, *Tetanocera fuscinervis*, *Tetanocera robusta*) were observed in a simulated natural interaction with honeydew producers. For aphid trials, one infested *C. annuum annuum* plant was taken from laboratory colonies and placed within a testing cage; in planthopper trials, a non-infested *C. annuum annuum* was placed in a trial cage and approximately 10-20 field-collected planthoppers were introduced. Hemipterans were given at least 24 hours to acclimate to cages before commencing trials. One adult marsh fly (taken from laboratory colonies) was introduced into each cage and their feeding behaviors and interactions with honeydew producers were recorded. Observations were made every 10 min for 5 hours, and included documenting the marsh fly's position (on plant or off plant) and behavior (resting, searching, interacting, indirect honeydew feeding, or direct honeydew feeding). Feeding was considered "indirect" if honeydew was on the leaf surface or bottom of the testing arena, and "direct" if honeydew was still in contact with the producer or colony at the time of feeding. Flies were scored as "interacting" with honeydew producers if they were observed in direct proximity to an individual

or colony while searching or palpating, but without the labellum extended to indicate feeding.

2.3.5. Statistical analyses

For general cafeteria experiments, dietary preference was evaluated by comparing differences between all options using Kruskal-Wallis tests, with *post-hoc* Nemenyi pairwise comparisons. Honeydew cafeteria results were analyzed with an overall Kruskal-Wallis test followed by a Dunn's *post-hoc* test for pairwise comparisons. *Post-hoc* tests were selected based on their appropriateness for the individual data sets. Across all trials, feeding results were also pooled where appropriate and feeding frequency compared versus the water control using a Wilcoxon-Mann-Whitney test. Analyses of marsh fly/hemipteran interactions were performed using Wilcoxon-Mann-Whitney tests. All statistics were performed using R (R version 3.0.2, R Core Team 2013, The R Foundation for Statistical Computing, Vienna, Austria) in R Studio.

2.4. Results

2.4.1. General cafeteria trials

In general cafeteria trials, Sciomyzidae fed at least once in 39 out of overall total of 57 trials, resulting in 126 individual feeding events (Fig. 2.1A, Appendix I.4). Flies fed on *D. reticulatum* 22 times (17% of the total number of feeding events), *L. stagnalis* 8 times (6%), honey-yeast 35 times (28%), glucose solution 50 times (40%), and water 11 times (9%). At family level, marsh flies demonstrated a significant preference between diets (P = 0.0212, Table 2.1), however the only significant pairwise comparison was a barely significant difference between feeding on glucose diet and *L. stagnalis* (P = 0.049). Examined by sex, males (*n* = 27) demonstrated a clear dietary preference (Kruskal-Wallis test, P = 0.01, K = 13.349) feeding on high-carbohydrate diets (glucose + honey-yeast) significantly more frequently than protein (slug + snail tissue) diets (P = 0.018, *post-hoc* Nemenyi test), while female Sciomyzidae (*n* = 30) seemed to have no significant preferences (P = 0.369, K = 1.994) according to Kruskal-Wallis analysis. At species level, *Tetanocera elata* were observed to feed a total of 83 times: 7 times on snail (8%), 32 times on

honey-yeast (39%), 40 times on glucose (48%), and 4 times on water (5%). Interestingly, no observations were made of *T. elata* feeding on slug tissue (from their larval host species). *Tetanocera elata* demonstrated a highly significant preference at species level ($P < 0.001$), with glucose ($P = 0.027$) and honey-yeast ($P = 0.014$) being fed on significantly more frequently than *D. reticulatum* (Table 2.1). When diet options were pooled categorically (Fig. 2.1B), Kruskal-Wallis tests revealed a highly significant difference ($P < 0.001$, $K = 20.291$) in *T. elata* feeding preference, with carbohydrate-rich diets (i.e., honey-yeast + glucose solution) being selected significantly more often than protein-based diets (*D. reticulatum* + *L. stagnalis*; $P < 0.001$) or the water control ($P = 0.017$) in a *post-hoc* Nemenyi test. No difference was observed in the amount of *T. elata* feeding between protein diets and water ($P = 0.79$).

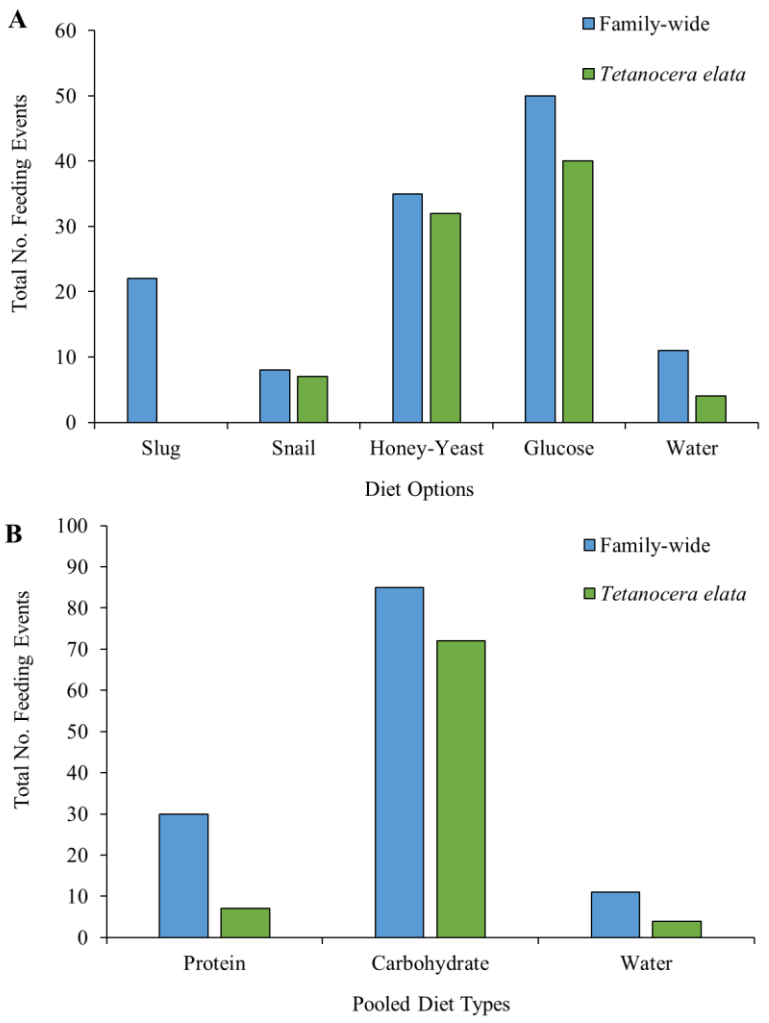

Figure 2.1. Total count of feeding events of Sciomyzidae in general cafeteria trials across all species (total number of trials = 57) and for *Tetanocera elata* (total number of trials = 25). Results given for (**A**) each individual food option and **(B)** diet categories pooled by type.

Table 2.1. Sciomyzidae feeding preferences observed in general cafeteria trials at family level (total number of trials = 57) and for *Tetanocera elata* (total number of trials = 25), displayed as mean (± SD) and median number of feeding events per trial, and pairwise comparisons of each diet choice.

	Family-Wide[1]					***Tetanocera elata***[2]				
	Slug (*Deroceras reticulatum*)	**Snail (*Lymnaea stagnalis*)**	**Honey-Yeast**	**Glucose**	**Water**	**Slug (*Deroceras reticulatum*)**	**Snail (*Lymnaea stagnalis*)**	**Honey-Yeast**	**Glucose**	**Water**
Mean ± SD	0.39 ± 1.24	0.14 ± 0.81	0.61 ± 1.43	0.88 ± 1.78	0.19 ± 0.48	0 ± 0	0.28 ± 1.21	1.28 ± 1.95	1.6 ± 2.40	0.16 ± 0.47
Median	0	0	0	0	0	0	0	0	0	0
Snail	0.724	-	-	-	-	0.973	-	-	-	-
Honey-Yeast	0.866	0.16	-	-	-	0.014*	0.091	-	-	-
Glucose	0.605	0.049*	0.992	-	-	0.027*	0.149	1	-	-
Water	1	0.777	0.823	0.545	-	0.924	1	0.151	0.234	-

[1]Kruskal-Wallis with Chi-square adjustment K = 11.531, P = 0.0212

[2]Kruskal-Wallis with Chi-square adjustment K = 20.853, P < 0.001

Values given for pairwise comparisons reflect significance levels yielded from post-hoc Nemenyi all-pairwise tests with chi-squared approximation. Statistically significant results are indicated at P < 0.05 (*)

2.4.2. Honeydew cafeteria trials

Of the 59 honeydew trials undertaken, feeding occurred at least once in 52 trials, yielding a total of 139 feeding events (Fig. 2.2A, Appendix I.5). Results from honeydew cafeteria trials support observations of the general feeding trials. At family level, 45%, 42%, and 13% of total feeding events were on honeydew solution, dry honeydew and water (respectively), with marsh flies demonstrating a highly significant preference of diet ($P < 0.001$, Table 2.2). Honeydew was highly preferred over water both as dry ($P < 0.001$) and solution ($P = 0.00152$) forms, and there was no significant difference in likelihood to choose dry or dissolved honeydew ($P = 1$). This same trend was mirrored at species level (Table 2.2, Fig. 2.2B), with *T. elata* feeding on honeydew significantly more frequently than the water control in both dry and dissolved forms ($P < 0.001$ and $P = 0.01969$, respectively). *Ilione lineata* did not show any strongly significant preferences in feeding choice (Table 2.2).

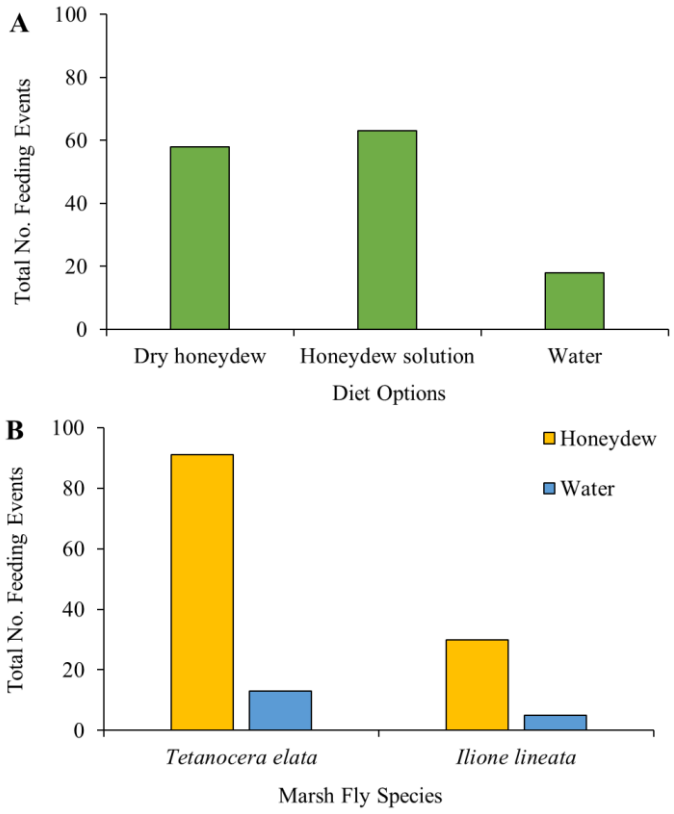

Figure 2.2. Total count of feeding events of Sciomyzidae in honeydew cafeteria trials. (**A**) Overall feeding occurrence (total number of trials = 59) on each food type at family level. (**B**) Feeding occurrence by species for *Tetanocera elata* (total number of trials = 13) on water (control) and honeydew (dry and aqueous solution options pooled).

2.4.3. Community level interactions

In interaction trials between Sciomyzidae and honeydew-producing Hemiptera, feeding occurred in 20 out of 42 trials (Fig. 2.3). Indirect feeding by marsh flies accounted for 88% (aphids) and 86% (planthoppers) of the three event categories recorded (i.e., indirect feeding, direct feeding, and interacting). Overall, feeding was observed significantly more frequently in aphid treatments than planthopper treatments ($P = 0.001$, $Z = 3.2409$), and indirect feeding occurred significantly more often in both aphid ($P < 0.001$) and planthopper ($P = 0.021$) treatments compared to direct feeding (Table 2.3).

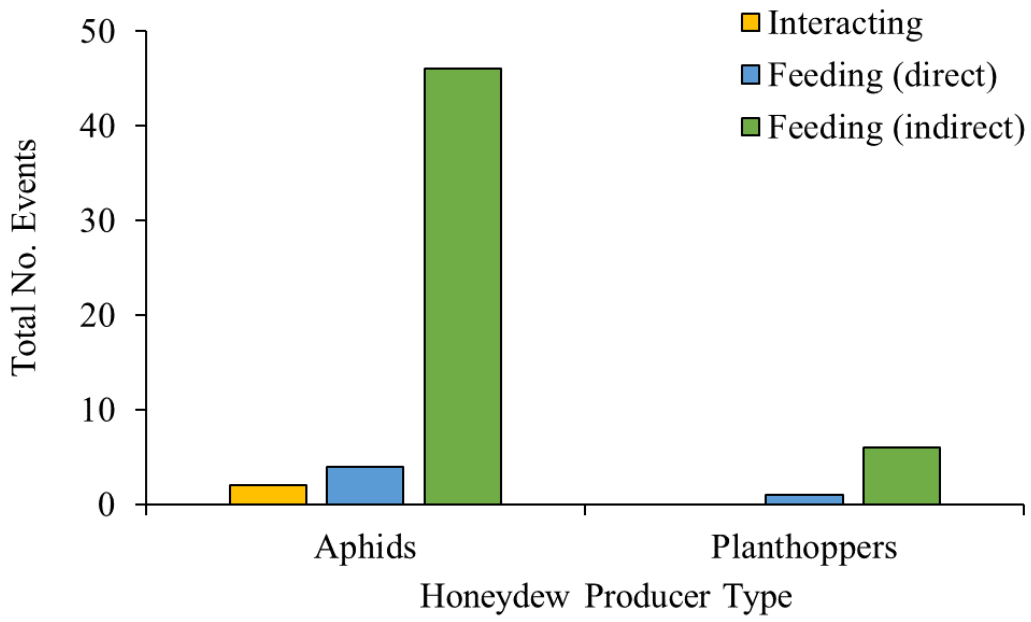

Figure 2.3. Total count of event categories (direct feeding, indirect feeding, interacting) for individual Sciomyzidae with honeydew-producing aphids (total number of trials = 21) or planthoppers (total number of trials = 21).

Table 2.2. Sciomyzidae feeding preference observed in honeydew cafeteria trials at family level (total number of trials = 59) and by species, *Tetanocera elata* (total number of trials = 46) and *Ilione lineata* (total number of trials = 13). Data are displayed as mean (± SD) and median number of feeding events per trial, and pairwise comparisons of each diet choice.

	Family-Wide[1]			***Tetanocera elata*[2]**			***Ilione lineata*[3]**		
	Dry Honeydew	**Honeydew Solution**	**Water**	**Dry Honeydew**	**Honeydew Solution**	**Water**	**Dry Honeydew**	**Honeydew Solution**	**Water**
Mean ± SD	0.98 ± 1.18	1.07 ± 1.54	0.31 ± 0.75	0.97 ± 1.06	1.00 ± 1.65	0.28 ± 0.78	1.00 ± 1.58	1.31 ± 1.11	0.38 ± 0.65
Median	1	0	0	1	0	0	1	1	0
Honeydew Solution	1	-	-	0.69194	-	-	0.742	-	-
Water	< 0.001**	0.00152*	-	< 0.001**	0. 01969*	-	0.644	0.049	-

[1]Kruskal-Wallis with Chi-square adjustment K = 19.361, P < 0.001

[2]Kruskal-Wallis with Chi-square adjustment K = 16.112, P < 0.001

[3]Kruskal-Wallis with Chi-square adjustment K = 5.7519, P = 0.05636

Values given for pairwise comparisons reflect significance levels yielded from *post-hoc* Dunn's all-pairwise tests with chi-squared approximation and Bonferroni correction. Statistically significant results are indicated at P < 0.05 (*) and P < 0.001 (**).

Table 2.3. Frequency of feeding and interaction events of individual Sciomyzidae with aphid (*n* = 21 trials) or planthopper (*n* = 21 trials) honeydew producers. Data are displayed as mean (± SD) and median number of feeding or interaction events per trial, and pairwise comparison of direct and indirect feeding frequency.

	Aphids			**Planthoppers**		
	Indirect Feeding	**Direct Feeding**	**Interacting**	**Indirect Feeding**	**Direct Feeding**	**Interacting**
Mean ± SD	3.07 ± 2.12	0.27 ± 0.59	0.13 ± 0.35	1.20 ± 0.84	0.20 ± 0.45	0 ± 0
Median	2	0	0	1	0	0
Direct Feeding	*-3.69***	-	-	*-2.6063**	-	-

Wilcoxon-Mann-Whitney Z values (given in italics) indicate significances between direct and indirect feeding on both types of honeydew producers at $P < 0.001$ (**) and $P < 0.05$ (*).

2.5. Discussion

Both sets of cafeteria trials confirm the preference of adult marsh flies for high-carbohydrate versus high-protein diets. These trials confirm observations from various small-scale and pilot studies (Naughton 2016) and resulting data seem to be in line with various anecdotal observations and the standard artificial diet regime for adult marsh flies (Berg & Knutson 1978; Knutson & Vala 2011). Honeydew is a common and abundant source of carbohydrates in nature, principally comprised of glucose, fructose, and sucrose at varying ratios, depending on both host plant and honeydew-producer species (Douglas 2006; Leroy et al. 2010; Golan & Najda 2011). While the results of this study indicate a preference for high-carbohydrate diets, other studies have shown that adult marsh flies receiving diets supplemented with crushed snails experienced higher fecundity compared with individuals reared on honey-yeast paste alone (Berg & Knutson 1978). Although female Sciomyzidae did not demonstrate a significant preference for any food type in our study, this may be explained by the potential combination of mated and unmated females in assays. Mated females may benefit from higher levels of protein consumption to promote oogenesis (Klowden 2007). In this regard, a natural diet of honeydew, which also contains levels of various proteins and amino acids, would be highly beneficial (Fischer et al. 2005; Golan et al. 2011; Sabri et al. 2013)

Numerous arthropod groups are known to utilize honeydew as a dietary resource, including Lepidoptera, Coleoptera, Hymenoptera, and many dipteran families (e.g., Syrphidae, Culicidae, Ceratopogonidae) (Foster 1995; Szadziewski et al. 1997; Douglas 2006; Leroy et al. 2010). Inherent to this varied range of insects which feed on honeydew is also the range of feeding behaviors and interactions with hemipteran honeydew-producers. Some insects, most notably ants (Hymenoptera: Dolichoderinae and Formicidae), have evolved highly sophisticated behaviors around honeydew exploitation. These complex interactions with Hemiptera, known as tending, is a mutualism which involves the honeydew feeder removing droplets of honeydew directly from the producer's anus and either consuming or storing it. This benefits the tender by providing a valuable food source (Folling et al. 2001; Douglas 2006). The honeydew producer also benefits indirectly in several ways. Tending species may guard a hemipteran colony from predators or parasites (Beattie & Hughes

2002; Diehl et al. 2013), and removal of the sugary excrement prevents hemipteran infection by fungi, microorganisms, and other pathogens (Hughes 1963; Bach 1991; Gaigher et al. 2011). In addition to ants, other eusocial Hymenoptera (Vespidae: *Polybia* spp.) and bark beetles (Coleoptera: Silvanidae) have been observed tending hemipteran populations for honeydew (Folling et al. 2001). A far more common approach to honeydew feeding than tending is opportunistic feeding. Rather than directly interacting with hemipterans, opportunistic honeydew feeders will consume honeydew that has fallen away from the producing colony, onto substrate or nearby plant surfaces. Feeding on honeydew in this manner requires far less investment of time and resources by the honeydew feeder, but the producing hemipterans are also not benefitting from protection from natural enemies as they would from tending species. This sort of opportunistic honeydew feeding, displayed by marsh flies in our feeding trials, may be even less resource-taxing than nectar feeding; because honeydew-producing hemipterans, such as aphids, are pervasive throughout numerous landscapes (in the case of marsh flies, wet and terrestrial grasslands and meadows), opportunistic honeydew feeders would not be required to invest high amounts of resources into searching for patches of nectar-producing flowers via visual or olfactory cues. They also avoid the risk of flower-based predators (e.g., spiders, predatory Hemiptera (Reduviidae), mantids, etc.) (Pellmyr 2002).

In our study, observations indicated that marsh flies fed on honeydew from both aphid and planthopper colonies, though the feeding frequency was higher in aphid trials. This may be due to the sheer volume of honeydew produced by the respective hemipterans, and the amount which had fallen away from the colonies. Aphids tend to produce copious amounts of honeydew and form denser colonies compared to the types of planthoppers examined (e.g., winged morphs or macropterous), which are largely solitary and highly mobile even in nymphal stages (Hughes 1963; Denno & Roderick 1990). The demonstrated preference of marsh flies for aphid honeydew compared to planthopper honeydew ($P = 0.0001$, represented by comparison of the frequency of feeding on each independently) may also suggest that the chemical components of the aphid-produced honeydew are more attractive to marsh flies or better fulfil their dietary requirements than planthopper honeydew. The sugar concentration of honeydew is influenced by a variety of factors (hemipteran species, host plant species, varying sugar concentration of phloem sap, minerality of soil [e.g.,

nitrogen content], etc.) (Pellmyr 2002; Douglas 2006; Golan & Najda 2011). However, all other variables being equalized in our experimental design, it seems that the difference in qualities and/or quantities of honeydew between aphids and planthoppers was consequential enough to affect feeding likelihood by marsh flies.

The types of relationships between honeydew producers and honeydew consumers also varies with the taxon of the producer. Stationary, colony-forming taxa, such as aphids or scale insects (Hemiptera: Sternorrhyncha: Coccoidea) are widely tended by other insects, whereas solitary or highly mobile taxa (e.g., leafhoppers and planthoppers) may not be subjected to the same frequency of engagement by honeydew consumers. Producers such as psyllids (Hemiptera: Sternorrhyncha: Psyllidae) are frequently tended by honeydew consumers in the nymphal stages, as they form stationary colonies on host plants, but direct harvesting of honeydew from highly-mobile adults is less common (Kistner et al. 2015). This difference was reflected in our community interaction trials, with direct feeding being more frequent in aphid treatments than planthopper treatments. Over the course of 21 trials per treatment, comprised of 30 observations each, there was only one recorded instance of interaction or direct feeding by marsh flies in planthopper treatments, compared with six interaction or direct feeding events in aphid treatments. Differences in honeydew constituent chemicals and sugar concentrations may have contributed to the differences in direct feeding frequency between treatments, however it is more likely that the highly mobile nature of planthoppers had a more cogent influence on marsh fly engagement.

Traditionally marsh flies in laboratory cultures have been fed on a high-carbohydrate artificial diet (honey and yeast), often supplemented with powdered milk or crushed snails to provide additional protein (Trelka & Foote 1970; Berg & Knutson 1978). In general cafeteria trials, while the number of feeding events was a significantly higher on options high in sugar (honey-yeast paste or glucose solution), some feeding was observed on protein options. In trials in which feeding occurred, approximately 30% of individuals consumed a protein option (i.e., mollusk tissue) either exclusively or in conjunction with a carbohydrate option. Interestingly, individuals which fed on either protein option always fed on the reciprocal type of protein which marsh fly larvae of the same species would consume (e.g., species whose larvae are obligate snail-feeders consumed only *D. reticulatum* slug tissue in

trials and vice-versa). Protein consumption was observed in four of the five species included in the study, and all displayed this avoidance of larval protein type. This suggests there may be resource partitioning between larval host or prey species and adult protein sources. Partitioning of this nature could be highly valuable as marsh fly populations are known to have very localized home ranges, necessarily creating a considerable overlap between larval and adult hunting/foraging area (Williams et al. 2009).

Protein consumption could also indicate a need for a low to moderate protein supplementation in an otherwise carbohydrate-based diet. Of the 12 individuals observed feeding on a protein option, three also consumed a carbohydrate option. In addition to being carbohydrate-rich, honeydew provides valuable amino acids and proteins (Auclair 1963; Fischer et al. 2005). In other dipteran families (e.g., Syrphidae), chemicals contained within aphid honeydew have been observed to promote and enhance oviposition (Verheggen et al. 2008; Leroy et al. 2010). Therefore, individuals requiring supplemental components in their diet may be able to get them largely from this single source, rather than expending extra energy foraging for dead or decomposing mollusks or arthropods. There is even some evidence that some species of Sciomyzidae may opportunistically feed on dead planthoppers (Zou et al. 2017), demonstrating another way in which honeydew-producers can benefit a marsh fly diet.

Illumination of these community-level interactions between marsh flies and honeydew-producers will have tangible benefits when considering marsh flies as natural enemies of mollusks for biological control. Because of their fragility as larvae (especially during the earliest instars), a combination augmentative and conservation biological control approach will likely be more feasible and effective than an inundative approach requiring mass-rearing (Hynes et al. 2014a). Such an approach assumes the persistence of an introduced population to an area of interest (e.g., arable field margins) without the need for continued augmentation and maintenance. In order for this type of approach to succeed, the landscape to which a natural enemy population is introduced must be one which will fulfil the natural enemy's biological and physiological requirements at all life stages. If honeydew is indeed a suitable dietary component for adult marsh flies, this will affect the management recommendations for important hemipteran pests such as cabbage aphid (*Brevicoryne*

brassicae L.), grain aphid (*Sitobion avenae* Fabricius), or peach potato aphid (*Myzus persicae* Sulzer) (Diehl et al. 2013; Ramsden et al. 2017). It may also affect the use of neonicotinoid insecticides, commonly used to control aphid infestations. Exposure to sub-lethal doses of such insecticides has been shown to significantly reduce feeding rate and honeydew production in aphids, and foliar application could leave residual amounts of insecticide on plant surfaces where marsh flies would be feeding, both of which would likely have detrimental effects on these natural enemies (Oliver et al. 2006; Shi et al. 2011).

Rather than eradicating hemipteran pests, growers should instead aim for control of these pests to below economic injury thresholds. Allowing for a small amount of crop damage or loss to hemipterans without incurring major economic losses could in turn reduce the amount of damage or loss to pestiferous mollusks by meeting the dietary requirements of their biological control agents (e.g., introduced *Tetanocera elata* populations). By identifying honeydew as a potential natural food source and shedding light on marsh fly-honeydew producer relationships, it should be possible to inform growers on how to best maintain field margins to sustain high abundances of these biocontrol agents, making their control of pest slugs more efficient. In this manner, a site-wide integrated pest management approach is more favorable than control of individual pest species (Chabert & Sarthou 2017).

Our study has also benefitted marsh flies which have the potential to be used as bioindicators by refining the knowledge base of marsh fly ecological interactions. Currently, adults of some marsh fly species can be used as a proxy for the biodiversity of wet grassland mollusks when the larvae of such species are closely linked with specific host mollusks. In this way, observed adult marsh fly biodiversity can be used alongside environmental features such as water depth and soil pH to represent the biodiversity of mollusks (Speight & Knutson 2012; Carey et al. 2017b). Likewise, presence and abundance of honeydew producers may now be considered to be useful in reflecting marsh fly presence. Especially in periods outside of the mating season, or in weather conditions unfavorable to adult marsh fly capture (e.g., heavy rains, strong winds, etc.), presence of honeydew producers in conjunction with host mollusks could be used to predict the presence of marsh flies within a site. This may prove valuable as adult marsh flies can be difficult to recover manually, especially within large sites. The nature of Sciomyzidae population structure, not forming dense aggregates while

having a very patchy and discrete distribution, can mean that no individuals are detected, even over the course of many sampling periods at a site where populations are known to occur. By evaluating a site based on other contingent taxa (e.g., host mollusks and honeydew producers), one may more easily assess whether marsh flies are likely to occur at a particular site and whether additional effort should be expended with future surveys.

The existence of aphids, as prolific honeydew producers, may be one such usable taxon. Depending on the species and climatic conditions, aphids may overwinter as eggs on a secondary overwintering host plant within the same geographic site (Diehl et al. 2013; Holman 2009) or, in more favorable conditions, they may remain obligately parthenogenetic, producing offspring year-round on primary host plants (Dedryver et al. 1998, Hughes 1963). If aphids are of the appropriate genotype or within such climatic conditions, they may be evaluated during periods when adult marsh flies are not easily found (e.g. while overwintering). For example, if attempting to select sites for surveys/collections in an off-season for action during the adult flight period, the co-occurrence of specific mollusk species and honeydew producers may indicate a more profitable habitat compared to one lacking honeydew producers.

While honeydew consumption is not novel amongst insects as a whole, this association has never before been observed for marsh flies and may offer key insights into their ecology in terms of being both biological control agents and bioindicators. This study offers the initial insights of the association between marsh flies and honeydew producers, but this line of enquiry could benefit from extensive additional research. Primarily, the authors feel that feeding trials comparing preference for honeydew from different producers, and especially a comparison between Auchenorrhycha and Sternorrhyncha produced honeydew, in conjunction with a nutritional analysis of each type of honeydew, will be highly beneficial for further refining the relationships between marsh flies and specific honeydew producers. Additionally, the dietary requirements of male and female adult marsh flies should be further investigated. Examining the specific metabolic requirements of males and females may reveal new trends, especially with regard to the physiological differences of varying female conditions (e.g., virgin, mated, gravid, etc.). It should also be noted that in our trials, the number of feeding events was very low compared to the total number of observations made (126 out of 1,710 for general cafeteria trials and 139 out

of 1,770 for honeydew trials). Further research into whether this is typical of feeding behavior for the family (e.g., intermittently) or whether it is an artifact of their crepuscular activity (resting more during the day but feeding more actively at dawn/dusk periods) may be of interest. Finally, field surveys should be carried out to examine population dynamics and relationships of marsh flies and honeydew producers across the range of marsh fly habitats, preferably over multiple years to observe any potential population density correlations between the two taxa.

2.6. Acknowledgements

The inspiration for this investigation came partially from experiments performed as part of an honors thesis project by David Naughton (Applied Ecology Unit, NUI Galway). Although the published study expanded in scope and intent since his thesis project, we are grateful to him for posing the initial question and getting people curious. Clémence Marchande and Simon Chapenoire provided valuable assistance in fieldwork and insect collection. Our sincere thanks to Green Earth Organic Farm (Corundulla, Co. Galway, Ireland) and the Clarenbridge Community Group (Clarenbridge, Co. Galway, Ireland) for allowing us access to their properties for insect collecting. Thanks also to Green Earth for their generous donation of aphid-infested plants. Funding for this research was provided by the Irish Research Council Government of Ireland Postgraduate Fellowship and the Thomas Crawford Hayes Research Fund (NUI Galway).

2.7. Statement of Author Contribution

A.B.E. and J.G.J.C. devised the main concepts for the project and developed the experimental procedures, while A.C. and M.F.D. primarily conducted the experiments. Data were analyzed by A.B.E. and A.C., and the manuscript was prepared by A.B.E. with support from A.C. and J.G.J.C. All research was supervised by M.J.G.

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Chapter 3 Nutritional ecology of predaceous *Tetanocera elata* larvae and the physiological effects of alternative prey utilisation

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3.1. 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, *A. 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

3.2. Introduction

Terrestrial molluscs, in particular slugs (MacDonald 2009; Douglas & 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 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 & 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, 2018) and metaldehyde has been banned from the UK from 2020 (HSE 2018). Even ferric phosphate, used in organic cultivation with variable success (Iglesias et al. 2001; Speiser & 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 & 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 & 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 does not guarantee a reduction of high-density slug populations below economic injury levels. Coupled with this are the issues of expense and shelf life of the biological control agent (MacDonald 2009; Glen & Wilson 1997; Grewal et al. 2005). In addition, while *P. hermaphrodita* parasitises a range of slug species, they are not universally effective (Dankowska 2006; Rae et al. 2007; Pieterse et al. 2017) with larger hosts often able to withstand or recover from infection (Speiser et al. 2001).

With this in mind, there has been considerable and ongoing research conducted to identify and evaluate other potential natural enemies that could be used as components of integrated slug pest management programmes. Sciomyzidae (Diptera) have been the topic of extensive research for the biological control of various terrestrial and semi-aquatic molluscs (Berg 1953; Knutson et al. 1965; Gormally 1988; Vala et al. 2000; Knutson & Vala 2011; Murphy et al. 2012; Hynes et al. 2014a). Numerous studies have suggested that the functional responses exhibited by many species of Sciomyzidae may demonstrate effective biological control of molluscs (Eckblad 1973; Haab 1984; Beaver 1989; Manguin & Vala 1989; Knutson & Vala 2011). Some species within the genus *Tetanocera* (Diptera: Sciomyzidae) have evolved as specialist predators of terrestrial slugs (Knutson et al. 1965; Berg & Knutson 1978). Specifically of interest for agriculture is *Tetanocera elata* (Fabricius), which has been shown to feed on the prominent agricultural pest *Deroceras reticulatum* Müller (Stylommatophora: Agriolimacidae) (Knutson et al. 1965). A multivoltine species producing two to three generations per year, *T. elata* undergoes three larval instars before pupating and becoming quiescent over winter. First and second instar larvae are obligate mesoparasitoids of *D. reticulatum*, and occasionally on closely related species such as *Deroceras laeve* Müller and *Deroceras invadens* Reise, Hutchinson, Schunack, & Schlitt (Knutson et al. 1965; D'Ahmed et al. 2019). Neonates burrow into the host either under the mantle near the 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 to generalism, 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 (Stylommatophora: Arionidae) and *Tandonia budapestensis* Hazay (Stylommatophora: Milacidae). All three species are widely distributed across Europe and are pestiferous species of economic importance (Douglas & Tooker 2012; Howlett 2012), commonly occurring in arable agroecosystems (Hunter 1968). Additionally, these species have adopted a global distribution associate with agricultural intensification, having been introduced into regions including North and South America, Australia and New Zealand. Larvae were presented with prey species in choice and no-choice assays, which were used to determine prey preference. Additionally, the current study examined, for the first time, the physiological effects of different prey species on developing *T. elata* larvae. Feeding efficiency, survivorship, and developmental rates were considered together to gauge suitability of the three potential prey species. The combination of prey suitability and preference provides valuable insight into the potential and realised prey range of predaceous *T. elata* larvae, which is an essential consideration to evaluate the potential for the use of *T. elata* as a biological control agent of slugs in European horticulture and agriculture.

3.3. Materials and Methods

3.3.1. Specimen collection and colony maintenance

Tetanocera elata colonies were established using field-collected adults to ensure the availability of larval instars as required. Adult *T. elata* were collected from dry grassland field sites in western Ireland (counties Galway, Clare, and Mayo) (Appendix II.1) from July to August 2017 by passing a heavy-duty sweep net (0.3 m long handle; 0.1 pore net; 0.5 m aperture) through tall vegetation. Specimens were identified in the field using morphology as described by Rozkoŝný (1984, 1987) and *T. elata* removed from sweep nets using acrylic barrel-style pooters (Watkins & Doncaster, The Naturalists, Hawkhurst, Kent, England) for transport 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 3.3.2).

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).

3.3.2. 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 (typically through the observation of exuviae), the remains of the neonate host carcass were removed, and larvae were maintained without food until larval gut was observed to be < 50% full at which stage the larvae were utilised for prey choice trials.

3.3.3. 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 (0.25g ± 0.04 SE, 0.25g ± 0.07 SE, and 0.39g ± 0.11 SE means for *D. reticulatum*, *A. hortensis*, and

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

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

3.3.4. Measurement of prey suitability

Immediately after the conclusion of each feeding trial, slugs were removed from experimental chambers and larvae were returned to colony rearing boxes along

with the prey individual on which they were feeding at the time of trial end. Larvae continued to receive their chosen prey in laboratory cultures *ad libitum* until the larva either died or began pupariation. Slugs provided for feeding were similar in size/weight, as was confirmed by statistical comparisons of the mean biomass given to each larva having no significant differences between prey species ($P = 0.15$, permutation F tests). If no feeding occurred during trials, larvae received *D. reticulatum* as the default prey species. Rearing boxes were checked every 2-3 days to assess survivorship as well as to perform enclosure maintenance and provide new prey as necessary. Development time of third instar larvae to pupariation, survivorship to pupariation, and the total number and biomass of prey provided to each larva was recorded for each individual to provide a measure of prey suitability. Pupariating larvae were considered dead if decomposition was observed. Three fully-formed pupae never produced adults. These puparia were allowed to remain undisturbed for approx. 9 months (into the subsequent summer season to account for the potential of the formation of an overwintering pupa), then dissected. All dissected puparia were confirmed to have degraded.

3.3.5. Statistical analyses

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

3.4. Results

3.4.1. Prey preference

Prey preference was measured by comparing the number of trials where feeding occurred with the number of trials where larvae did not feed for each slug species. Across all choice levels (e.g., no-choice, two-choice, and three-choice) feeding occurred on all potential prey species during the three hour observation period. Naïve *T. elata* larvae attacked prey slugs at least once in 91% of all trials, with successful feeding occurring in 74% of all trials. Statistical comparisons were only made between species at the same choice level (i.e., predation frequency was compared between species in no-choice trials, a separate comparison was made for two-choice trials, and another comparison was made for three-choice trials).

In no-choice trials, all *D. reticulatum* specimens (100%) exposed to *T. elata* were fed on successfully by larvae in comparison to just 67% and 46% for *T. budapestensis* and *A. hortensis*, respectively (Table 3.1). Larvae demonstrated significant differences in feeding rates ($P = 0.017$), with *D. reticulatum* being predated significantly more frequently than not ($P = 0.042$). No significant differences in predation frequency were observed for *A. hortensis* or *T. budapestensis*. In two-choice trials when the data are combined for each slug species tested (Fig. 3.1), *D. reticulatum*, with a 52% success predation rate, was again the slug species most successfully preyed upon by *T. elata* larvae in comparison to *A. hortensis* (44%) and *T. budapestensis* (25%). In addition, the number of successful feeding events by *T. elata* larvae on *A. hortensis* was significantly greater ($P = 0.0484$) than on *T. budapestensis* in the *A. hortensis* / *T. budapestensis* two-choice trial (Table 3.1). In contrast, although no significant differences were detected in the three-choice trials, it is interesting to note that when *T. elata* larvae had a choice between the three slug species, *D. reticulatum* was predated upon least frequently (14%) in comparison to *A. hortensis* (36%) and *T. budapestensis* (21%) (Table 1). In addition, as the treatments progressed from no-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. 3.1).

3.4.2. 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).

Table 3.1. 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. slugs exposed	No. successful feeding events	% successful feeding events	P-value
No-choice				**0.017***
D. reticulatum	10	10	100	**0.042***
A. hortensis	13	6	46	0.35
T. budapestensis	15	10	67	0.09
Two-choice				
D. reticulatum | *A. hortensis*	12	6 | 4	50 | 33	0.34
D. reticulatum | *T. budapestensis*	11	6 | 4	55 | 36	0.34
A. hortensis | *T. budapestensis*	13	7 | 2	54 | 15	**0.048***
Three-choice	14	-	-	0.54[†]
D. reticulatum		2	14	-
A. hortensis		5	36	-
T. budapestensis		3	21	-

[†] Because results for three-choice prey preference omnibus tests were non-significant, pairwise comparisons were not made.

3.4.2.1. Efficacy of attack and feeding

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 (Appendix II.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. 3.2).

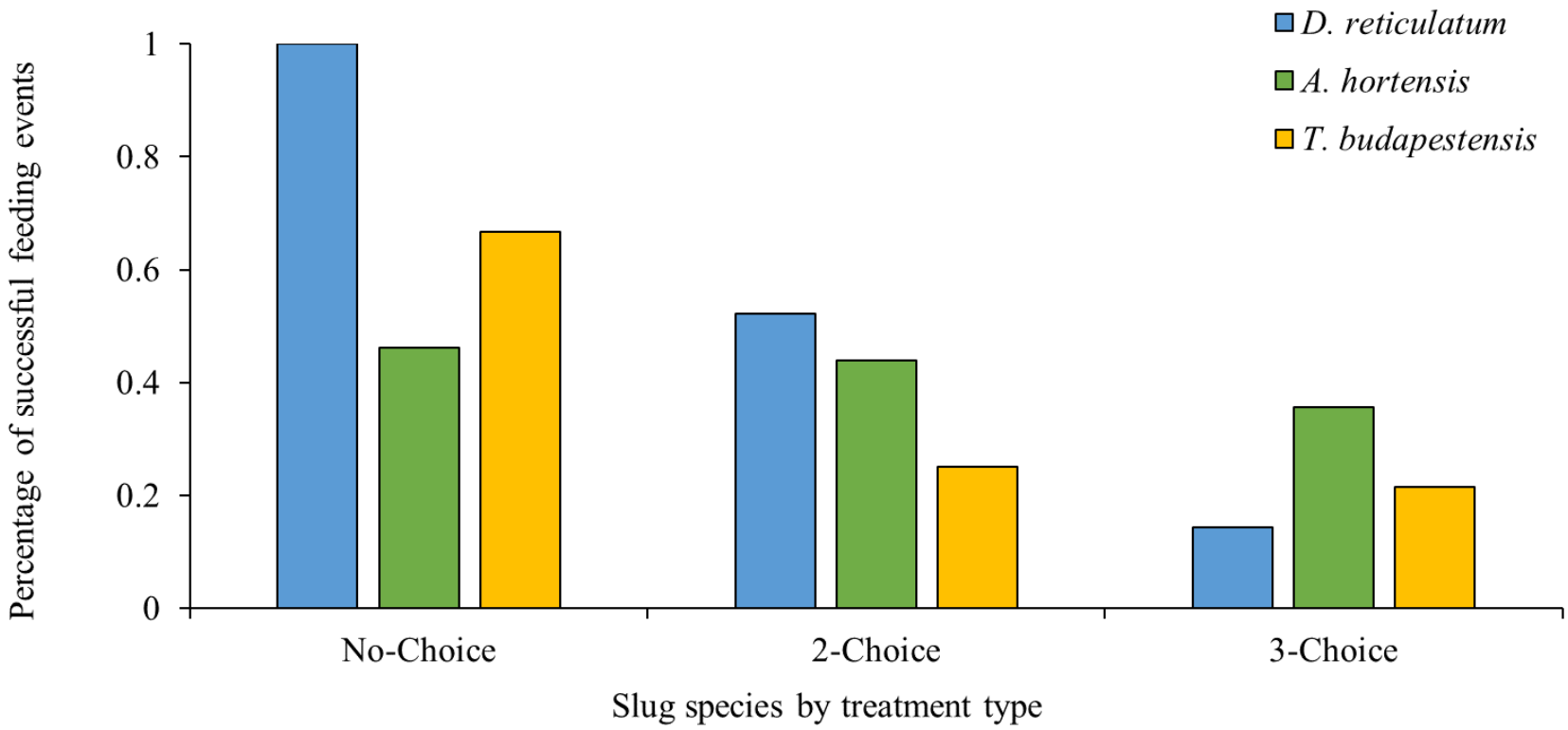

Figure 3.1. Percentage of successful feeding events by third instar *Tetanocera elata* larvae on each of three prey species in no-choice, two-choice, and three-choice feeding trials. Data for pairwise two-choice trials are pooled to illustrate percentage successful feeding events on each slug species overall.

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, χ^2 = 11.258, df = 2) between the three potential prey species (Table 3.2). 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 3.2).

Table 3.2. Median and range (min – max) of the number of attacks preceding successful feeding events undertaken by *T. elata* larvae for each prey species in no-choice, two-choice, and three-choice treatments. Statistical comparisons were made using Kruskal-Wallis tests with post hoc Dunn's tests.

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

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

3.4.2.2. 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 evaluated concurrently to reflect overall larval survivorship to the beginning of pupariation, which was significantly affected by prey species (P = 0.0435, χ^2 = 9.8221, df = 4) (Table 3.3). 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 3.3). All other pairwise comparisons between prey

species and pupariation success relevant to the study were non-significant (Appendix II.3).

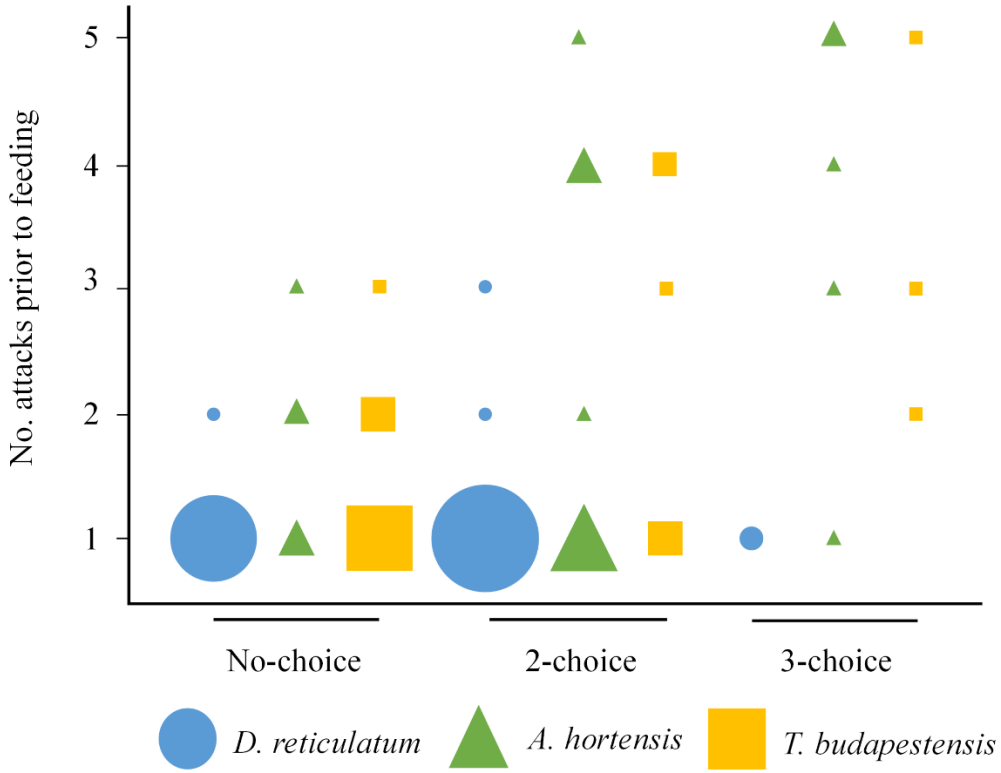

Figure 3.2. Number of attacks (i.e., handling time) of *Tetanocera elata* larvae on each prey species across choice levels. Markers are scaled to reflect the number of observations.

One adult female and one adult male, reared as larvae on *D. reticulatum* and *T. budapestensis* respectively, successfully eclosed (Table 3.3), but no adults eclosed from *A. hortensis*-reared pupae. When comparing rates of full pupariation, larvae reared on *A. hortensis* showed slightly higher survivorship (n = 4, 25%) than *D. reticulatum* (n = 9, 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 (n = 37, 64%) compared to those reared on *A. hortensis* (n = 8, 50%) or *T. budapestensis* (n = 5, 25%). It is worth noting that a considerable majority (84%) of all pupariation attempts resulted in death before successful pupariation was accomplished for larvae reared on all prey species combined.

Table 3.3. Development time in days (d) and survival rates of third instar *Tetanocera elata* larvae reared on *Deroceras reticulatum*, *Arion hortensis*, or *Tandonia budapestensis*. Numbers of replicates for Mean developmental rates are the same n listed for corresponding Survivorship 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	3

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

3.4.2.3. 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 ($P = 0.4574$, $F_{5,42} = 0.9529$) (Fig. 3.3). Of the larvae which successfully pupariated, those reared on *D. reticulatum* reached pupariation at a similar rate (60.44 d ± 8.13 SE) as those reared on *A. hortensis* (63.00 d ± 1.78 SE) (Table 3.3). The single larva to complete pupariation on *T. budapestensis* (45 d) fell between the minima for development time on *D. reticulatum* (25 d) and *A. hortensis* (58 d) (Table 3.3). There was no observed difference in development time to full puparia between larvae reared on *D. reticulatum* and *A. hortensis* (Fig. 3.3). As with larvae which successfully completed pupariation, prey species had no significant effect on the development rate of larvae only achieving partial pupariation (Fig. 3.3). Developmental rate to successful puparia could not be statistically compared for larvae reared on *T. budapestensis* because only a single puparium was formed. 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).

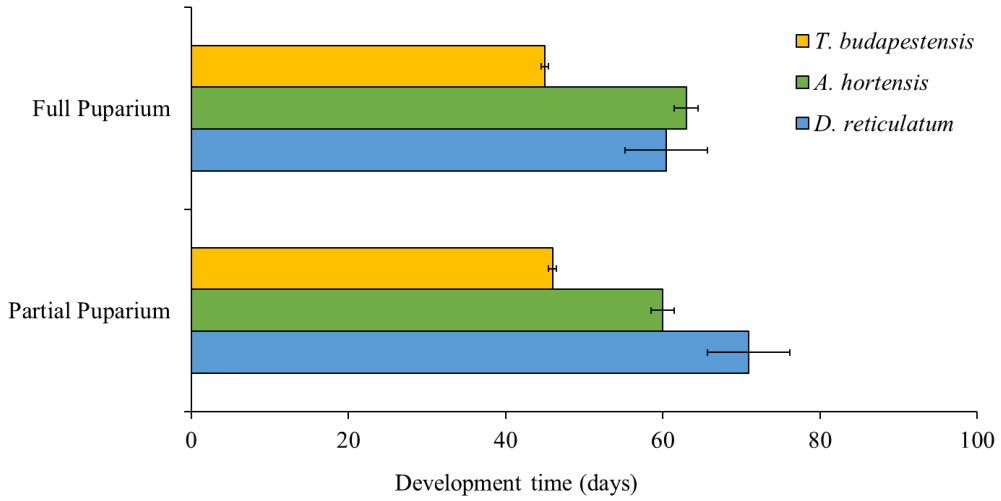

Figure 3.3. Mean developmental rates (± SE) of third instar *Tetanocera elata* larvae reared on *Deroceras reticulatum*, *Arion hortensis*, or *Tandonia budapestensis*. Larvae are separated by survivorship types: partial puparium (e.g., those that died while pupariating) and full puparium (e.g., those that successfully completed formation of a puparium).

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

Feeding by larvae in no-choice trials demonstrated a significant affinity for *D. reticulatum*, representing the only observed instance of 100% feeding rate in the trial. Likewise, in pairwise trials where *D. reticulatum* was an option, it was fed on at higher (though non-significant) frequencies than other prey options. The elevated rates of feeding on *D. reticulatum* may be the result of a number of pre-existing conditions. First, *D. reticulatum* is the optimal neonate host (Knutson et al. 1965; D'Ahmed et al. 2019), and the species on which all larvae used in trials were reared in the parasitoid first and second instars. While the third instar larvae used in trials were considered

naïve, as they had not been given any slug meal once they matured to the free-living predaceous stage, they did have some prior association with *D. reticulatum* as this was the host on which they were raised, and they were allowed to continue feeding on the original neonate host carcass for a short period after maturing to third instar. This may have 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.

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 in part 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, as larvae undergo pupariation with too little biomass accumulation, and suffer fatality as a result. 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 biological control for pestiferous slugs in European horticulture that should continue to be explored. 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 spatial aggregation of *D. reticulatum* populations closer to the soil surface making them easily accessible (Hunter 1966) and synchronicity with *T. elata* life history (Speight & Knutson 2012), could make large prey shifts 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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Chapter 4 Characterisation of habitat requirements and natural history of *Tetanocera elata* (Diptera: Sciomyzidae) for the development of a self-sustaining conservation biological control programme for agriculturally pestiferous slugs

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4.1. Abstract

Terrestrial slugs are pervasive pests of agriculture throughout temperate regions and have the potential to disrupt the germination of seedlings, cause damage to fruiting bodies of crops, and vector plant pathogens. *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae), a widely distributed Palaearctic species, is an obligate mesoparasitoid and predator of highly pestiferous slugs such as *Deroceras reticulatum* (Müller) (Stylommatophora: Agriolimacidae). It has the potential to be developed as a native natural enemy in a conservation biological control programme as an alternative to chemical molluscicides. To better understand the ecological requirements of this species for the more effective targeted engineering of semi-natural habitats in agroecosystems, a detailed observational study was conducted at a site in the west of Ireland possessing naturally occurring *T. elata* populations. Taller dead vegetation was associated with greater rates of *T. elata* presence throughout the site (as indicated by resolution of $R^2 > 0.02$ in PCA graphs), and within the area of greatest *T. elata* aggregation, there was a significantly greater percentage cover of dead vegetation in plots where *T. elata* were present ($P = 0.0172$, $W = 2268$). Abundance of *T. elata* adults was also significantly correlated to hedgerow proximity ($P = 0.03$, $\chi 2 = 4.7055$, $df = 1$). Comparison of local patches where *T. elata* were recovered revealed no apparent association with plant community composition. The compilation of *T. elata* collection records across multiple years suggest the possibility of this species being univoltine in Ireland. Results of the study presented here are directly applicable for the design of a conservation biological control programme which effectively satisfies the principal habitat requirements of *T. elata* populations.

Key words: agroecology, conservation biological control, ecological engineering, hedgerow, mollusc, vegetation

4.2. Introduction

Throughout the world, terrestrial slugs (Mollusca: Gastropoda) are serious pests of tillage agriculture. One species in particular, *Deroceras reticulatum* (Müller) (Stylommatophora: Agriolimacidae), has spread from its native Palaearctic range (Wiktor 2000) and established populations on all continents except Antarctica (Smith 1989; Robinson 1999). While large populations of *D. reticulatum* are regularly associated with agricultural land (Howlett 2012) they can also successfully occupy a wide range of other habitats (South 1992). This ability to disperse into other habitats and form aggregations outside of cropping areas makes *D. reticulatum* a particularly difficult pest to target and control. Damage incurred by *D. reticulatum* and other slug pests has been valued at £8 and £10 million (GBP) in the UK (MacDonald 2009), primarily from feeding damage to seedlings causing crop failure (Hunter 1968). In the EU there are currently three chemicals approved for slug control: methiocarb, metaldehyde, and ferric phosphate (European Commission 2016a,b,c). These chemicals are typically administered in pellet form which slugs ingest. However, methiocarb use has recently been restricted by the EU (European Commission 2014; European Commission 2018) and metaldehyde will no longer be available for use in the UK beginning in 2020 (HSE 2018), due to their detrimental effects on non-target species and especially their ability to contaminate waterways (South 1992; Cloyd 2012; Howlett 2012). There is also mounting evidence that ferric phosphate, currently approved for organic farming, is not effective at significantly reducing slug feeding damage to crops (Iglesias et al. 2001; Speiser & Kistler 2002; Rae et al. 2009) and may have negative non-target effects on soil arthropods caused by excess iron build-up, especially when coupled with chelating substances (Langan & Shaw 2006; Edwards et al. 2009).

Biological control of slugs offers an alternative to chemical pesticide that can be used in both conventional and organic agriculture. Currently the only widely available biocontrol agent of slugs is the parasitic nematode *Phasmarhabditis hermaphrodita* (Rhabditida: Rhabditidae) (Glen & Wilson 1997), marketed under the name Nemaslug (BASF, Ludwigshafen, Germany). While *P. hermaphrodita* is useful in slug control, it is limited in its use as an inundative biological control agent and, as such, is prone to the same shortcomings of many such “single use” natural enemies which do not

persist in the agroecosystem, including high expense, labour-intensive application multiple times per growing season, variable success rates and control levels, and short shelf life (Glen & Wilson 1997; Speiser et al. 2001; Rae et al. 2007; Howlett 2012), rendering them an unsustainable long-term solution (Michaud 2018).

In an effort to both develop alternative slug control programmes and advance sustainable agriculture practices, recent research has focused on the potential use of *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae) in a conservation biological control programme. The larval stages of *T. elata* are specifically associated with terrestrial slugs (Knutson et al. 1965). First and second instar larvae parasitise *D. reticulatum* and *Deroceras laeve* Müller as mesoparasitoids which kill the host by the end of the second instar, at which point the larvae become free-living and active predators which feed on an expanded range of slug species (Knutson et al. 1965; Hynes et al. 2014a; D'Ahmed et al. 2019; Bistline-East et al. 2019). Predaceous larvae have the capacity to kill up to 12 prey slugs before beginning pupariation (Hynes et al. 2014a), making them potentially valuable natural enemies. Due to their particular biological requirements and life cycle (e.g., current challenges in mass rearing [Hynes et al. 2014b,c], lengthy developmental time [Knutson et al. 1965; Hynes et al. 2014c]), and the nature of cropping areas where slugs are in need of control (open cropping fields from which aerial natural enemies may disperse), *T. elata* may not be viable for use in inundative biocontrol programmes in the same way as *P. hermaphrodita*. Rather, this Palaearctic species is an excellent candidate for conservation biological control. The aim of such a programme would be to enhance agroecosystems in such a way that populations of natural enemies are self-regulating and self-sustaining, providing constant and recurring pest control (Holland et al. 2016). For *T. elata*, this would be accomplished by managing agricultural landscapes which can meet the essential requirements to complete its life cycle after initial populations are introduced.

There are numerous approaches to landscape management practices in conservation biological control (Crowder & Jabbour 2014; Holland et al. 2016; Begg et al. 2017; Landis 2017), however application can vary widely depending on the desired output (i.e., level of pest suppression) and the specific taxon of interest. In the case of conservation biological control with a single target species, it is likely best to maximise the biological control output by specifically designing habitat features that meet the biological and ecological requirements of that natural enemy (Van Driesche

& Bellows 2001; Ramsden et al. 2015; Holland et al. 2016). While there has been much research focusing on the biology, physiology, and biological control potential of *T. elata* in recent years (Hynes et al. 2014a, b, c; Bistline-East et al. 2018; D'Ahmed et al. 2019; Bistline-East et al. 2019), the only habitat data for this species to date are provided by a limited number of individual collection records or species lists for the family Sciomyzidae (Chandler 1972; Blackith et al. 1991; Williams et al. 2007; Speight & Knutson 2012).

The current study aims to identify specific habitat characteristics important to *T. elata* populations by undertaking an extensive examination of local habitats within a field site where *T. elata* are regularly observed. The authors also sought to confirm the flight period of *T. elata* adults in Ireland, as phenology of the target species is another important aspect of a successful conservation biological control programme, providing temporal management recommendations in addition to physical management.

4.3. Materials & Methods

4.3.1. Study site

Surveys of habitat characteristics took place at Cow Park, Clarenbridge, Co. Galway, Ireland (ITM 541725.671, 720345.825) where *T. elata* adults had been recovered in previous years. The field site (bordered by a river and deciduous woodland on the eastern side and surrounded by hedgerows/scrub; Fig. 4.1) was comprised largely of a dry meadow with a patch of wet grassland to the north-east of the site (GS2 and GS4 respectively after Fossitt [2000]).

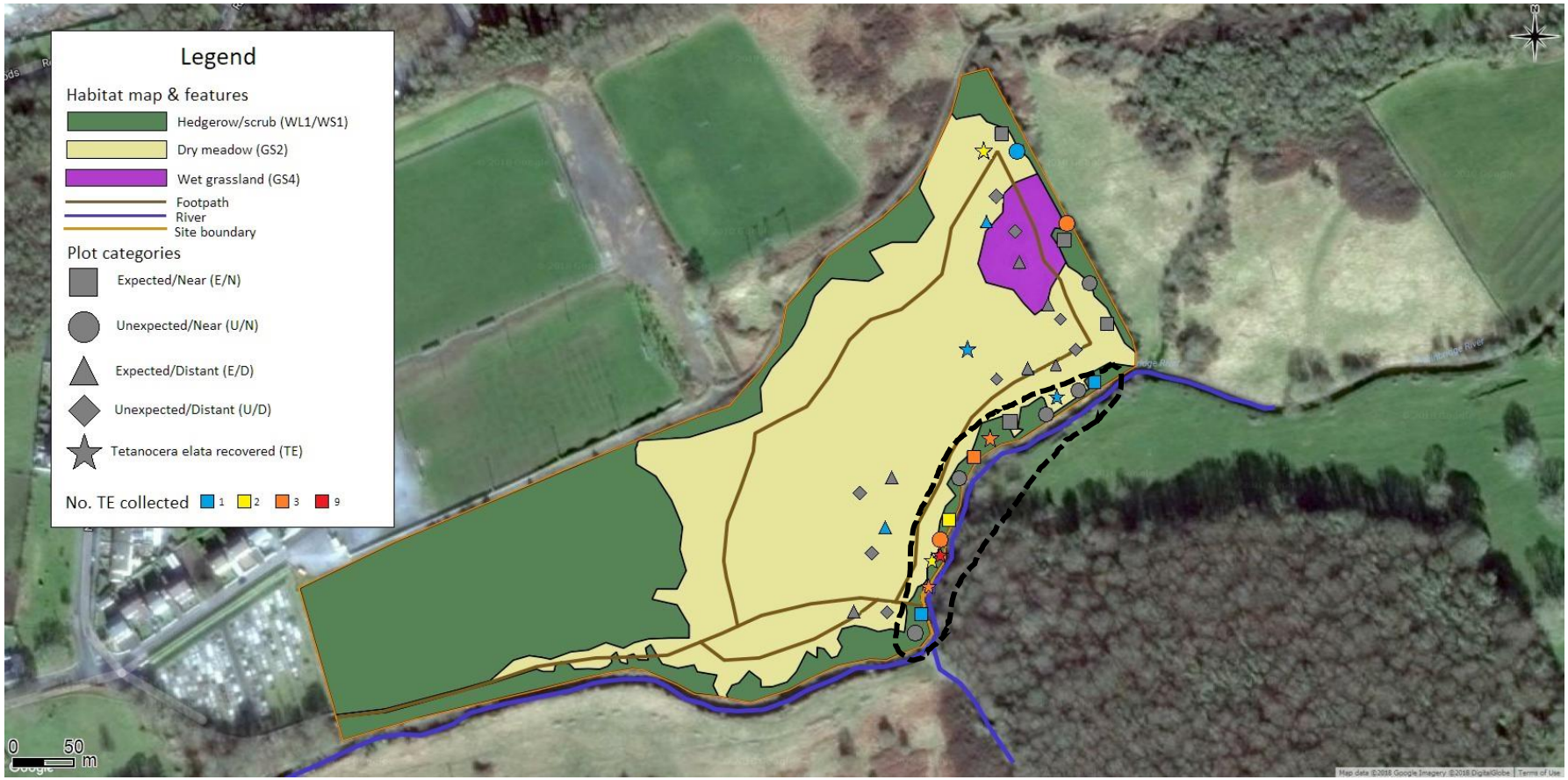

Figure 4.1. Habitat map of vegetation study site, Cow Park, Clarenbridge, Co. Galway. Plots comprising the E/SE subset indicated by a dashed line. Habitat types listed as per Fossitt (2000).

4.3.1.1. Local habitat selection

The survey was comprised of a series of 0.5 m x 0.5 m plots of four categorical types placed semi-randomly throughout the study site according to category criteria. Categories of local habitats measured using these observation plots were comprised of areas that appeared visually similar (e.g., had a similar appearance in plant community and structure) to where *T. elata* adults had been collected in previous years ("expected"), and plots that appeared visually dissimilar to areas where *T. elata* adults had previously been recovered ("unexpected"). Expected plots were identified by structure and species, consisting typically of thick graminoid tussocks in tall stands, while unexpected plots possessed less densely growing vegetation (or even displayed gaps in growth) and were lower growing. Each of these categories was replicated at near (< 5 m) and distant (> 10 m) proximities to a hedgerow boundary, to provide four categorical types of observational plots (expected/near [E/N], unexpected/near [U/N], expected/distant [E/D], and unexpected/distant [U/D]). Each category had 8 replicates, resulting in 32 observational plots. In addition to the above plots which were selected in May 2017 (before the *T. elata* adult flight period), subsequent plots were included in the study as *T. elata* specimens were recovered throughout the summer (see Section 4.3.2), regardless of vegetation appearance or proximity to hedgerow, to comprise a fifth treatment category ("TE"). On occasions where *T. elata* collection coincided with previously designated plots, such plots were both assigned a "TE" plot number as well as maintaining their original designation. In this way, observations were made in both *T. elata*-positive and *T. elata*-negative plots. All plots were marked with a bamboo garden stake which remained in place throughout the study to ensure identical areas were observed each time. The boundaries of observational plots were marked using a 0.5 m x 0.5 m wire frame quadrat oriented using the bamboo stake and closest linear feature (e.g., hedgerow or other boundary).

4.3.2. Invertebrate sampling

Sampling for *T. elata* specimens and their associated slug larval hosts (*D. reticulatum*) were conducted to determine what effect, if any, the vegetative habitat parameters being monitored had on the presence and abundance of these species.

Surveys for invertebrates only took place within the active summer season (June – August 2017).

4.3.2.1. *Tetanocera elata* adults

Adult *T. elata* were collected by passing a heavy-duty sweep net (45 cm diameter, mesh size 0.1 mm) in a figure-of-eight motion through vegetation. Collections were made using short transects (approx. 2 m) in randomised directions to cover the entire study site (i.e., sampling observation plots and all other vegetation on site). Transects did not run through observation plots to avoid trampling vegetation, but sweeps covered vegetation in plots by extending sweep nets into plots as transects ran adjacent. Recovered individuals were collected in barrel-style pooters (Watkins & Doncaster, The Naturalists, Hawkhurst, Kent, UK) and returned to laboratory facilities at the National University of Ireland Galway for examination. Sciomyzidae were examined using a dissecting stereomicroscope (Olympus SZ40, X6.7 to X40 magnification) and species and sex were confirmed morphologically (Rozkoŝný 1984, 1987). Field locations where *T. elata* were collected were marked as a "TE" plot using a bamboo stake (as per local habitat selection, Section 4.3.1.1) and numbered chronologically as individuals were recovered throughout the flight period. Where *T. elata* were recovered within approx. 0.5 m of a designated (*a priori*) observation plot, the existing plot was given an additional "TE" designation.

4.3.2.2. Terrestrial slugs

Pitfall traps were deployed at the end of the summer season (10 August 2017) to survey for terrestrial slug species associated with *T. elata* habitats. To minimise disturbance to study areas, traps were placed at the exterior margins of each observational plot and baited with the intention of attracting slugs from within and around observational areas. Plastic cups (180 ml) were buried with the lip placed level at topsoil and contained a bait comprised of cotton wool soaked in dark beer (Smith & Boswell 1970). Traps were covered with a corrugated plastic square (10 cm x 10 cm) held in place by two 150 cm nails to keep out any precipitation and prevent potential disturbance by foraging small mammals. Four pitfall traps were set for each plot, one along each edge. Traps were deployed overnight for approximately 18 hours, when slugs are most active (Douglas & Tooker 2012) and therefore most likely to be trapped. All slugs collected in traps were recorded, including any slugs recovered on

the exterior of the cup or on the corrugated plastic cover. Slugs were recorded as either "*D. reticulatum*" or "other". Abundance of ground beetles (Coleoptera: Carabidae), numerous species of which are known to prey on slugs, were also recorded.

4.3.3. Ecological measurements

Measurement of plant community composition consisted of both percentage cover and abundance measurements. Percentage cover of plant species was assessed first by making a visual estimation of the proportion of the plot within the quadrat frame occupied by each species visible when viewed top-down (Sutherland 2006). A species list of plants was then generated for each plot by hand-searching within the delineated area, including both visible and understorey plants, and morphologically identifying present species using vegetative keys (Streeter et al. 2009; Clark 2015; Price 2016). The relative abundance of each species identified when hand-searching was also ranked and scored using the DAFOR scale (e.g., dominant, abundant, frequent, occasional, or rare [Sutherland 2006]). Plant community observations were made bi-weekly during the summer flight period (June – August 2017).

Vegetation structure was comprised of measurements of the height of live and dead vegetation as well as the depth of the detritus layer in the understorey. Heights of both live and dead vegetation were taken for each plot by lowering a metre stick through vegetation until the base rested on but did not penetrate the topsoil, and then taking the maximum measurement of live (growing) vegetation and dead vegetation *in situ* height at that point. Points were taken near each corner of the quadrat and at a random location within the frame and measurements were subsequently averaged to yield a mean live and mean dead vegetation height per plot. The detritus layer was measured similarly using a tapered garden stake (to better penetrate detritus to reach topsoil without compressing the layer) marked with corresponding measurements (cm). These measurements were completed in five random locations throughout the plot and pooled to give a mean detritus depth per plot. Unlike plant community analysis, structural features were measured only at the start and end of the adult flight season to track the seasonal growth of plants in survey plots.

Finally, vegetation "openness" (i.e., thickness or thinness of growth) at each plot was measured at the beginning (16 June) and end (10 August) of the sampling period

using the golf-ball method for rangeland assessment described by Schultz *et al.* (2017) to compare changes in plant structure over the peak growing period. Ten golf balls (fluorescent orange for easier visual detection [Links Choice, VA, USA]) were dropped perpendicularly into each plot from a height of approximately 2 m and each was assigned a score based on visibility of the golf balls. Lower scores (e.g., fewer golf balls visible) correspond to a "closed" vegetation structure suggesting more dense growth, while plots with high scores (e.g., higher visibility of golf balls) indicate a more "open" structure. The exception to this was where vegetation had such a closed structure that the balls could not penetrate; in these cases, golf balls were assigned a score of 0 associated with extremely dense vegetation (Schultz et al. 2017).

After the end of both peak summer growth season and *T. elata* flight period (September 2017), observations continued for major primary parameters (percentage cover and height of live and dead vegetation) on a cycle of 6 to 8 weeks, dependent on weather conditions. The 2017 winter season experienced numerous storms in western Ireland, and observations were occasionally delayed when weather conditions were considered to be hazardous or when vegetation was not accessible (e.g., under snow). Observations continued until May 2018, to complete a 12 month survey period of primary parameters. Measurements of plant community composition and structure in off-season periods were also continued to compare stages of vegetation structure and community progression with *T. elata* life history and provide a synchronicity estimate for habitat features and *T. elata* development.

4.3.3.1. Indirect parameter assessment

In addition to direct measurements, other ecological parameters were calculated and examined for their potential influence on *T. elata* presence and abundance. Four criteria were generated: light penetration (L), soil moisture (F), reaction/pH (R), and nitrogen content (N). These criteria were generated using Ellenberg index values for vegetation (Equation 4.1), adapted by Hill *et al.* (1999) for use in the UK. A weighted average for each plot was produced using flight period measurements, as adapted by Diekmann and Grerup (1998) where x = the median percentage cover for a species of plant in that plot over the duration of the study, y = the Ellenberg index value for the plant. Ellenberg values were calculated using measurements taken during *T. elata* flight period.

$$WA = \frac{(x_1 \times y_1) + (x_2 \times y_2) + \cdots + (x_n \times y_n)}{(x_1 + x_2 + \cdots + x_n)} \qquad \textbf{Eq. 4.1}$$

4.3.4. Natural history of Tetanocera elata *in Ireland*

A description of *T. elata* adult phenology and habitat range was compiled through first-hand observational data supplemented by records from the literature and museum specimens. Original data were gathered by the Applied Ecology Unit (National University of Ireland Galway) through active and passive collection of *T. elata* at 38 sites in the west of Ireland (Table 4.1) throughout the summer flight period between 2006 and 2018. Collections began as early as May and continued until mid-August to early September, depending on sampling year and the associated study. Habitat classification for such primary specimens were based on direct observation of plant community (Fossitt 2000). Records of *T. elata* collections from literature (Table 4.2) were compiled by conducting a literature search on Web of Science and Google Scholar, using search terms "*Tetanocera elata*" + "Ireland". Information from museum specimens was taken directly from collection catalogues (Table 4.2).

4.3.5. Statistical analyses

Analysis of categorical environmental factors (i.e., distance category from hedgerow and *T. elata* presence/absence) not appropriate for use in linear models were completed using a Chi-square test. Comparisons of invertebrate collections in pitfall traps were conducted using Wilcoxon rank-sum or t-tests, as appropriate based on normality of data sets. Similarity of plant community (e.g., percent cover) and structure (e.g., height, depth of detritus, density) were compared between treatment types using individual measurements over 12 months using Kruskal-Wallis tests, with *post-hoc* Dunn tests with Bonferroni adjustment for multiple comparisons where Kruskal-Wallis were significant. Comparison was also made between plots pooled by *T. elata* presence or absence in a subset of plots along the east/southeast field margin near the hedgerow boundary ("E/SE subset") (Fig. 4.1). This subset was isolated because plots along the eastern site margin accounted for nearly all *T. elata* presence, and restricting the analysis to these plots limited potential complicating variables, namely aspect. Additionally, data were restricted to "near" plots due to a significant

Table 4.1. Collection records of *T. elata* (TE) adults in Ireland, including location, sampling period, year, method of collection, and description of the habitat where specimens were recovered. Broad habitat types are also noted in descriptions. Specimens included in this table were directly gathered by the authors or researchers associated with the Applied Ecology Unit, NUI Galway.

Site	Collecting Period	Year(s)	No. TE Collected	Collection Method	Collector	Habitat Description
Cow Park	May – August	2016 – 2018	63	SW	ABE	Dry grassland with riparian border and wet grassland mosaic (**G**)
Menlo	May – August	2016 – 2017	6	SW	ABE	Wet grassland along river (**W**)
Green Earth Organic Farm	May – August	2011 – 2013, 2016 – 2018	14, 17	SW, E	ABE, TMH	Abandoned grass field adjacent to agriculture (tillage and grazing) (**G**)
Sligo farm 1	June – September	2018	5	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 2	June – September	2018	5	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 3	June – September	2018	4	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 4	June – September	2018	2	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 5	June – September	2018	13	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 7	June – September	2018	2	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 9	June – September	2018	1	M	KDA	Variable agricultural grassland with linear feature (**G**)

Sligo farm 10	June – September	2018	1	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 11	June – September	2018	1	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 13	June – September	2018	2	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 14	June – September	2018	2	M	KDA	Variable agricultural grassland with linear feature (**G**)
Sligo farm 15	June – September	2018	2	M	KDA	Variable agricultural grassland with linear feature (**G**)
Burren	June – July	2017	4	SW	ABE	Unmanaged calcareous grassland surrounded by hazel (**G**)
Mulranny	June – July	2017	10	SW	ABE	Unmanaged humid grassland near carpark (**G**)
NUIG	June – August	2017	10, 28	SW	ABE, KDA	Margin of improved amenity grass field, near mixed hedgerow (**G**)
Site 04	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Site 05	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Site 06	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Site 11	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Site 12	July – September	2014	6	M	JGJC	Wet grassland, Fossitt GS4 (**W**)

Site 16	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Site 19	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Site 24	July – September	2014	1	M	JGJC	Wet grassland, Fossitt GS4 (**W**)
Hare Island	July – August	2011 – 2013	8	E	TMH	Abandoned grassland surrounded by hedgerow (**G**)
Kilcolgan	July – August	2011 – 2013	5	E	TMH	Ungrazed grassland alongside hedgerow (**G**)
Beechlawn Organic Farm	July – August	2011 – 2013	13	E	TMH	Uncultivated field margin of tilled organic field (**T**)
Cummer	July – August	2011 – 2013	1	E	TMH	Uncultivated margin of potato field (**T**)
Caherlistrane†	September	2002, 2006	2	SW	CDW/ RJMcD	Ungrazed wet grassland (**W**)
Connemara National Park†	June, August	2007, 2006	2	SW	CDW/ RJMcD	In *Juncus*-rich field, track edge (**W**)
Killeenavarra†	August	2006	1	SW	CDW/ RJMcD	Poor grassland at edge of turlough (**G**)
Spiddal†	September	2006	1	SW	CDW/ RJMcD	Edge of Shannawoneen Wood (**G**)

Newbridge†	September	2006	1	SW	CDW/ RJMcD	Fallow potato field (**T**)
Skealoghan turlough†	July, September	2006, 2004	2, 2	P, SW	CDW/ RJMcD	Edge of turlough, 1 each in zones of: *Eriophorum angustifolium* (**W**), unflooded calcareous grassland (**G**); 2 in poor grassland zone (**G**)
Ardkill turlough†	August	2004	1	SW	CDW/ RJMcD	Edge of turlough, *Phalaris arundinacea* zone (**W**)
Lough Nanannagh†	August	2002	1	SW	CDW/ RJMcD	Edge of lake (**W**)

†Published record (Williams et al. 2007)

Collection Method abbreviations: SW = sweep net; E = emergence trap; M = malaise trap; P = pan trap.

Collector initials: TMH = Tracy M. Hynes; JGJC = John G.J. Carey; RJMcD = Rory J. McDonnell; all other listed collectors are authors

Habitat type: G = grassland; W = wet grassland; T = adjacent to tillage field

correlation (P = 0.03, χ^2 = 4.7055, df = 1) between distance category and *T. elata* presence (as concluded by Chi-square analysis). These comparisons were performed using Wilcoxon rank-sum tests with continuity correction and approximate P-values to account for rank ties.

Principle components analysis (PCA) plotting environmental variables against *T. elata* presence/absence was carried out using PC-ORD v.6 (McCune & Mefford 2011). The primary matrix was comprised of direct and indirect quantitative environmental variables, including: percent cover of the five most abundant plant species (*Dactylis glomerata* L. [Poales: Poaceae], *Festuca pratensis* Huds. [Poales: Poaceae], *Poa pratensis* L. [Poales: Poaceae], *Centaurea nigra* L. [Asterales: Asteraceae], and *Plantago lanceolata* L. [Lamiales: Plantaginaceae]), percent cover of dead vegetation, mean height of live and dead vegetation, vegetation thickness, depth of detritus layer, and Ellenberg calculated values (light, moisture, reaction [pH], nitrogen). Measurements were entered as the mean value per plot over the 12 month trial period. The categorical variable of *T. elata* absence (0) or presence (1) comprised the second matrix and was used as a grouping factor. A preliminary PCA was conducted at a site level including all plots, and secondary PCAs were run subsequently using data from the E/SE subset for environmental variables recorded during the flight period and for the 12 month trial. All PCA analyses used a variance/covariance centred matrix with scores calculated based on distance biplot.

Following PCA analysis, environmental variables were examined for their influence on *T. elata* abundance in the E/SE subset with a multiple regression analysis, using values of Principle Components 1 and 2 as the independent variables and *T. elata* abundance as the dependent variable. Analyses (with the exception of PCA) were conducted using R (R version 3.2.5, R Core Team 2013, The R Foundation for Statistical Computing, Vienna, Austria) in R Studio.

Table 4.2. Collection records of *T. elata* (TE) adults in Ireland as described in published literature or museum catalogues. Habitat descriptions are excluded because collection location was very infrequently listed per species/individual in literature or documented for museum specimens.

Collecting Date/Period	Year(s)	No. TE recorded	Source	Accession No.
12 June	1901	1	Dublin NHM†	NMINH:1902:29.1
27 June	1901	1	Chandler 1972	-
03 July	1969	1	Chandler 1972	-
07 July	1909	1	Chandler 1972	-
11 July	1982	1	Dublin NHM	NMINH:1988.54.1
11 July	1989	1	Dublin NHM	NMINH:2008.37.1
12 July	1901	1	Chandler 1972	-
18 July	1978	1	Dublin NHM	NMINH:1986.19.2
20 July	1901	1	Chandler 1972	-
22 July	1978	1	Dublin NHM	NMINH:1986.19.1
25 July	1940	1	Chandler 1972	-
25 July	1950	1	Chandler 1972	-
28 July	1901	1	Chandler 1972	-
28 July	1911	1	Dublin NHM†	NMINH:1913.54.1
July	1940	1	Chandler 1972	-
July	1948	1	Chandler 1972	-
04 August	1924	1	Dublin NHM†	NMINH:1924.11.1
05 August	1893	1	Dublin NHM†	NMINH:1893.188.1
08 August	1906	1	Chandler 1972	-
August	1943	1	Chandler 1972	-
12 September	1978	1	Dublin NHM	NMINH:1986.19.3
October	1989	1	Blackith et al. 1991	-
May – August	1940	1	Chandler 1972	-
May – September	2000 – 2003	9	Speight 2004a*	-
June – August	1997	1	Speight 2004b*	-
July – August	1978	1	Blackith et al. 1991	-
unspecified	1833	1	Chandler 1972	-
unspecified	1871	1	Chandler 1972	-
unspecified	1882‡**	1	Dublin NHM	NMINH:1882.1.1
unspecified	1912	1	Chandler 1972	-
unspecified	1910 – 1920	1	Dublin NHM	NMINH:1913.54.2
unspecified	1970	3	Chandler 1972	-

†Record also published in Chandler 1972

‡ Exact collection date not recorded; date listed is year of entry into museum catalogue

* Sciomyzidae comprised at least one focal group of the study and were specifically targeted for collection/identification

** Possibly collected from Northern Ireland

4.4. Results

4.4.1. Invertebrate sampling

During the summer flight period, a total of 32 individual *T. elata* were collected from 13 discrete plots within the field site (Table 4.3). Of the 13 plots where *T. elata* were recovered, seven plots were unique (54%) and six (46%) coincided with both expected and unexpected pre-designated survey plots (Table 4.3). Only two TE plots occurred distant from the hedgerow, with 85% of TE plots occurring at "near" proximity. Four individuals (12.5%) were recovered at plots designated as having a superficially dissimilar physical appearance ("unexpected") to where *T. elata* had been previously recovered, however 96% of these were collected near the hedgerow (Table 4.3), indicating the importance of this feature. Overall, there were significantly greater numbers of *T. elata* captured in "near" proximity to the hedgerow ($P = 0.03$, $\chi^2 = 4.7055$, $df = 1$), and the majority of captures (87.5%) occurred near the hedgerow comprising the east/southeast margin of the site (Fig. 4.1). Based on these collection numbers, environmental factors were examined both at site level and for this E/SE subset specifically.

Pitfall trapping returned 31 slugs, including one *D. reticulatum*, in addition to 93 carabid specimens (Appendix III.1). The only *D. reticulatum* recovered was found near the hedgerow. Within the E/SE subset, presence of *T. elata* was not associated with variability in slug density ($P = 0.9572$, $t = -0.48617$), however there were significantly fewer carabids recovered in plots where *T. elata* were present than in other plots ($P = 0.002$, $t = -3.4378$), potentially indicating a negative association.

Table 4.3. Plot classification and *T. elata* collection locations in Cow Park during the trial period (June – August 2017). Specimens that were recovered in the absence of designated plots were used to establish "unique" plots; others which were recovered within approx. 0.5 m of an *a priori* selected plot were associated with that plot as well as being given a TE designation. For these co-occurring plots, the associated plot is listed.

TE plot	No. *T. elata* collected	Hedgerow proximity	Unique plot?	*a priori* plot designation
TE 1	3	Near	Y	-
TE 2	2	Near	Y	-
TE 3	9	Near	Y	-
TE 4	3	Near	N	U/N
TE 5	2	Near	N	E/N
TE 6	1	Distant	N	E/D
TE7	3	Near	N	E/N
TE 8	3	Near	Y	-
TE 9	1	Near	Y	-
TE 10	1	Distant	Y	-
TE 11	1	Near	N	U/N
TE 12	2	Near	Y	-
TE 13	1	Near	N	E/N
Site total	32			

Plot category abbreviations: E/N = expected/near; U/N = unexpected/near; E/D = expected/distant; U/D = unexpected/distant; TE = plot where *T. elata* was recovered

4.4.2. Characterisation of vegetation

4.4.2.1. Plant community

At site level, a total of 35 plant species/groups were identified across the plots during the 12 month period (Appendix III.3). Grasses occurred at the highest frequency across all plots, the most frequent being *P. pratensis* (100%), *D. glomerata* (97%), and *F. pratensis* (72%). The most frequently occurring forb species throughout the site were *Centaurea nigra* (54%) and *P. lanceolata* (36%) (Appendix III.3). Significant differences were observed between categories for each of these most frequently occurring species (Appendix III.4), however *post-hoc* pairwise comparisons between plot types showed no consistent trends either across species or with respect to *T. elata* presence (Appendix III.5). *Dactylis glomerata* cover was the

most variable, while *F. pratensis* showed the least variability across plots (Appendix III.5). The plant community composition described using the DAFOR scale was highly variable between plots (Appendix III.3), and the species present where *T. elata* was recovered varied considerably between plots. Graminoids and forbs comprised similar proportions in all plot categories at site level, while scrub (*Rubus fruticosus*) and other vegetation types (e.g., *Pteridium aquilinum*, lichens and mosses) were present only in those categories of plots occurring near a hedgerow (Fig. 4.2A).

Vegetation in the E/SE subset demonstrated similar patterns to site-wide frequency and percentage cover. The E/SE subset of observational plots demonstrated similar relative frequencies of these dominant species (100%, 100%, 81%, 38%, and 38% respectively), and dead vegetation was again observed in all plots. Dead vegetation comprised a mean percentage cover of 40.9 ± 3.0 SE. The other most dominant species provided similar coverage across plots pooled according to *T. elata* presence/absence (Table 4.4). As observed at site scale, graminoid and forb vegetation types were represented at similar levels in E/SE subset plots when pooled according to *T. elata* presence/absence (Fig. 4.2B). When pooled in this manner, plots where *T. elata* were present had significantly higher percentage cover of dead vegetation and *P. lanceolata* ($P = 0.0172$, $W = 2268$ and $P < 0.001$, $W = 2743.5$, respectively); no significant differences were observed in the remaining dominant species (Table 4.4).

4.4.2.2. Local structure

The majority of observational plots (62%), representing plots from every treatment category, received the maximum possible vegetation closeness score (0). The remaining plots were distributed in incrementally increasing categorical scores, corresponding to increasing vegetation openness (Table 4.5). In plots where *T. elata* specimens were recorded, the median vegetation closeness score was 0 and ranged from 0 to 3.5. A significant difference was observed in median vegetation openness between plot types ($P = 0.0303$, $\chi^2 = 10.692$, $df = 4$) with unexpected/distant plots having more open structure than any other plot type (Table 4.5), but not when pooled by *T. elata* presence/absence in the E/SE subset ($P = 0.7855$).

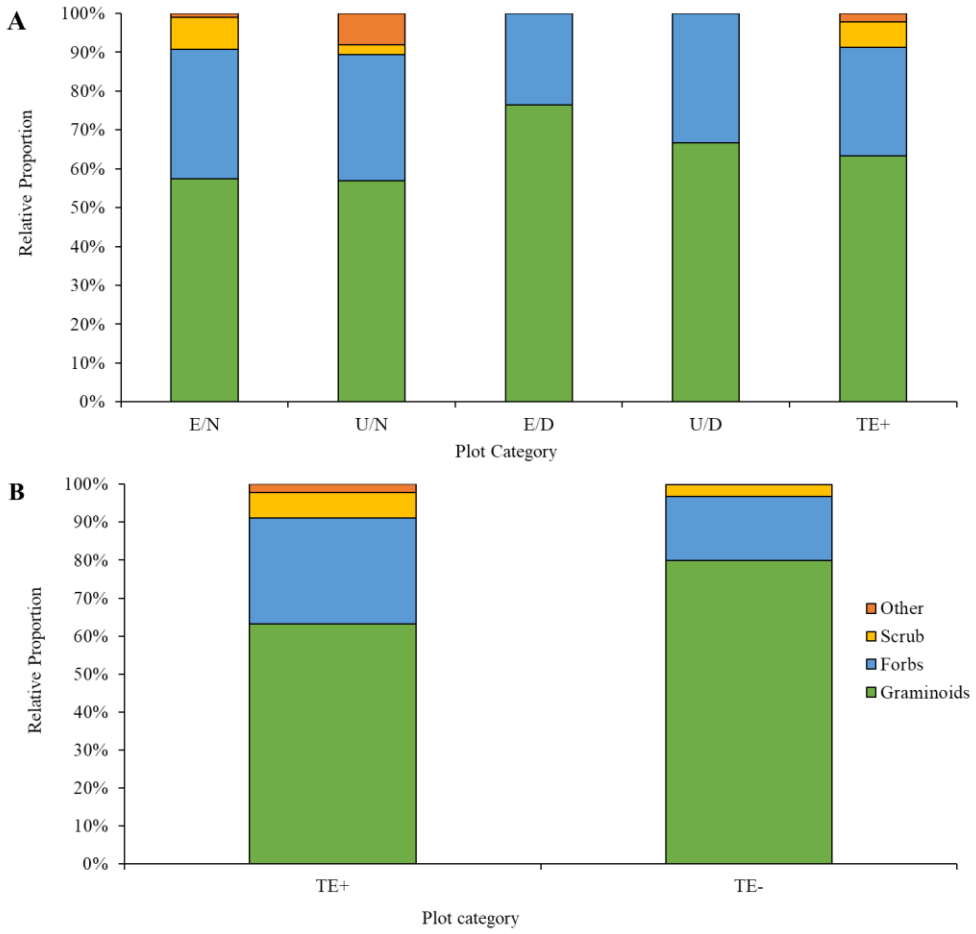

Figure 4.2. Mean proportion of graminoids, forbs, dead vegetation, and other growth (*Pteridium aquilinum*, *Rubus fruticosus*) per plot category at site level (**A**) and in the E/SE subset (**B**) over 12 months.

At site level, maximum live vegetation height ranged from 1 cm to 150 cm, with a site-wide mean height of 46.8 cm ± 1.8 SE. In the plots where *T. elata* were recorded, live vegetation height ranged from 3 cm (overwinter) to 139 cm (peak summer). Maximum height was highly variable across plots and treatment types, with a mean live vegetation heights of 53.56 cm ± 11.4 SE (expected/near), 40.21 cm ± 9.5 SE (unexpected/near), 55.00 cm ± 12.0 SE (expected/distant), 44.84 cm ± 9.4 SE (unexpected/distant), and 42.18 cm ± 8.0 SE (TE) (Table 4.5). Live vegetation height differed between plot categories ($P = 0.0133$, $\chi^2 = 12.62$, $df = 4$), but the high variance of heights within and between plot categories rendered pairwise comparisons nonsignificant when adjusted (Bonferroni) for multiple comparisons.

Maximum dead vegetation height at site-level ranged from 1 cm to 184 cm, with a site mean height of 33.24 cm ± 1.8 SE (Table 4.5). In plots where *T. elata* specimens were recovered the average dead vegetation height was 34.01 cm ± 8.9, with a range from 2 cm to 184 cm, representing the second shortest and tallest dead vegetation measurements at the site. The mean height of dead vegetation was greater in areas where *T. elata* were collected during the flight period than in most other plots (Table 4.5). Dead vegetation height varied significantly between plot categories ($P = 0.0056$, $\chi^2 = 14.607$, $df = 4$), with significant pairwise differences observed between expected and unexpected/near plots ($P = 0.0026$) as well as expected/near and unexpected/distant ($P = 0.0137$). When plots were examined based on *T. elata* presence in the E/SE subset, mean dead vegetation height was greater and live vegetation was shorter on average in plots where *T. elata* was recovered (Fig. 4.3), though neither difference was statistically significant ($P = 0.2621$ and 0.3573, respectively).

Detritus depth had a site-wide mean of 5.7 cm ± 0.4 SE. In the plots where *T. elata* were recorded, the average depth was 6.00 cm ± 1.0. Measurements of detritus depth varied less than measurements of vegetation height, however there was still enough variability within plots of the same category to render comparisons between categories non-significant ($P = 0.1567$, $\chi^2 = 6.6312$, $df = 4$) (Table 4.5). In the E/SE subset, there was no difference in detritus depth between plots pooled by *T. elata* presence/absence ($P = 0.7855$) (Fig. 4.3).

4.4.2.3. Indirect parameters

Ellenberg values showed small variations between plots due to differences in light, moisture, pH, and nitrogen, however when means were calculated for each treatment there was little variation evident (Table 4.5). No significant differences existed for indirect parameters between plot types.

Table 4.4. Mean percentage cover of the five most abundant plant species and dead vegetation in E/SE subset, pooled based on *T. elata* presence or absence. Statistical comparisons are given per plant species between *T. elata* present and absent plots based on two-sample Wilcoxon tests, and indicated as significant (* P < 0.05) and highly significant (** P < 0.001).

	Mean Cover (%) ± SE			**P-value**	**Test statistic (W)**
	Subset total	***T. elata* present**	***T. elata* absent**		
Dead vegetation	40.9 ± 2.6	44.6 ± 4.0	34.0 ± 2.9	0.0173 *	2268
Dactylis glomerata	29.2 ± 2.0	28.6 ± 3.3	28.2 ± 3.3	0.6260	1250.5
Poa pratensis	12.1 ± 1.7	12.7 ± 2.5	12.0 ± 3.4	0.9960	1941.5
Festuca pratensis	11.6 ± 1.7	9.5 ± 1.5	13.7 ± 5.0	0.5013	2140
Centaurea nigra	1.7 ± 0.8	2.2 ± 1.4	1.0 ± 0.8	0.6940	2085.5
Plantago lanceolata	1.3 ± 0.5	1.6 ± 0.7	0 ± 0	< 0.0001 **	2743.5

Table 4.5. Summary of vegetation structure by plot category, including mean live and dead vegetation height (12 month and flight period means; min/max), mean depth of detritus layer, median vegetation thickness score (Schultz et al. 2017), and mean calculated Ellenberg values (Hill et al. 1999). Statistically significant differences between plot categories per variable are indicated by the same superscript letter. Pairwise differences are the result of *post-hoc* Dunn tests with Bonferroni adjustment for multiple comparisons following significant Kruskal-Wallis tests for each variable.

Plot category	Mean height (cm) ± SE (min – max)				Mean detritus depth (cm) ± SE	Median structure score	Mean Ellenberg values			
	Live vegetation		Dead vegetation							
	12 mo.	Flight Period	12 mo.	Flight Period			Light (L)	Soil moisture (F)	pH (R)	Nitrogen (N)
E/N	53.56 ± 11.4 (8 – 150)	90.97 ± 9.1 (8 – 150)	43.15^{A,B} ± 12.2 (3 – 184)	59.75 ± 14.4 (8 – 184)	5.65 ± 1.0	0	7.03 ± 0.2	5.10 ± 0.1	6.52 ± 0.2	5.38 ± 0.2
U/N	40.21 ± 9.5 (1 – 125)	69.70 ± 8.0 (2 – 125)	26.75^{A} ± 9.9 (1 – 121)	34.27 ± 12.0 (1 – 121)	5.39 ± 1.6	1.3	7.03 ± 0.1	5.22 ± 0.1	6.25 ± 0.1	5.23 ± 0.2
E/D	55.00 ± 12.0 (3 – 140)	91.86 ± 9.6 (5 – 140)	36.62 ± 11.5 (1 – 136)	52.09 ± 13.5 (1 – 136)	6.79 ± 1.6	0	7.16 ± 0.1	5.21 ± 0.1	6.71 ± 0.1	5.62 ± 0.1

Plot category	Mean height (cm) ± SE (min – max)				Mean detritus depth (cm) ± SE	Median structure score	Mean Ellenberg values			
	Live vegetation		Dead vegetation							
	12 mo.	Flight Period	12 mo.	Flight Period			Light (L)	Soil moisture (F)	pH (R)	Nitrogen (N)
U/D	44.84 ± 9.4 (7 – 110)	71.66 ± 6.4 (9 – 110)	27.09[B] ± 8.2 (1.5 – 128)	34.94 ± 9.8 (1.5 – 128)	5.11 ± 1.6	0	7.06 ± 0	5.60 ± 0.2	5.96 ± 0.2	5.02 ± 0.1
TE	42.18 ± 8.0 (3 – 139)	78.35 ± 7.3 (8 – 139)	34.01 ± 8.9 (2 – 184)	56.34 ± 10.9 (4.5 – 184)	6.00 ± 1.0	0	7.17 ± 0.1	5.25 ± 0.1	6.61 ± 0.1	5.58 ± 0.2
Site	46.80 ± 1.8	80.48 ± 2.25	33.24 ± 1.8	47.01 ± 3.76	5.97 ± 0.4	0	7.07 ± 0.1	5.27 ± 0.1	6.40 ± 0.1	5.39 ± 0.1

Plot category abbreviations: E/N = expected/near; U/N = unexpected/near; E/D = expected/distant; U/D = unexpected/distant; TE = plot where *T. elata* was recovered

[A] P = 0.0026

[B] P = 0.0137

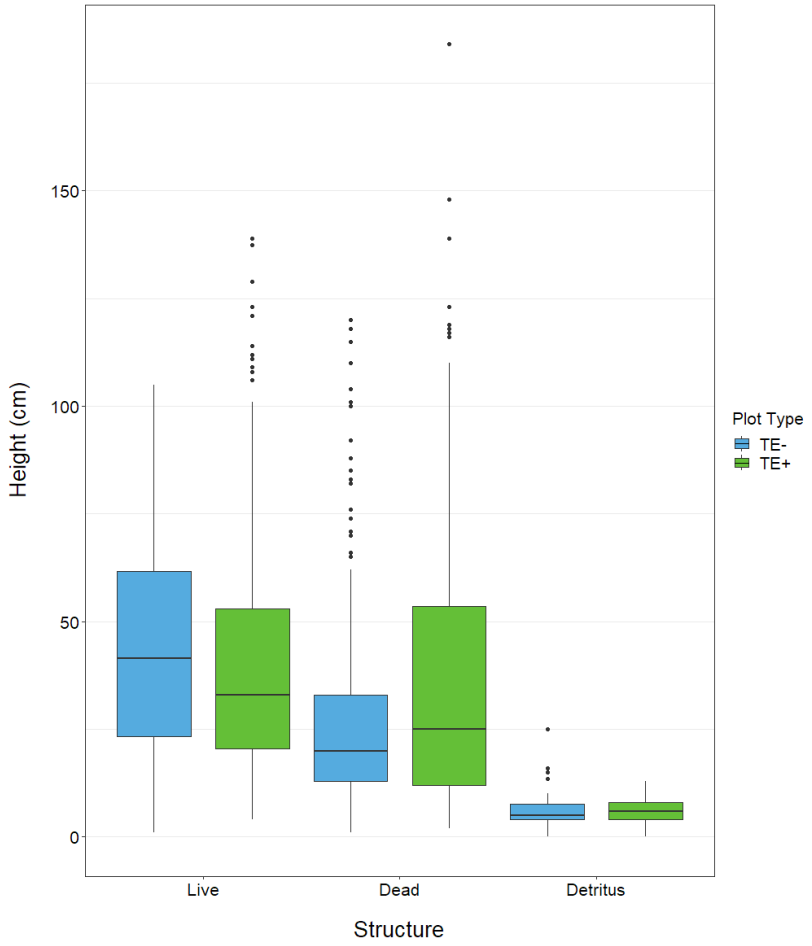

Figure 4.3. Mean and range of vegetation heights (live and dead) and detritus depth over 12 months in plots from the E/SE subset. Plots are pooled by presence or absence of *Tetanocera elata* (TE).

4.4.3. Effects of environmental factors on Tetanocera elata *populations*

At the site level, significantly greater abundance ($P = 0.03$, $\chi^2 = 4.7055$, df = 1) of *T. elata* was recorded close to the hedgerow in comparison to distant plots. Results of site-level PCA produced two axes which accounted for over 97% of observed variation (83.5% and 13.8% on PC1 and PC2, respectively) in environmental factors, with near complete overlap between plots when grouped by *T. elata* presence/absence. When considering data from only the E/SE subset, there was a much clearer separation of groups based on *T. elata* presence/absence. During the flight period, PCA ordination accounted for over 99% of variation, with 82.9% attributed to PC1 and 16.3% explained by PC2. Similarly, ordination of mean data over 12 months explained over 98% of variation; 86.2% represented on PC1 and 11.9% on PC2. Environmental factors related to structure (live and dead vegetation height, detritus

depth, and density) resolved with R^2 values > 0.2 and oriented on PC2 in all cases, indicating the presence of *T. elata* may be positively correlated with the height of dead vegetation and negatively correlated to live vegetation height. A multiple linear regression using the results of the E/SE subset PCA with 12-month data indicated significant differences in *T. elata* abundance with PC2 ($P = 0.02733$, $t = 2.512$), however the non-significant global values for the model ($P = 0.07594$, $F_{2,12} = 3.22$) suggest a lack of power to this relationship.

4.4.4. Natural history of Tetanocera elata *in Ireland*

Collection records were obtained for 292 specimens of *T. elata* adults from across Ireland, spanning from 1833 to 2018. Of these, 86% were primary collection records (66%) by authors or secondary collection information (20%) recorded by other members of the Applied Ecology Unit (Table 4.1). Published (11%) and museum (3%) data comprised the remaining records (Table 4.2). The earliest specific date of collection of *T. elata* in Ireland is 12 June, with the last individual collected on an unspecified date in October (Table 4.2). Cumulatively, the greatest number of *T. elata* have been collected in July; this proportion is also similar across various years (Tables 4.1, 4.2). Based on the collected data reviewed here, adult populations typically begin to eclose in mid to late June, and population density steadily increases and peaks in late July. There appears to be a dip in the first half of August, with another lower peak in population density in late August, after which numbers steadily decrease through late September (Fig. 4.4).

Of the locations where *T. elata* adults have been recorded, there are numerous descriptions of various habitats. A considerable number have been recovered from unmanaged dry and seasonally wet or humid grasslands. Additionally, *T. elata* adults have been collected in the following habitats in Ireland: agricultural land of varying usage (intensive, intermediate, or extensive pasture/sileage, field margins with/without hedgerows, and disused/fallow land); unmanaged grassland with hedgerow; residential garden; near turloughs (seasonal lakes); beside rivers or streams; within or beside woodland (including gorse thickets, *Salix* stands, and *Pinus* plantations); blanket bog; and acidic fen (Table 4.1).

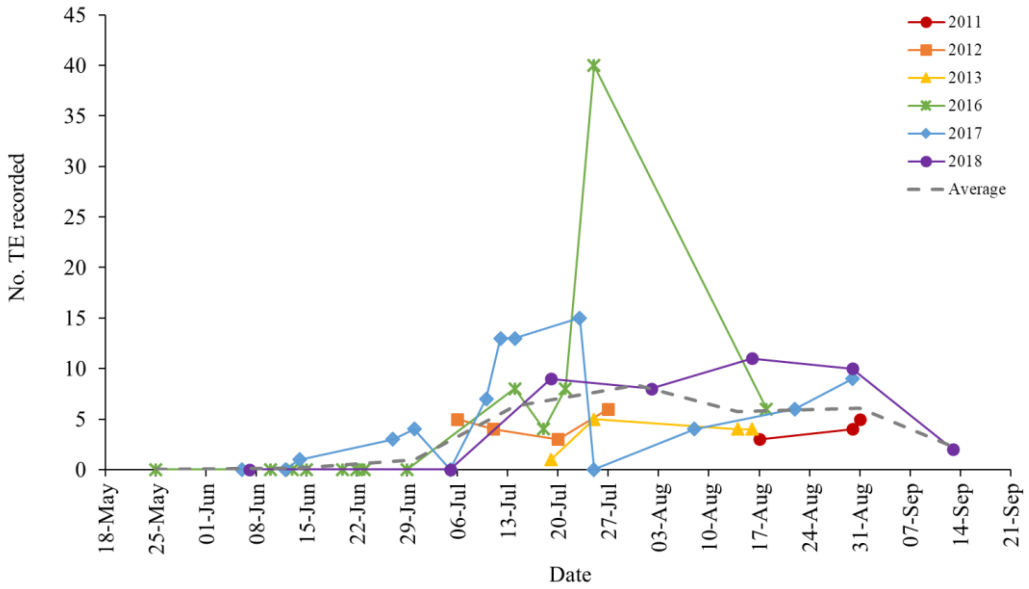

Figure 4.4. Number of *T. elata* adults (TE) recorded by year in Ireland from continuous sampling schemes at the Applied Ecology Unit (National University of Ireland Galway). The number of *T. elata* collected were averaged across years and is overlaid (dashed line) to demonstrate average population trends.

4.5. Discussion

The primary finding of the current study into essential habitat features is that hedgerow proximity is significantly associated with *T. elata* populations. The authors have observed anecdotally that hedgerows may be important features in habitats when collecting *T. elata*, and data presented here quantitatively supports this assertion. These results support the rapidly growing body of evidence that presence and proximity of hedgerows have significant positive effects on the abundance of natural enemies of invertebrate crop pests, with a higher abundance of beneficial species closer to the hedgerow (Morandin et al. 2014; Holland et al. 2016; Garratt et al. 2017). A large proportion of *T. elata* specimens recovered in the study were collected near the south-eastern hedgerow border of the site, in a grouping comprising the E/SE subset (Fig. 4.1). There are several possible explanations for this including hedgerow quality, a lack of connectivity between hedgerow sections (e.g., gaps in hedgerows for human or livestock movement or thin growth of hedgerow species), or hedgerow orientation relative to wind patterns and sun exposure at the study site. Although specific hedgerow characteristics were not quantified, the hedgerow bordering areas where most *T. elata* were recovered was characterised by a mixture of

tall tree species (*Fraxinus* spp., *Acer pseudoplataus*, *Fagus* spp.) commonly found in woodland habitats and smaller hedgerow species (*Crataegus monogyna*), compared to the other hedgerows in this study which were dominated by a single smaller species (*C. monogyna*). Hedgerow quality has been shown to influence invertebrate communities, with richness and abundance of beneficial invertebrate species (e.g., natural enemies and pollinators) increasing with increasing hedgerow quality, such as increased diversity of woody species and trees, hedges laid to prevent gapping, and the maintenance of a healthy understory (through reduced mowing and herbicide/pesticide application) (Garratt et al. 2017). Localisation of *T. elata* around the south-east hedgerow, which had the highest density of *T. elata* at the study site, could also be attributed to a lack of connectivity between hedgerows. Several gaps were observed in the hedgerow at the study site, primarily caused by a footpath leading from the grassland into adjacent woodland on the distal south-east margin, and a large gap in the north-east corner of the site used for livestock passage to adjacent fields. Patchy or uneven growth was also noted along the northern margin. Due to the sedentary behaviour of adult Sciomyzidae, which restrict themselves to favourable areas (Williams et al. 2009a, b), the dispersal of *T. elata* to other hedgerows across the site may have been hampered by the lack of a continuous hedgerow, as has been observed for other invertebrates with limited dispersal capacity (Holland et al. 2016).

The study also revealed the positive association between *T. elata* presence and dead vegetation height, and an inverse relationship with live vegetation height. This contrasts somewhat with previous research, which while also finding vegetation structure to be an important factor influencing sciomyzid communities (Williams et al. 2009a, b), the structure discussed in literature likely refers to the height of living vegetation. In the current study, while plots in the E/SE subset did not significantly differ from one another in composition/structure based on *T. elata* presence, there was a significantly greater coverage of dead vegetation in plots where *T. elata* were recovered, potentially indicating a build-up of dead vegetation over an extended period of time. This observation coincides with records of *T. elata* being collected from ungrazed or abandoned agricultural land (Knutson et al. 1965; Speight 2004a; Speight & Knutson 2012). When considering these findings in a three-dimensional scale, the combination of high cover and height suggests a greater overall volume of dead vegetation, again potentially indicative of grazing which is of low intensity or absent.

This may be important for a number of reasons, such as providing a more sheltering canopy allowing for development of a mesocosm within, which is more buffered against changes in temperature, humidity, or light penetration (Gontijo 2019). The larger amounts of decaying vegetation may also have an effect on soil and invertebrate communities. Such areas may also provide desirable shelter and alternative food sources for slugs (Godan 1983), including *D. reticulatum*; it would, therefore, benefit *T. elata* larvae greatly to also inhabit a similar spatial niche. Adult *T. elata* are known for having high patch fidelity and low dispersal rates of only around 20 metres (Williams et al. 2009a; Speight & Knutson 2012), which suggest if these areas do provide ideal juvenile habitats, the adult life stage will likely remain closely associated. There was no impact detected from detritus levels or vegetation openness as influencing *T. elata* presence, however it should be noted that the majority of plots throughout the study site were dominated by *D. glomerata*, a species well known for forming dense tussocks over several growing seasons. Tscharntke and Greiler (1995) have suggested that tussock-forming grasses are important for providing shelter for ground-dwelling arthropods, especially as they build up and degrade in regular cycles. Terrestrial slugs, such as *D. reticulatum*, are also known to associate with tussock-forming grasses for the shelter they provide (South 1965). This tussock structure, combined with high volumes of dead vegetation, could provide valuable refuges for developing *T. elata* larvae.

While some significant differences were identified between plot categories in the coverage of the most abundant species, plant community composition did not show any consistent trends with relation to where *T. elata* were present throughout the study site. Although collection numbers showed highest *T. elata* abundance clustered in the E/SE subset, the collected individuals were distributed across both *a priori* treatment types (expected/near, unexpected/near) possessing variable habitat parameters. This clustering may be explained in part by the site being bordered to the east by a river (Fig. 4.1), which likely created a physical boundary for distribution. Conversely, the north border of the site was adjacent to additional grass and pasture fields separated by gappy hedges, which may have allowed for more distribution if *T. elata* populations were present in that area.

Plant community composition in wet and dry grasslands can be confidently excluded as an important indicator of the presence and abundance of *T. elata*

populations. This result was not unexpected as *T. elata* have historically been recorded in a wide variety of habitats (Speight 2001; Knutson et al. 1965; Williams et al. 2009a), which in itself suggests that *T. elata* are able to survive in a range of habitats and do not depend on any particular host or shelter plant associations. This lack of association between *T. elata* and plant species may be explained by the distribution and feeding habits of its larval host/prey species. *Deroceras reticulatum*, on which first and second instar *T. elata* are highly host-specific (Knutson et al. 1965), is a generalist herbivore which selects food sources based on nutritional status (Cook et al. 2000). It is also one of the most widely distributed terrestrial slug species, occupying a range of habitats from frequently disturbed tillage fields to intensively managed agricultural land to abandoned fields (Hunter 1966; South 1992). This association is widened still further as *T. elata* larvae mature to predaceous third instars, at which point they are capable of utilising several other slug species as prey options (Knutson et al. 1965; Knutson & Vala 2011; Giordani et al. 2014; Bistline-East et al. 2019), none of which have a specialist diet (Hunter 1968; South 1992). In addition to the larvae feeding on generalist molluscs, adult *T. elata*, which rely on a largely carbohydrate-based diet, are not known to associate with any particular nectar-producing plants and have not been observed feeding on floral or extrafloral resources. Recent research has demonstrated that adult Sciomyzidae including *T. elata* may associate opportunistically with specialist and generalist insect herbivores (Hemiptera) to fulfil part or all of their dietary requirements by consuming honeydew (Bistline-East et al. 2018). Because such herbivores would be expected to have a fairly uniform distribution within a site such as the one in which the current study was conducted, *T. elata* likely were not restricted in their distribution based on this trophic association.

When the Ellenberg index values for secondary habitat parameters were compared based on *T. elata* presence/absence, adult *T. elata* adults did not appear to have any preference for light penetration, soil moisture, soil pH, or nitrogen content throughout the site. However, as these values were calculated indirectly based on plant community, there may be some difference in actual environmental conditions within the understorey in locations where *T. elata* occurred compared to the wider site. Future research should aim to measure such factors directly (e.g., through the use of environmental data loggers).

The adult flight period of *T. elata* in this study was observed to occur from July through August, which agrees with published records for Ireland (Speight & Knutson 2012). In other collecting years, the earliest record of *T. elata* in Ireland occurred on 12 June, with the latest on an unspecified date in the month of October (Table 2). Population densities demonstrated similar relative patterns across years and collectors. In contrast to literature where *T. elata* is considered a bivoltine species, in Ireland the population dynamics may not support this. While the highest densities in Ireland are most often seen in late July, followed by a second smaller peak in mid to late August, this may not allow enough time for a generation to complete development under field conditions, especially considering most contemporary observations have recorded the first adult eclosions at the end of June or early July (ABE unpublished data) (Knutson et al. 1965). This pattern may, instead, be an artefact of differential development rates of offspring from the previous year. Individuals eclosing early in the flight period may be of the cohort of eggs laid earliest in the year prior, and had the most time to complete development. Those eclosing late in the season (e.g., during the August peak or later) may be those which developed from eggs laid at the end of the previous season. These individuals would likely have experienced slower development rates as larvae due to the declining temperatures and availability of host/prey individuals. It may also be that these late-occurring individuals are less robust than those completing development earlier in the season. Anecdotal experience indicates that these adults collected late in the flight season also do not survive as long under laboratory conditions as those collected in July. It could be argued that these individuals are simply adults from the July cohort which are reaching the end of their oviposition period, however this does not explain the observed gap in collection numbers between early and late adults, and would also not coincide with oviposition periods observed in recent research (Hynes et al. 2014b). Additional observations of *T. elata* phenology could further clarify this pattern and perhaps identify with more certainty whether this species is undergoing a shift in voltinism.

The findings of this study (e.g., little to no association with particular plant communities) may in fact be promising for the potential for *T. elata* to be established as a self-sustaining natural enemy in a conservation biological control scheme because it means that the vegetation composition of arable field margins will not likely have to be heavily manipulated to provide a suitable habitat. The correlation of *T. elata*

presence to hedgerow proximity is both promising and challenging for its potential future use in biological control. The findings of this study further validate the importance of maintaining hedgerows in field margins, in this case for the benefit of *T. elata* populations, but hedgerows are also widely acknowledged for their importance for other natural enemies, pollinators, and biodiversity within agroecosystems (Thomas & Marshall 1999; Holland et al. 2016; Garratt et al. 2017; Van Vooran et al 2017). In addition, hedgerows have been also shown to provide beneficial habitats for birds, mammals, and woodland plants (Wehling & Diekmann 2009; Brien et al. 2016; Garratt et al. 2017; Heath et al. 2017). As a result, providing a habitat for *T. elata* can be multifunctional by also increasing biodiversity at the agroecosystem level. Within agroecosystems lacking hedgerows, it can now be recommended with confidence that establishment of a hedgerow is likely essential to support populations of this natural enemy for biological control. Although the planting of hedgerows is profitable over time, initial investment is high and may prove prohibitive for growers. It also takes time to establish a hedgerow which may result in *T. elata* populations introduced for slug control not initially being self-sustaining. Alternatively, *T. elata* population establishment may be more likely to succeed when introduced into sites which already benefit from well maintained, mature hedgerows. The association of *T. elata* with hedgerows also presents some potential challenges. The current study recovered two individuals in the central part of the site, which indicates *T. elata* adults may not be inclined to disperse away from the hedgerow with which they are associated. Translated into a biological control context, this could result in high levels of pest suppression around the edges of a crop field (e.g., the margin(s) nearest a hedgerow) but insufficient control of pests within the field itself. This could potentially be counteracted by establishing low hedgerows within tillage fields, similar to what is done with intercropped beetle banks, however this adds a degree of logistical difficulty with regard to the continued tillage and harvest of the field.

When considering the implication of vegetation structure for the use of *T. elata* as a self-sustaining biological control agent of slugs, it is important to note that the vegetation structure in the study site, which had not been mown or grazed for at least three years (E. O'Riordan, *pers. comm.*), differs from what would be found in and around tillage fields in agroecosystems. Specifically, the importance of land being undisturbed for extended periods should not be underestimated. Plots where *T. elata*

adults were recovered were all characterised by tall, thick stands of dead vegetation, which can only occur if grasses have been allowed to grow and die back over several cycles. If *T. elata* populations are to be established, then, the landscape requires areas of abandonment (e.g., unmanaged field margins or fallow portions of tillage fields), which may prove difficult or undesirable for some growers. Under the current Green Low-carbon Agri-environmental Scheme (GLAS) in Ireland, farmers are required to graze, mulch, or mow such areas at least once per year to be eligible to receive payment (DAFM 2015); additionally, to receive payment under the Basic Payment Scheme (BPS) farmers are required to keep their land in "good agricultural and environmental condition", which also requires mowing or grazing of grass margins at least annually (DAFM 2017). Ultimately, these characterisations of important habitat features for *T. elata* populations should be fairly easily applied to existing agroecological landscapes, especially those which already have some areas of semi-natural habitat, but some accommodations will likely need to be made with regard to the criteria of agri-environmental support schemes.

The identification of these features is the first step to developing a viable conservation biological control scheme (Holland et al. 2016). By quantifying habitat features and phenology of Irish *T. elata* populations, this study lays the groundwork for future conservation biological control development. However, while this is a valuable first step, the authors acknowledge the small scale of the study. It should, therefore, be viewed as a preliminary case study and proof of concept and be used to conduct further wider scale studies in future. While a mild negative correlation was observed in the current study, between carabids and *T. elata*, this was the result of only a single night of sampling. For any true association to be made, a more robust survey of carabid populations is required, preferably across the entire flight period. Further research is also needed to examine what impact the slug communities have on *T. elata*. It was beyond the scope of this study to directly survey the honeydew producers in the site. It has previously been shown that the chemical composition of honeydew can vary between species of producers. Consequently, honeydew with a different chemical composition could impact the fitness of adult *T. elata*. The presence of a hedgerow rather than a grassy verge at field margins has previously been shown to increase densities of the honeydew producers such as aphids (Hemiptera: Aphidoidea) (Van Vooran et al. 2017) and should be further explored. Hedgerow characteristics were not

examined in this study although this could now (based on the results of this project) be an important factor for the success of *T. elata* as a biological control agent.

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Chapter 5 General Discussion

5.1. Summary of Major Findings

For any organism to be effectively utilised in the biological control of a pest, a thorough understanding of the candidate's biology, physiology, and ecological interactions is required. While some studies addressing foundational concepts exist for *Tetanocera elata* (Knutson et al. 1965; Knutson & Vala 2011; Hynes et al. 2014a, b, c), much remains uninvestigated between the theoretical knowledge base and an applied output. The research reported in this thesis aims to bridge that gap (at least partially) between theoretical and applied. Through the studies described herein, ample information was gained which can contribute to the development of an integrated control scheme for terrestrial slug pests in agriculture. The general discussion for this thesis is structured using sub-headings representing the key findings under which a detailed discussion of the results, implications, limitations, and related future research are presented. This is followed by three sections which discuss: a) additional avenues for research; b) the feasibility of using *Tetanocera elata* as a biological control agent; and c) the overall context in which conservation biological control sits, particularly with reference to important links between biological control optimisation and biodiversity in agriculture.

5.1.1. Adult Sciomyzidae feed on hemipteran honeydew

By presenting multiple food options simultaneously in cafeteria trials, a potential natural diet for *T. elata* adults was identified for the first time. Aside from simply determining the utilisation of carbohydrate-rich food sources (as is commonly observed throughout the Insecta), this study also revealed the previously unknown community interaction between Sciomyzidae and honeydew-producing Hemiptera. Sciomyzidae at family and species level showed significant preference for honeydew over the standard artificial diet used in laboratory rearing. Honeydew can be a valuable nutritional resource, especially for species not specialised for floral nectar access, providing not only high amounts of sugars but also amino acids and proteins (Auclair 1963; Fischer et al. 2005) which have been demonstrated to increase oviposition rate and quality in Diptera (Verheggen et al. 2008; Leroy et al. 2010). This has implications for improving adult fecundity in laboratory cultures. Another interesting pattern observed in general cafeteria trials was what may be resource partitioning, with *T.*

elata adults (which have slug specialist larvae) consuming only the snail tissue protein. This pattern did not produce statistically significant differences between rates of feeding on slug or snail tissue and it is possible that this could have simply been the result of low numbers of flies which fed on any protein option (mean number of feeding observations on snail tissue was just 0.28 ± 1.12 SE). However, if resource partitioning is, in fact, occurring, it could suggest that adults have the ability to obtain protein from molluscs that are not necessary for larval survival (i.e., the obligate larval host). This supports the hypothesis that *T. elata* adults do not disperse from their natal patch (as evidenced by documented dispersal capacity by Williams *et al.* [2009]), as they are able to utilise existing resources without needing to forage long distances.

These data may put to rest contention over the potential use of various food items (gastropod mucus, dead and dying insects, floral resources) suggested by earlier studies (Berg & Knutson 1978; Knutson & Vala 2011), and better inform biological control programmes and ecological surveys for Sciomyzidae. Because this association is now known, management strategies for conserving *T. elata* populations in agroecosystems can include such measures as retaining grass strips that are excluded from pesticide application and which provide habitat and alternative non-crop host plants for Hemiptera between cropping cycles. This would in turn ensure that sufficient resources are maintained to support *T. elata* adult populations.

Studies of dietary preference may be warranted to explore the role satiation plays in feeding, and how various diet options (e.g., protein vs. carbohydrate) affect the rate of satiation when feeding. Adult diet also has the potential to affect both longevity and fecundity. While *T. elata* showed preference for hemipteran honeydew over the water control, it was outside the scope of the study to track long-term effects of diet type. In addition to comparing artificial diet to honeydew, a third treatment of floral nectar, extracted from abundant species in hedgerow understoreys or field margins or common cover crop/intercrop plants, would also be worthwhile and potentially provide further insights into ecological interactions and habitat resource utilisation. Further studies could investigate factors relating to potential so-called ‘domestication phenomena’ (Michaud 2018), such as the effect of diet of laboratory strain founders on offspring (i.e., fitness) or whether laboratory-reared individuals sired by adults kept under certain conditions demonstrate behavioural differences of parasitism/predation over multiple generations. Finally, molecular gut analysis of field-collected

individuals has the potential to reveal food sources actually utilised by adult Sciomyzidae in nature.

5.1.2. Predaceous larvae do not demonstrate preference of prey slug species

A primary desire of most biological control programmes is to employ natural enemies which are highly host-specific specialists, so as to incur as few unintended non-target effects as possible (Murdoch et al. 1985; Van Driesche & Bellows 2001; Hoddle 2016). Species are generally considered specialists if they rely on a single species or limited group of closely taxonomically related species to complete their life cycle. In contrast, generalists may be considered at several levels, including 'true generalists' (e.g., carabids which can utilise anything from molluscs to arthropods to dry pet food as a food source) or those which can target a range of species of a certain size (e.g., *Chrysoperla carne*a [Neuroptera: Chrysopidae]) or based on behaviour (e.g., spiders) (Symondson et al. 2002). Further confusing trophic categorisation is the existence of intermediate terms including 'polyphagous', 'stenophagous', and 'oligophagous', which can all be interpreted in different ways depending on the context of research and researcher (Symondson et al. 2002). The genus *Tetanocera* is regarded as oligophagous by Knutson & Vala (2011) and studies reviewed therein, which likely refers to the ability of larvae to feed on a range of prey species while remaining restricted to gastropods. Addressing the lack of clarity regarding the predating ability of *Tetanocera* spp., including *T. elata*, is therefore, of vital importance if *T. elata* is to be considered as a biological control agent.

When assessing prey range, Evans *et al.* (1999) employ the terminology of 'essential' prey to describe a species which a predator requires as at least as a portion of its diet, with 'alternative' prey referring to species that may comprise varying levels of the diet or have the potential to do so. This terminology is utilised throughout the discussion of *T. elata* prey range here to lend clarity. *Deroceras reticulatum* is considered essential prey as it is the associated host species for the parasitoid life stage and early third instar larvae frequently use the host carcass as their first meal. Any other prey species are regarded as alternative species until such point they are determined to be a required dietary component. The results of the third instar prey choice study did not determine a clear preference between *D. reticulatum* and alternative prey species *Arion hortensis* or *Tandonia budapestensis* with regard to the

number of individuals of each species which were attacked and fed on, which support initial observations by Knutson *et al.* (1965) that predaceous *T. elata* larvae fed on a range of commonly-occurring slug species. However, when attacking prey, larvae required fewer attacks before successful feeding on *D. reticulatum* than either alternative species. This may be a reflection of co-evolution of parasitoids to counteract the host immune defences of *D. reticulatum* (Charnov & Skinner 1983; Beckage 1985; Kawecki 1998) that has carried over into the predaceous larval stage. On the other hand, both alternative prey species could possess more effective antipredator strategies which required larvae to make additional attacks before feeding was successful. In this regard, it is interesting to note that O'Hanlon *et al.* (2018) observed that *D. reticulatum* relies on calcium-thickened mucus secretion to deter predators but its mucus is not as viscous as other species observed (namely *Geomalacus maculosus* Allman [Sylommatomorpha: Arionidae], which produces mucus with extremely high viscosity). While predaceous *T. elata* larvae have also been documented feeding on *G. maculosus* in no-choice laboratory trials (Giordani et al. 2014), no record was made of attack efficacy (see Section 5.2 for further discussion of non-target predation of *G. maculosus*).

Third instar larvae in trials frequently demonstrated behaviours characteristic of 'search and wait' or 'wait' responses described by Hynes *et al*. (2014a) and confirmed by D'Ahmed *et al.* (2019), in which larval attack on prey individuals were largely the result of slugs incidentally encountering a stationary larva. Encounters of slugs with stationary or randomly-moving larvae described in this study may have been an artefact of probability – especially in choice arenas – where higher prey density in arenas of the same size increased the likelihood of slugs encountering larvae by chance. As such, true patterns of prey preference may have been masked, and it is strongly encouraged that further testing incorporate adjusted arena size. Arenas could be scaled based on the average movement per unit time of each slug species (e.g., if *D. reticulatum* moves 10 cm per hour on average, this arena could be made half the size as an arena for *A. hortensis* moving at 20 cm per hour) to reduce undue variability caused by prey movement and reveal clearer patterns of predator/prey interactions. Further exploration of prey preference by *T. elata* larvae in field trials, or at least in semi-natural terraria maintained under field conditions, may result in a more accurate reflection of what can be expected in an actual *T. elata* introduction for biological

control. Associated with this could be studies which investigate the success rate of neonate parasitism of *D. reticulatum* under field conditions. This could provide insights into the levels of host parasitism that may be expected from a biological control programme. Surveys of *D. reticulatum* in *T. elata* habitats would also add further knowledge of naturally-occurring parasitism rates. Larval survivorship may also be studied in more detail, especially with regard to the high mortality during pupariation witnessed in this study, as well as by Hynes et al. (2014c). Hungering/starvation of larvae could have contributed to increased third instar mortality, as has been documented for neonate larvae (D'Ahmed et al. 2019), and warrants further investigation.

Additional laboratory-based predator/prey studies can also build on preliminary predating behaviour described by Hynes *et al.* (2014a). Larvae may express variable behaviours based on prey species which could in turn account for differing rates of predation. Additionally, prey species may exhibit different activity levels, spatial utilisation within arenas, or predator avoidance or evolved defence strategies that may affect predation rates. This has yet to be described or quantified with slug species interacting with *T. elata* larvae and could provide valuable insights into community interactions.

5.1.3. Prey species utilisation may have physiological consequences

While no behaviour or prey choice indicated preference, there seems to be, at least some physiological detriment to third instar larvae when reared solely on alternative prey. Larvae reared on *D. reticulatum* had a higher survivorship to pupariation, although developmental rate was slower than for larvae receiving alternative prey. Because larvae were fed these species without choice of alternative species, a shorter developmental period could reflect an attempt to pupariate earlier after failure to locate better prey (as demonstrated for the single adult that eclosed from the *T. budapestensis*-reared cohort), rather than attempting prolonged feeding on suboptimal species. Conversely, numerous studies have observed prolonged development time on suboptimal prey (Albuquerque et al. 1997; Vivan et al. 2003; Ferrer et al. 2008; Chen et al. 2018). A potential trade-off, therefore, is presented as to whether it is more beneficial for the larva to continue to remain in the third instar, a vulnerable and delicate life stage, and continue to expend energy and hunt for more

optimal prey to reach a certain nutritional or biomass threshold, or to risk pupariation. Pupariation and successful pupation numbers across all three prey species were low. Survivorship of even larvae reared on *D. reticulatum* further illustrates the difficulty of rearing *T. elata*, which may prove a complicating factor in the context of feasibility of an augmentative biological control agent.

Hynes *et al.* (2014b, c) improved rearing success by determining optimal development temperatures, and it was outside the scope of this research to further refine rearing methods. Larvae were reared specifically for use in behavioural and physiological experiments and not with the objective of improving maximised output. High mortality occurred as third instar larvae matured to pupariation and was observed in multiple cultures across multiple years. This transition seems to be a particularly vulnerable period for *T. elata* and should be studied further. Yield would likely benefit from rearing eggs and larvae within environmental chambers which can fully regulate temperature, relative humidity, and photoperiod to ensure optimal conditions are always maintained. In addition to larval survivorship, laboratory colonies consistently display prolonged pupal periods and short lifespan of laboratory-eclosed adults. Future research should endeavour to determine whether such prolonged pupation is a result of overwintering quiescence and, if so, attempt to overcome this delay through such artificial manipulations as cold shock or maintaining colonies consistently under summer temperatures and photoperiods (Hynes et al. 2014b).

5.1.4. Habitat structure influences Tetanocera elata *presence and abundance*

This part of the study was initially designed to test whether it was possible to visually identify locations within a site where *T. elata* was likely to be present, thus making possible pre-flight period scouting of field sites and enabling more efficient sampling. The results of these observations are more intricate than was expected, with categories of designated observation plots differing from others in no discernible pattern (with regard to either plot category or *T. elata* presence). One consistent factor of importance was proximity to a hedgerow, with significantly more *T. elata* adults recovered within 5 m of the hedgerow than anywhere else at the site. This is likely important for ecological management of *T. elata* populations in agroecosystems. Populations of *T. elata* adults at the study site were heavily clustered along the east/south-east margin. This could be a direct result of gaps of different sizes in the

hedgerow impeding movement (Garratt et al. 2017). Because *T. elata* is not a strong disperser (Williams et al. 2009), this alone may have been enough to prevent movement throughout the rest of the site and an important factor when considering conservation measures of semi-natural habitats in agroecosystems.

Another important factor reflecting *T. elata* presence was the height of dead vegetation. Coupled with significantly greater percentage cover of dead vegetation in plots where *T. elata* was present (namely the E/SE subset), there may be a relationship between the volume of dead vegetation on a three-dimensional scale. This is certainly indicative of abandoned grassland, or at least one which has not been actively managed (e.g., grazed or mowed) for many years, which supports many records of habitats in Ireland where *T. elata* has been collected (Knutson et al. 1965; Speight 2004a, b; Speight & Knutson 2012). Such vegetation build-up could create beneficial shelters for developing *T. elata* larvae in the soil, as well as provide attractive food and shelter for *D. reticulatum* hosts (South 1992; Gontijo 2019).

This investigation was the first of its kind for *T. elata*, and the first to evaluate the relationship of these flies with habitat features in any detail. Many avenues could be explored in future studies, but the most important currently seem to be gaining an understanding of how hedgerow orientation, quality, and connectivity influences *T. elata* abundance and dispersal, and determining with greater clarity how *T. elata* adults utilise habitat features. The former will require similar observations as were conducted in the Cow Park case study replicated at multiple field sites with varying quality and configuration of hedgerows. Several approaches can be used to accomplish the latter task, but perhaps most enlightening would be the use of protein markers and enzyme-linked immunosorbent assays (ELISA) applied to specific regions of a habitat (e.g., hedgerow, margin, field, etc.), and screening *T. elata* adults as they are collected (Hagler & Naranjo 2004).

5.1.5. Tetanocera elata *may be univoltine in Ireland*

The phenology assembled for *T. elata* both aligns with previous knowledge and presents interesting new possible interpretations. Averaged across collection years where precise collection dates are known, the observed flight period begins in late of June and typically extends into mid-September. One historical record exists for *T. elata* collected in October, however the date was not specified so there is

no way of determining if this occurred at the beginning or end of the month. This is slightly abbreviated from the European flight period (April – October) (Knutson & Vala 2011). Adult populations seem to reach peak density in Ireland around the third week in July, followed by a marked decrease and second smaller peak over the following three weeks. While previous accounts have described *T. elata* as multi- or bivoltine across its range (Knutson & Vala 2011; Speight & Knutson 2012), the compiled phenology reported here for Ireland seems to indicate only a single generation (univoltine). The secondary population peak in August could be explained by a second generation but the time from oviposition to eclosion is estimated as 53 days at its fastest, with the potential to take over 75 days, under optimised laboratory rearing conditions (Knutson et al. 1965; Hynes et al. 2014c). It is possible that a female eclosing in early June could produce offspring that develop to adult stage by late August, however first-hand records from the Applied Ecology Unit (NUI Galway) have no accounts for *T. elata* before 30 June. This also assumes that larvae experience no time delay in finding a neonate host, and does not take into account mating and pre-oviposition time for eclosing females.

5.2. Additional Avenues of Research

A population genetic study may be of interest comparing individuals from different populations throughout Ireland, and even across their entire native distribution. Such a study could be accomplished using common mitochondrial segments such as COI isolated by standard arthropod primers. Results of Irish population comparisons would provide insight into the isolation of populations from one another, and if populations are isolated, could give estimates of allelic frequency differences and inbreeding measures. Evaluation of the genetic diversity of *T. elata* populations would allow for examination of the evolutionary history and biogeography of the species throughout its distribution, expanding the knowledge base of this under-studied species. It would also ensure that cultures reared for use in biological control programmes will have sufficient levels of genetic diversity to prevent genetic bottlenecks or inbreeding depression, and maintain functional populations when released in the field.

Investigations into the various community interactions of *T. elata* may reveal valuable information directly applicable to a developing biological control programme. The effects of multiple natural enemies targeting the same pest species have the potential to work synergistically, enhancing overall pest control, or antagonistically and in competition with one another resulting in less efficient pest control (Thies et al. 2011). A recent study by D'Ahmed *et al.* (2019) demonstrated the first instance of interaction between *T. elata* and the parasitic nematode *Phasmarhabditis hermaphrodita*, both natural enemies of *D. reticulatum* and other pestiferous terrestrial slugs. While larvae preferentially targeted nematode-infected slugs for prey, there also appeared to be a negative effect on larval survivorship and fitness. Because *P. hermaphrodita* is a widely-used slug control treatment, a more detailed evaluation of the interactions between *T. elata and P. hermaphrodita* should be undertaken before implementing new biological control programmes. Ideally, this would entail both trials to study the effect of *P. hermaphrodita* on *T. elata* larval development when infected and uninfected prey are both provided *ad libitum*, as well as field-scale experiments to study localisation effects and spatial resource partitioning between the two natural enemies. Additional interactions to be explored are the relationships between *T. elata* larvae and generalist predators (e.g., carabids) or parasitoids. Some Sciomyzidae are known to be hosts of various hymenopteran parasitoids, including *Tetanocera ferruginea* Fallén in North America (Knutson & Vala 2011). While there are no records of such natural enemies for *Tetanocera* spp. in Europe, the parasitisation of such a closely-related species warrants further investigation for *T. elata*. As most parasitoids target sciomyzid eggs (as per numerous studies reviewed by Knutson & Vala [2011]), this should be the focus of any survey for *T. elata* parasitoids. This can be easily accomplished with the use of sentinel egg cards deployed in various habitats where *T. elata* occur, as well as within agroecosystems where there is interest for introduction.

Finally, a life table study of pestiferous slugs could maximise the efficacy of future biological control programmes. By releasing and monitoring a pre-determined cohort of slug eggs, mortality factors can be identified at each life stage. This could include natural parasitism rates of *T. elata*, predation/parasitism by other natural enemies, and mortality incurred by other control methods. If such factors are identified, they may be intentionally manipulated for improved slug control.

5.3. Feasibility of *Tetanocera elata* as a Biological Control Agent

Sciomyzidae have long been recognised as having many favourable attributes of effective biological control agents, and the studies presented in this thesis seem to support the same for *T. elata*. Populations of *D. reticulatum* can form aggregates of up to approximately 150/m^2 in arable fields in Ireland (Glen et al. 2006). Female *T. elata* can lay 200 – 400 eggs (Hynes et al. 2014b; D'Ahmed et al. 2019); assuming a maximum 52% hatch rate, as observed by Hynes *et al.* (2014b), this alone provides 156 neonates to potentially parasitize slug hosts. Based on further observations of larval survivorship by Hynes *et al.* (2014c), it may be extrapolated that 107 of this cohort would survive through the parasitoid phase, with a total of 95 individuals surviving as third instar larvae. In this predatory phase, larvae have the capacity to predate 9 – 12 slugs (Knutson & Vala 2011). With a conservative estimate of 10 slugs killed per larva, this amounts to 950 slugs killed. Including those slugs killed by parasitoid larvae, the total yield of biological control by a single *T. elata* female may be estimated to be over 1,000 slugs.

While it cannot be said with certainty whether predaceous *T. elata* larvae are generalists as of yet, results of prey choice and physiology trials seem to indicate that they retain some optimisation for predation on *D. reticulatum* while also having the ability to utilise some alternative slug prey species without immediate deleterious effects. If a biological control agent is somewhat plastic in its prey utilisation, this may enhance its survivorship and contribute to a more stable, sustainable population. Once a biological control agent is released at a site, prey populations should be expected to decrease. If this decrease is severe, biological control agents may experience a shortage of acceptable hosts/prey; in this manner, the ability to utilise alternative hosts or prey may be highly beneficial for the biological control agent, as well as having the additional benefit of controlling a secondary or tertiary pest species (Chen et al. 2018). In addition to host specificity, effective biological control agents should have a synchronous biology with the target pest(s) and demonstrate high reproductive potential and efficient prey-finding behaviours (Murdoch et al. 1985; Mair and Port 2001). Previous studies have observed *Tetanocera* species, including *T. elata*, to be highly fecund (with females able to produce over 400 eggs) (Knutson et al. 1965; D'Ahmed et al. 2019). Neonate larvae are largely ambush parasitoids which conserve energy by lying in wait for a host individual to come across them, then efficiently

attacking and parasitising the target (Knutson et al. 1965). There is some evidence of predaceous third instar larvae utilising a similar tactic as well as direct searching behaviour (Hynes et al. 2014a; D'Ahmed et al. 2019). This is likely an efficient host/prey-finding tactic, as it is believed female *T. elata* oviposit in or around substrate where *D. reticulatum* aggregate (Knutson et al. 1965).

While predators are typically able to utilise a wide array of food items, there is ample evidence that generalist insect predators regularly exhibit preferential prey choice (Gilbert 1990; Jackson and Rundle 2008; Noriyuki and Osawa 2012). However, other studies have suggested that within a prey range, predators do not necessarily use prey quality as the most important criterion for prey selection and that this relationship may actually be a more complex mixture of factors, including prey mobility, energetic requirement of predation, size, palatability, and others (Eubanks & Denno 2000). Additionally, some insect larvae experience a dietary expansion as development progresses which allows later larval instars to prey on a wider range of food than early instars (Scriber & Slansky 1981; Barton Browne 1995; Lundgren & Weber 2010). Based on evidence from feeding trials, however, *T. elata* larvae are unlikely to undergo a drastic shift in nutritional requirement as they develop from parasitoids into the predaceous third instar. Additionally, the first meal of larval Sciomyzidae influences subsequent prey choice (Dillon et al. 2014), which in this case would apply to parasitoid *T. elata* larvae feeding on a *D. reticulatum* host. While third instar *T. elata* larvae have been observed predating a variety of slug species including *Tandonia* and *Limax* species (Knutson et al. 1965; Barker et al. 2004; Speight & Knutson 2012), it is unclear whether these observations were made in the absence of alternative prey species, and whether larvae fed solely on these alternative prey species successfully completed development. It is possible that these observations were another example of larval utilisation of alternative prey in the absence of their preferred prey species. In order for *T. elata* to be considered a true generalist, it would be expected that larvae would perform equally well on any prey species consumed and experience little to no trade-offs between prey species (Levins 1968; Noriyuki & Osawa 2012). This was not the case, however, with significantly lower survivorship to partial pupariation observed for larvae reared on *T. budapestensis* than those on *D. reticulatum*. Therefore *T. elata* likely should continue to be considered oligophagous, feeding primarily on a limited range of prey species with some plasticity in predation

range. This lower suitability of alternative prey may reflect a restricted realised prey range (Noriyuki & Osawa 2012).

Based on survivorship observed in prey choice and physiology trials, larvae seem able to survive on alternative slug species for at least short periods, and the consumption of *A. hortensis* or *T. budapestensis* tissue at least does not appear immediately detrimental to larval physiology (see Section 5.1.2 for detailed discussion). Preference aside, it is possible that *T. elata* larvae could survive on or benefit from a mixed diet in nature, where both *D. reticulatum* and alternative prey are consumed. While not necessarily offering the full nutritional complement necessary for larvae to continue to develop and pupate, utilising alternative prey species would meet the larva's energetic requirements and prevent starvation, as has been observed in other insects (Evans et al. 1999), while they continue to search out essential prey. If abundance of the essential prey becomes restricted, the ability of larvae to predate other available species, until such time where *D. reticulatum* once again becomes available, would be a highly beneficial trait. Due to the inherent ephemeral nature of agroecosystems where *T. elata* could be employed for pest slug control (e.g., disturbance due to tilling, harvest, crop rotation, weed control, etc.), such demonstrated prey-switching ability in the absence of optimal prey is of great benefit (Levins 1968; Murdoch 1969; Noriyuki & Osawa 2012; Faria et al. 2014; Chen et al. 2018).

The question, then, becomes one of prey specificity and potential non-target effects on other species in and around the agroecosystem where *T. elata* may be established. While observations from the current study demonstrated the ability of *T. elata* larvae to attack and feed on slug species other than *D. reticulatum*, it could be argued that larvae possess a considerable preference for their neonate host species simply based on higher attack efficacy and lower energetic costs associated with feeding. It is likely that in agroecosystems where *D. reticulatum* is abundant and aggregated (Hunter 1966; Douglas & Tooker 2012) thereby increasing the likelihood of larval encounters with prey individuals, *T. elata* may feed on alternative slug species but at very low levels. Additionally, feeding on these other species which are also pestiferous will be beneficial if occurring at measurable levels. However, it is more likely that feeding by *T. elata* larvae would be associated with prey availability, and (as with all predator/prey and parasitoid/host systems) synchronicity between

consumer and prey is essential (Van Driesche & Bellows 2001). Luckily, agroecosystems regularly support dense populations of *D. reticulatum* (Hunter 1966; Douglas & Tooker 2012). The population dynamics of the three slug species examined in the current study are offset with one another and cyclical. *Deroceras reticulatum* typically has two generations per year, the first of which occurs around May in the UK and Ireland (Hunter 1966). In July and August, when neonate *T. elata* typically hatch (Speight & Knutson 2012), Hunter (1966) reported that 77% of *D. reticulatum* observed (averaged across years) were ≤ 100 mg. This smaller host size may be easier for neonate *T. elata* to parasitise. These slugs continue to develop and grow over the course of the summer months, and body size ratio of *D. reticulatum* in mid- to late-summer corresponds well for *T. elata* larval predation. In contrast, *A. hortensis* has only one generation per year (hatching in July) and *T. budapestensis* takes approximately 18 months to turn over a new generation (hatching between May and August) (Hunter 1966).

This means that *A. hortensis*, though fed on readily in trials, may not be synchronous enough to provide adequate prey for developing *T. elata* larvae, with individuals being either very small or twice the size of most *D. reticulatum*. *Tandonia budapestensis*, on the other hand, may show some favourable synchronicity for *T. elata* larvae, but its longer generation time means that there will likely be lower densities available for predation (Hunter 1966). Though seasonal weight observations by Hunter (1966) indicate that, during periods where *T. elata* larvae are active (July through September), both *D. reticulatum* and *T. budapestensis* of appropriate size may be available, larvae in feeding trials fed on *D. reticulatum* at higher numbers than *T. budapestensis*, though not significantly so. Higher aggregations of *D. reticulatum* could likely translate to this composing the bulk realised prey selection under field conditions.

When environmental conditions are favourable (e.g., temperate and humid), *D. reticulatum* develop and reproduce so rapidly they become dominant in terms of population density when compared with other co-occurring slug species (Hunter 1966, 1968). As this also corresponds with the flight and reproduction period of *T. elata* (Speight & Knutson 2012), the high availability of *D. reticulatum* makes them likely to be utilised primarily compared to other sympatric slug species and it appears unlikely that *T. elata* larvae would undergo a marked prey shift from second instar to

the predatory third instar stage. *Arion hortensis* can become dominant over winter months, when *D. reticulatum* numbers decline due to mortality incurred by frost and cold temperatures, but as *T. elata* is quiescent as a pupa during this time (Speight & Knutson 2012), this would have little bearing on larval feeding habits.

Also important for consideration is the ecology of potential prey species. *Deroceras reticulatum* has a vertical distribution typically within 8 – 10 cm from the soil surface (Moens & Glen 2002) in comparison to *A. hortensis* or *T. budapestensis* which generally exist deeper within soil strata (Hunter 1966). This proximity makes *D. reticulatum* more accessible to *T. elata* larvae which do not burrow deep within the soil but rather tend to hunt at the soil surface (Knutson et al. 1965; Knutson & Vala 2011). Within agroecosystems, *A. hortensis* and *T. budapestensis* are largely restricted to areas of disturbed or cultivated ground, such as cropping areas that are regularly tilled and planted. While *D. reticulatum* also occurs in these areas in abundance, it does not experience the same restrictions and can survive well in other natural and unmanaged areas (Van den Bruel & Moens 1958; Hunter 1966). This may be an added benefit for the utilisation of *T. elata* in conservation biological control. As crops are constantly being turned over and arable fields themselves are constantly in flux, any natural enemy would require the use of integrated refugia to sustain populations. Such refugia may come in the form of unmanaged field margins, hedgerows, wildflower strips, beetle banks, or adjacent land or fields left fallow. It is beneficial, therefore, to know that such refugia critical to sustaining natural enemy populations (e.g., *T. elata*) can also support the natural enemy's ideal prey. Because *A. hortensis* and *T. budapestensis* tend not to occur in these habitats at great densities (if at all) *T. elata* will largely be associated with their essential host/prey species which will benefit introduced populations. Predation on other slug species commonly occurring in grassland habitats (e.g. arionid, milacid, or limacid species) by *T. elata* larvae may occur and should be investigated, but it is anticipated that these results will likely mirror results in this and prior studies (Knutson et al. 1965).

The topic of most concern for any proposed biological control agent is the potential for non-target effects on species which are not pestiferous in the system to which the biological control agent is introduced (Van Driesche & Bellows 2001; Perrings et al. 2010; Hoddle 2016; Warner 2016). As discussed previously, because *T. elata* is a native natural enemy this risk is greatly reduced, but should not be altogether

discounted without consideration. The previously mentioned instance of a predaceous *T. elata* larva feeding on the EU protected *G. maculosus* (Giordani et al. 2014) occurred under laboratory conditions when no other prey was available and the larva had been starved. This may further indicate that *T. elata* is able to utilise alternative prey species, even those taxonomically distant from its co-evolved hosts when conditions are poor, not necessarily that they will utilise this species by choice in any natural setting. The risk of non-target predation is further reduced when considering that the distribution of *G. maculosus* is restricted to bog and woodland/forest habitats, habitats where *T. elata* are unlikely to occur in large numbers. Additionally, *T. elata* is known to demonstrate very high patch fidelity and is not a strong disperser, moving up to a maximum of perhaps 20 metres (Williams et al. 2009). There may be some cases where an agroecosystem borders or shares land with *G. maculosus* habitats, however *T. elata* is highly unlikely to disperse and threaten this protected species. Additionally, most blanket bogs in Ireland are located in the west, when the majority of arable agriculture is concentrated in the south and east.

Because any targets of a slug biological control programme would all be native or naturalised species, utilising native natural enemies would be the most favourable approach. Rather than purely augmentative or inundative control methods, which are ideal in glasshouses or polytunnel enclosures, a conservation biocontrol approach would seek to alter the ecological landscape in and around agriculture to promote populations of native natural enemies in open arable fields. It is possible to maximise the effect of conservation biological control by specifically engineering the ecosystem around the requirements of the natural enemy of interest (Van Driesche & Bellow 2001; Ramsden et al. 2015; Begg et al. 2017). *Tetanocera elata* does not occur commonly in crop fields, instead being predominantly distributed in wet and dry grasslands (Speight & Knutson 2012). Therefore, populations would need to be explicitly introduced onto farms after being collected and reared from neighbouring habitats in an initial augmentative release as a part of such a biocontrol programme. This species is known to be able to survive at low to moderate population densities in a variety of habitats (Williams et al. 2009; Knutson & Vala 2011; Carey et al. 2015) so the likelihood is good that they would be able to establish in ecologically-managed agroecosystems and provide pest control.

Habitat structure is an essential aspect to consider when designing ecological intensification for many natural enemies, and field observations confirm the same is also true for *T. elata*. Shelters, which can be comprised of anything from tussock-forming grasses in field margins to patches of semi-natural woodland, provide alternative habitats and resources to natural enemies. Live and dead vegetation build-up in concentrated areas has been shown to form microhabitats for small terrestrial arthropods, each possessing its own microclimate. Interior microclimate is widely affected by shelter, including the mediation of temperature swings, the maintenance of constant relative humidity, reduced light penetration, and protection from wind (Gontijo 2019). This could be an important feature, especially to developing *T. elata* larvae in the soil, receiving shelter from a closed vegetation structure. Proximity to hedgerows was also significantly correlated to *T. elata* abundance, indicating that these structures, too, are likely an essential component of an engineered *T. elata* habitat.

Hedgerows are widely accepted to be beneficial to biodiversity (Ramsden et al. 2015; Begg et al. 2017; Holland et al. 2016; Garratt et al. 2017; Gontijo 2019), and *T. elata* are yet another species that would benefit from their presence in agroecosystems. Garratt *et al*. (2017) found that the ideal implementation of hedgerows for ecological pest control was a continuous row composed of a diverse community of woody hedge and small tree species. Continuity of hedgerows, and their orientation so as not to impede the movement of natural enemies, is well supported (Ramsden et al. 2015; Tscharntke et al. 2016; Garratt et al. 2017); this is an especially important consideration for *T. elata*, which is not a strong disperser and would likely rely on hedgerow continuity for movement. Also because of this limited movement, proximity of the hedgerow to the crop itself is important. While no measurement of 'ideal' hedgerow distance exists (Tscharntke et al. 2016), the proximity preference of *T. elata* indicates that hedges or other shelters would likely have to be immediately adjacent to crops, or distributed within the field (i.e., intercropping). Floral and structural resources provided by a robust understorey below hedgerows have been shown to increase the abundance of natural enemies (Ramsden et al. 2015; Garratt et al. 2017), with hedgerows paired with high coverage grass margins the most effective (Garratt et al. 2017). *Tetanocera elata* would be well supported by a semi-natural habitat such as this, as thick grass tussocks would form shelters for larvae while hedgerows would

serve adults in the same way. Some consideration, however, must also be given to the potential for slugs to utilise these habitats. While it would not likely affect abundances of *A. hortensis* or *T. budapestensis*, as these species rely on tillage to break up soil to facilitate their colonisation, *D. reticulatum* is adept at occupying both agricultural and natural habitats (Hunter 1966). *Arion* spp. have not been found to benefit from semi-natural habitats, but *D. reticulatum* aggregations have been found at high densities in herbaceous field margins (Fusser et al. 2017). Semi-natural habitats for *T. elata* conservation, therefore, require careful and specific engineering so that they can meet the ecological needs of *T. elata* but do not exacerbate pest densities or damage incurred by *D. reticulatum*.

A final point of consideration for *T. elata* as a biological control agent is associated with the source of said natural enemy for introduction into engineered ecosystems. Laboratory rearing of *T. elata* remains labour-intensive and low-yield. Until more efficient rearing techniques can be developed (see Section 5.1.3 for recommendations), this remains a potential limitation. Additionally, originators must be sourced for every laboratory strain, most effectively from local naturally-occurring populations. This presents somewhat of an ethical problem, as to whether mass harvesting of populations is detrimental to natural ecosystems and if the value of that impact is overshadowed by the benefit gained through pest slug suppression (Michaud 2018). High genetic diversity needs to be maintained to prevent inbreeding depression and maximise the fitness of laboratory-reared insects (Coelho et al. 2016; Stouthamer 2017), which would likely require collections from multiple populations of *T. elata* across Ireland. While this may not be problematic for such natural enemies as hymenopteran parasitoids that occur at very high density, *T. elata* have most frequently been encountered at frequencies of fewer than 10 individuals per active sampling event. Any reduction in population, therefore, may be highly destructive to local population persistence. A careful balance will need to be calculated with regard to capture, rear, and release in a biological control context.

5.4. Increasing Biodiversity, Increasing Pest Control

The current production of global agriculture is not sufficient to feed the ever-growing human population (United Nations 2019) and as the population continues to expand, so too does the amount of Earth's terrestrial area dedicated to food production. Current estimates state that almost 12% of area on land is currently dedicated to arable farming and increasing, while agriculture as a whole comprises 38% (Foley et al. 2011; Tscharntke et al. 2016). This rapidly increasing need for greater amounts of crop output makes it easy to justify the use of high-intensity agriculture, vast swathes of monocropped fields dedicated solely to food production. To ensure the greatest possible yield, these crops are regularly sprayed with pesticides before any damage can be done, and the margins kept bare to ward off pests and disease (or taken over entirely to crop production). It is easy to believe that all of these modern advances in chemistry and engineering maximise the food we get out of each field, but the evidence is mounting that this is hardly the case. Intensively managed fields actually suffer reductions in yield amount and quality compared to farms that let their field margins grow (Tscharntke et al. 2005; Nicholls & Altieri 2007; Jonsson et al. 2015). Aside from being financially and ecologically unsustainable (Foley et al. 2011; Michaud 2018), intensive agriculture is also less efficient (Thies et al. 2011). To truly maximise food production, we need to start working with nature instead of against it.

As early as 1995, the loss of biodiversity incurred by agricultural intensification and expansion had been acknowledged (Stanners & Bordeau 1995). Across Europe, heavy use of fertilisers and chemical pesticides have degraded environments within cropping fields, and the destruction of grassland meadows, hedgerows, and woodland to accommodate intensive tillage practices have resulted in a severe loss of biodiversity and acute simplification of landscape complexity (Tscharntke et al. 2005; Chaplin-Kramer et al. 2011; Thies et al. 2011; Jonsson et al. 2015). One landmark publication by Stern *et al.* (1959) who reviewed various studies which were conducted as early as Wigglesworth (1945) recognised issues arising from contemporary agricultural practices. These included unsustainable use of pesticides, and warnings of the development of resistance to pesticides by arthropod pests, growing intensity of secondary outbreaks, and harm caused to humans, animals, and the surrounding environment. Sixty years later, we can affirm the voracity of these sentiments. If we

want to ensure continued food security to meet global demand, sustainable management strategies are the best and perhaps only option.

Rather than stripping nature away to give greater area to intensive crop fields, the practice of ecological intensification uses the inherent assets of a functioning ecosystem for the benefit of agriculture (Thies et al. 2011; Bommarco et al. 2013; Pywell et al. 2015). Specifically of use here is the concept of utilising in-built ecological interactions for crop pest control, commonly referred to as 'ecological pest control', and linked with conservation biological control (Van Driesche & Bellows 2001). There are many approaches to the ultimate goal of pest control, but all take advantage of existing natural enemies (predators and parasitoids, generalists and specialists alike) by ensuring habitats around and within crop fields provide resources to sustain functioning populations of these natural enemies (Nicholls & Altieri 2007; Jonsson et al. 2015). As an added benefit, ecological intensification practices can increase both biodiversity and crop yield, improve water quality, provide carbon sequestration, and improve the aesthetic or recreational value of farmland (Landis et al. 2000; Pywell et al. 2015). Numerous studies have demonstrated the benefits of semi-natural habitat presence in agroecosystems, with many citing significant decreases in pest density or damage as a direct result of increased density and richness of natural enemy species (reviews by Thies et al. 2011; Pywell et al. 2015). Generally, conservation biological control is accomplished through such methods as limiting the frequency and area of pesticide application, reducing the disturbance of natural enemy communities, and offering resources such as food and shelter outside of cropping fields (Landis et al. 2000; Nicholls & Altieri 2007; Crowder & Jabbour 2014; Gontijo 2019).

This is not to say, however, that conservation biological control efforts are always successful. The results of conservation biological control in any given system can be unpredictable and it can be difficult to relate causal agroecological conservation efforts to pest control effects (Gontijo 2019), owing in no small part to variation in climate and region in which such management is applied (Thies et al. 2011; Crowder & Jabbour 2014; Tscharntke et al. 2016; Gontijo 2019). Additionally, the benefits of ecological intensification can be slow to develop (Pywell et al. 2015). Tscharntke *et al.* (2016) discussed a number of potential shortcomings of ecological pest management, including the capacity for semi-natural habitats in agroecosystems to

harbour greater levels of pests and plant pathogens than their corresponding natural enemies (essentially introducing additional pests into crops), or the inability of some semi-natural habitats to support adequate densities of natural enemy populations. Substantial investment may be made by farmers to construct hedgerows for their perceived benefit to natural enemy conservation, only to find that their orientation or design in fact limits the ability of natural enemies to disperse and access pests in crop fields (Gontijo 2019). In addition, conservation biological control efforts may not be sustainable if they must be supported by constant ongoing augmentative releases of natural enemies (Michaud 2018).

Ultimately these limitations can be distilled into a single fundamental principle: to employ ecological intensification and conservation biological control successfully, there must first be a deep understanding of the system to which it is being applied, along with all the underlying ecological functions therein. Understanding the ecology and phenology of specific natural enemies of target pests can ensure semi-natural habitats are properly oriented and in effective proximity to crops, and understanding of the enemy's spatial orientation should guarantee its overwintering habitat requirements are met (Nicholls & Altieri 2007; Ramsden et al. 2015; Tscharntke et al. 2016; Gontijo 2019). Knowledge of alternative host plant utilisation by pest species (such as *D. reticulatum* maintaining higher populations in field margins dominated by herbaceous plants compared to woody margins [Fusser et al. 2017]) can limit the inclusion in semi-natural habitats of species favourable to pests (Tscharntke et al. 2016). If the dispersal capacity of natural enemies (e.g., that specialist parasitoids tend to be spatially limited while generalist predators have the capacity to work at landscape level) is considered during the design of semi-natural habitats, hedgerows, beetle banks, and other such shelters can be constructed in a proximity and with connectivity that allows for effective pest control (Nicholls & Altieri 2007; Thies et al. 2011; Jonsson et al. 2015; Ramsden et al. 2015; Garratt et al. 2017). Finally, it should be acknowledged that management of landscapes surrounding conserved semi-natural habitats are likely to impact natural enemy conservation efforts. Intensive agricultural practices of frequent tillage, pesticide application, and the use of susceptible crop strains can mediate the beneficial effects and numbers of natural enemies in conserved areas (Jonsson et al. 2015; Tscharntke et al. 2016), and conservation biological control

of many serious pest species is rendered ineffective by monocropping and the ephemeralness of annual crop cycles, both of which favour pests (Michaud 2018).

Conservation biological control through ecological intensification of agricultural landscapes can be effective if employed intelligently (Van Driesche & Bellows 2001; Chaplin-Kramer et al. 2011; Crowder & Jabbour 2014). Indeed, Pywell *et al.* (2015) demonstrated that the removal of up to 8% of 'productive' land for the formation of semi-natural habitats showed no net decrease in crop yield in sites across Europe. Maintenance of semi-natural habitats and increases in biodiversity can have numerous unintended benefits as well (Pywell et al. 2015), including increases in the biodiversity of taxa other than, and in addition to, the intended natural enemy (Altieri & Nicholls 2007). The positive effects of naturally-occurring enemies resulting from conservation biological control coupled with integrated pest management schemes have been reviewed by Ramsden *et al.* (2015), and Tscharntke has a long history of documenting agricultural benefits as a result of ecological management (Tscharntke & Greiler 1995; Thies & Tscharntke 1999; Tscharntke et al. 2005; Tscharntke et al. 2007; Tscharntke et al. 2012).

Despite overwhelming evidence that biological control approaches are effective and generally more environmentally-friendly than conventional agricultural practices, public interest in such programmes are decreasing (Brodeur et al. 2018). Some farmers still consider land set aside to regenerate semi-natural habitats – and, in turn, increased natural biological control – as a waste of money, land, or both, as well as impeding the use of modern technology (e.g., tractor and machinery access) (Tscharntke et al. 2016). However, the proportion of academic studies devoted to biological control has remained relatively steady over the past 25 years, confirming the importance and use of the practice in a variety of systems and for the achievement of a multitude of various goals (Brodeur et al. 2018).

The failing, then, seems to rest with biological control researchers and practitioners. While high-quality science with a robust number of replicates will always be necessary, we perhaps do not often acknowledge the importance of the public to our research. Public engagement and outreach is essential for communicating important findings not only to farmers who may potentially implement these strategies, but also for the general public to raise the opinion and perceived value of

biological control. As unfortunate as it often is, public opinion drives politics, and with the majority of biological control studies and applications funded by governments or associated organisations (Brodeur et al. 2018), public opinion may ultimately dictate the amount of continued research in the field. If biological and ecological pest control are valued more highly by the public, it is likely that the demand for crops grown in such a manner will increase and with it, the number of farmers willing to apply these methods to meet that demand.

No good science can exist in a vacuum, and biological and ecological pest control are no exception. It is, after all, for the good of humanity that we endeavour to improve agricultural output and sustainability.

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APPENDICES

APPENDIX I Supplemental Information for Chapter 2

Appendix I.1

Representative botanic species comprising marsh fly habitats, with notes on nectar availability. Prevalence is represented across all marsh fly collection sites (see Supplemental Table S2) and is estimated as overall biomass abundance across sites. Nectar availability descriptions are taken from personal observations at field sites (ABE) or adapted from Pellmyr 2002 and Hicks *et al.* 2016.

Botanic species		**Prevalence at sites (DAFOR scale)**	**Nectar availability**
Common name	**Latin name**		
Graminoids			
Cock's foot	*Dactylis glomerata* L.	Dominant	Little/no nectar
Soft rush	*Juncus effusus* L.	Dominant to occasional	Little/no nectar
Sweet vernal grass	*Anthoxanthum odoratum* L.	Frequent	Little/no nectar
Fescue	*Festuca* spp.	Occasional	Little/no nectar
Yorkshire fog	*Holcus lanatus* L.	Occasional	Little/no nectar
Forbs			
Buttercup	*Ranunculus* spp.	Frequent	Some nectar
Common field speedwell	*Veronica persica* Poir	Rare	Little nectar
Thistle	*Cirsium* spp.	Rare	Considerable nectar, but not easily accessed
Common knapweed	*Centaurea nigra* L.	Frequent	Considerable nectar, but not easily accessed
Meadow vetchling	*Lathyrus pratensis* L.	Occasional	Some nectar
Bush vetch	*Vicia sepium* L.	Occasional	Some nectar
Silverweed	*Potentilla anserina* (L.) Rydb.	Occasional	Little nectar
Bird's foot trefoil	*Lotus corniculatus* L.	Rare	Little nectar
Lady's bedstraw	*Galium verum* L.	Rare	Little nectar
Orchids	Orchidaceae	Rare	Non-rewarding nectar
Hedgerow plants			
Bramble	*Rubus fruticosus* L.	Dominant to abundant	Some nectar
Hawthorn	*Crataegus monogyna* Jacq.	Abundant to Occasional	Little nectar
Blackthorn	*Prunus spinosa* L.	Abundant to Occasional	Little nectar

Appendix I.2

Sciomyzidae collection site data. Habitat classifications made using Fossitt 2000.

Site No.	Site Name	Location (GPS)	Primary Habitat Classification
1	Menlo	53.301150, -9.076884	Wet grassland (GS4)
2	Green Earth Organic Farm	53.393769, -8.977851	Dry meadow (GS2); formerly cultivated land (BC1 or BC2) that has been unmanaged and recolonized
3	Cow Park	53.229550, -8.873727	Dry meadow (GS2)

Appendix I.3

Results of screening trial of glucose concentration preference in marsh flies, *Ilione lineata*, *Tetanocera arrogans*, and *Pherbina coryleti*. These data were used to determine the concentration of glucose solution used in general cafeteria trials.

Glucose concentration	No. Feeding Events	No. Individuals Feeding
0%	0	0
5%	0	0
10%	6	3[†]
25%	4	1
50%	5	1

[†]The greater number of individual flies feeding at 10% glucose concentration was determined to be more useful than the number of feeding events alone in determining the most desirable concentration to use in general cafeteria trials.

Appendix I.4

Choice observations of individual Sciomyzidae in 57 general cafeteria trials, 39 of which had at least one feeding event (shown below).

Species	No. times feeding occurred				
	Slug	**Snail**	**Honey-Yeast**	**Glucose Solution**	**Water**
Ilione lineata	0	0	0	1	0
Ilione lineata	1	0	0	0	0
Ilione albiseta	0	0	0	0	1
Pherbina coryleti	1	0	0	0	1
Pherbina coryleti	4	0	0	0	0
Pherbina coryleti	6	0	0	0	0
Pherbina coryleti	1	0	0	0	0
Pherbina coryleti	0	1	2	0	1
Tetanocera arrogans	1	0	0	0	0
Tetanocera arrogans	0	0	0	2	0
Tetanocera arrogans	0	0	1	0	0
Tetanocera arrogans	0	0	0	3	0
Tetanocera arrogans	1	0	0	1	0
Tetanocera arrogans	0	0	0	0	2
Tetanocera arrogans	0	0	0	0	1
Tetanocera arrogans	0	0	0	0	1
Tetanocera arrogans	0	0	0	1	0
Tetanocera arrogans	1	0	0	0	0
Tetanocera arrogans	0	0	0	2	0
Tetanocera arrogans	6	0	0	0	0
Tetanocera elata	0	0	2	0	0
Tetanocera elata	0	0	0	1	0
Tetanocera elata	0	0	4	0	0
Tetanocera elata	0	0	1	7	0
Tetanocera elata	0	0	0	4	0
Tetanocera elata	0	0	2	0	0
Tetanocera elata	0	0	0	0	1
Tetanocera elata	0	0	0	4	0
Tetanocera elata	0	0	0	4	0
Tetanocera elata	0	0	0	6	0
Tetanocera elata	0	1	0	4	0
Tetanocera elata	0	0	6	0	0
Tetanocera elata	0	6	1	0	0
Tetanocera elata	0	0	5	0	2
Tetanocera elata	0	0	4	0	0
Tetanocera elata	0	0	0	6	0
Tetanocera elata	0	0	2	0	0
Tetanocera elata	0	0	5	4	0
Tetanocera elata	0	0	0	0	1
Total feeding events:	22	8	35	50	11

Appendix I.5

Choice observations of individual Sciomyzidae in 59 honeydew cafeteria trials, 52 of which had at least one feeding event (shown below).

Species	No. times feeding occurred		
	Dry Honeydew	Aqueous Honeydew	Water
Ilione lineata	6	1	0
Ilione lineata	1	0	0
Ilione lineata	1	1	0
Ilione lineata	1	0	0
Ilione lineata	1	2	0
Ilione lineata	0	0	1
Ilione lineata	1	2	1
Ilione lineata	0	3	0
Ilione lineata	1	2	0
Ilione lineata	1	2	0
Ilione lineata	0	3	2
Ilione lineata	0	1	1
Tetanocera elata	4	3	0
Tetanocera elata	1	0	0
Tetanocera elata	3	0	0
Tetanocera elata	2	0	0
Tetanocera elata	0	4	0
Tetanocera elata	3	1	0
Tetanocera elata	1	1	0
Tetanocera elata	0	1	0
Tetanocera elata	2	0	0
Tetanocera elata	0	1	0
Tetanocera elata	3	1	0
Tetanocera elata	1	0	1
Tetanocera elata	1	0	0
Tetanocera elata	1	4	0
Tetanocera elata	2	0	0
Tetanocera elata	1	0	0
Tetanocera elata	0	0	4
Tetanocera elata	3	1	0
Tetanocera elata	0	1	0
Tetanocera elata	1	0	0
Tetanocera elata	1	2	0
Tetanocera elata	1	2	0
Tetanocera elata	0	6	0
Tetanocera elata	2	0	0
Tetanocera elata	0	0	3
Tetanocera elata	1	1	0
Tetanocera elata	1	0	0
Tetanocera elata	0	2	0
Tetanocera elata	2	2	0
Tetanocera elata	0	0	1
Tetanocera elata	0	7	0
Tetanocera elata	1	4	0
Tetanocera elata	1	0	1
Tetanocera elata	2	0	0
Tetanocera elata	1	0	0
Tetanocera elata	0	1	1
Tetanocera elata	0	1	0
Tetanocera elata	2	0	1
Tetanocera elata	1	0	0
Tetanocera elata	0	0	1
Total feeding events:	58	63	18

Appendix I.6 References

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APPENDIX II Supplemental Information for Chapter 3

Appendix II.1

Locations and description of field sites where *Tetanocera elata* 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♀

Appendix II.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	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

Appendix II.3

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

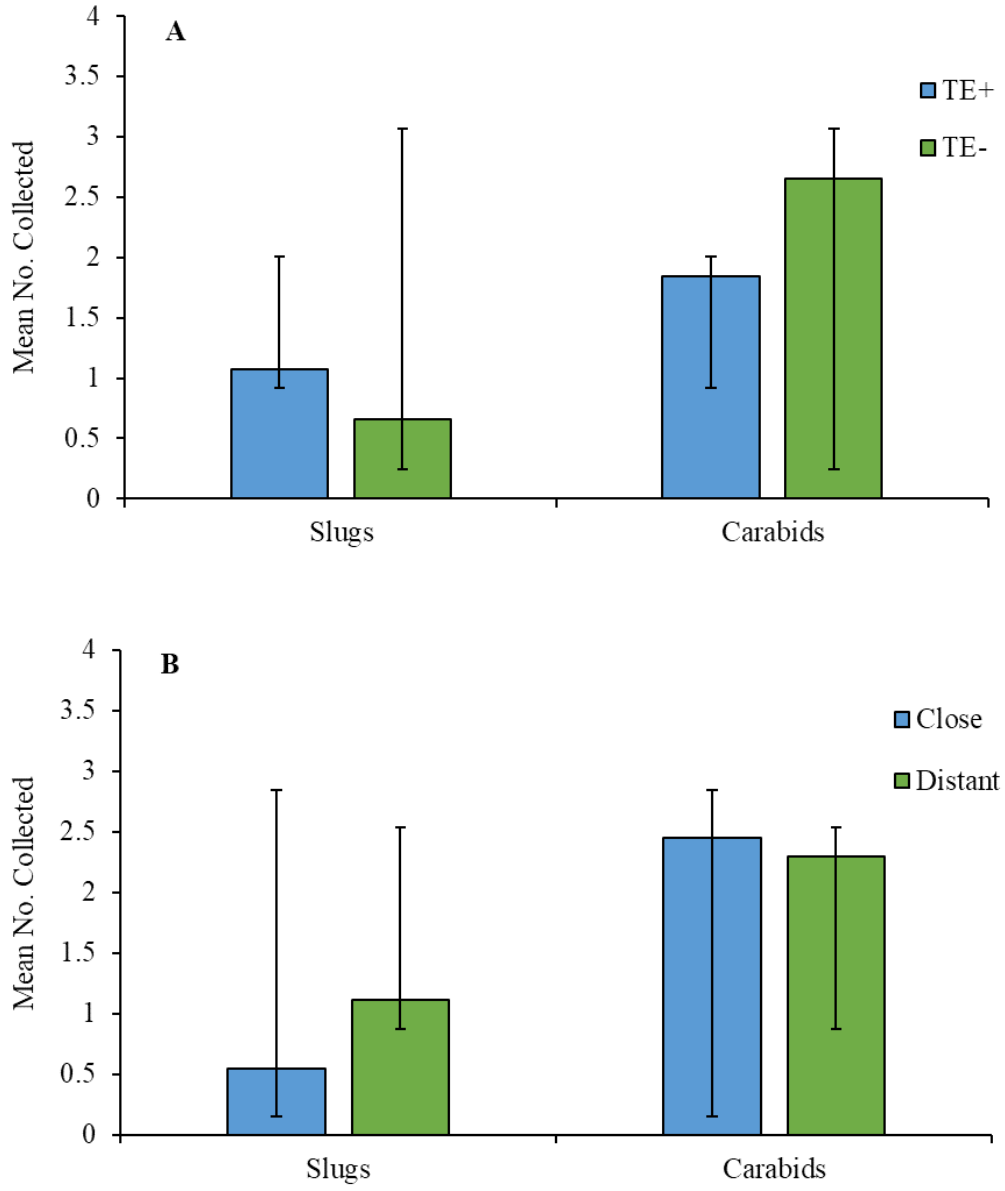
		No pupariation			**Partial pupariation**			**Full pupariation**	
		DR	**AH**	**TB**	**DR**	**AH**	**TB**	**DR**	**AH**
No pupariation	*A. hortensis*	0.2183							
	T. budapestensis	0.3977	0.3020						
Partial pupariation	*D. reticulatum*	0.3977	0.1489	0.3020					
	A. hortensis	0.1217	0.3487	0.1821	0.0769				
	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.

APPENDIX III Supplemental Information for Chapter 4

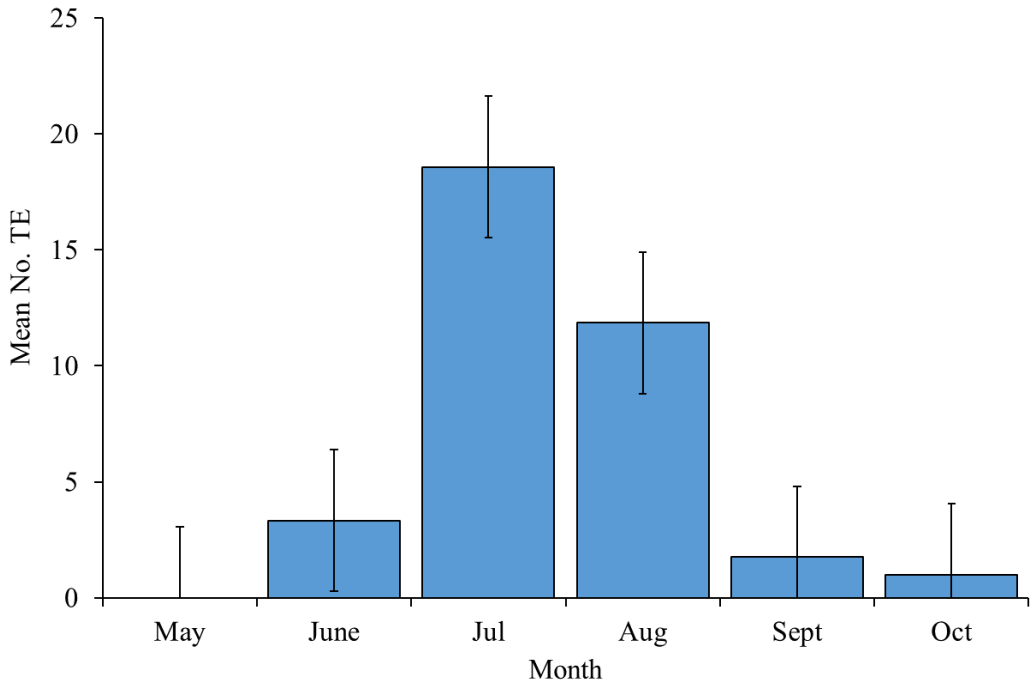
Appendix III.1

Mean collection numbers of slugs and carabids from pitfall traps. Numbers are grouped by (**A**) *Tetanocera elata* presence/absence and (**B**) proximity to hedgerow. The single *D. reticulatum* captured in pitfall traps was collected from a near/unexpected plot where *T. elata* was absent. No significant differences were detected for slugs or carabids when compared based on *T. elata* presence or hedgerow proximity.



Appendix III.2

Average number of *Tetanocera elata* recovered per recorder per month in Ireland.



Appendix III.3

Plant species recorded at Cow Park site scale and mean percentage cover per plot category for the summer flight period (June – August) and over 12 months. Mean abundance per species (*n* = 48 each *a priori* plot category, *n* = 43 TE), per plot category is indicated by DAFOR for summer flight period only (5 = Dominant, 4 = Abundant, 3 = Frequent, 2 = Occasional, 1 = Rare, 0 = Singleton/no record). Frequency reflects the proportion of all observation plots (*n* = 39) in which each species was observed at least once.

Species	Mean Cover (%) ± SE										Median Abundance (DAFOR)					Frequency (%)
	12 mo.					Flight Period										
	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	
Dead vegetation	50.6 ± 5.4	30.2 ± 1.0	28.7 ± 1.0	28.6 ± 1.5	41.5 ± 2.0	48.1 ± 10.8	16.0 ± 1.2	14.8 ± 1.0	13.3 ± 1.9	16.0 ± 2.3	5	4	5	4	5	100
Poa pratensis	20.3 ± 4.6	16.6 ± 1.4	12.4 ± 1.5	16.2 ± 1.6	13.1 ± 1.5	24.1 ± 7.7	14.3 ± 1.4	9.4 ± 1.5	14.2 ± 1.3	10.6 ± 1.6	2	3	3	4	3	100
Dactylis glomerata	44.8 ± 4.5	19.0 ± 2.2	39.8 ± 3.0	14.2 ± 1.4	25.6 ± 2.0	59.0 ± 8.6	23.0 ± 3.2	51.2 ± 2.3	17.3 ± 2.2	35.8 ± 3.5	5	4	5	3	4	97
Festuca pratensis	19.6 ± 3.5	9.2 ± 1.4	9.8 ± 2.2	7.0 ± 1.3	10.4 ± 1.6	32.3 ± 6.0	11.9 ± 2.3	12.8 ± 3.4	7.1 ± 1.5	17.2 ± 2.5	3	2	0	0	4	72

Species	Mean Cover (%) ± SE										Median Abundance (DAFOR)					Frequency (%)
	12 mo.					Flight Period										
	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	
Centaurea nigra	8.7 ± 3.1	6.5 ± 1.6	2.6 ± 0.7	7.9 ± 1.9	3.5 ± 1.1	13.8 ± 4.8	9.9 ± 2.4	4.0 ± 1.2	12.9 ± 3.1	7.4 ± 1.9	0	1	0	2	0	54
Plantago lanceolata	0.6 ± 0.2	1.6 ± 0.4	0.4 ± 0.1	1.2 ± 0.3	1.5 ± 0.3	0.8 ± 0.3	2.8 ± 0.7	0.4 ± 0.1	1.8 ± 0.6	2.2 ± 0.6	0	0	0	0	0	36
Agrostis stolonifera	4.1 ± 1.6	1.7 ± 0.5	0.2 ± 0.1	2.9 ± 0.8	0.3 ± 0.2	5.5 ± 2.1	2.1 ± 0.6	0.2 ± 0.1	3.6 ± 1.1	0.4 ± 0.2	0	0	0	0	0	31
Anemone ranunculoides	3.4 ± 1.5	1.8 ± 0.8	0.3 ± 0.1	1.1 ± 0.5	-	4.4 ± 2.0	2.8 ± 1.3	0.5 ± 0.1	1.8 ± 0.8	-	0	0	0	0	0	15
Anthoxanthum odoratum	1.4 ± 0.4	2.6 ± 0.4	2.0 ± 0.2	5.0 ± 0.6	1.4 ± 0.4	1.4 ± 0.4	1.3 ± 0.3	0.9 ± 0.2	3.2 ± 0.4	0.3 ± 0.1	0	0	0	1	0	64
Arrhenatherum elatius	1.1 ± 0.5	2.8 ± 0.9	-	1.5 ± 0.7	0.5 ± 0.3	1.8 ± 0.8	4.7 ± 1.4	-	2.7 ± 1.2	1.2 ± 0.7	0	0	0	0	0	15
Cerastium arvense	-	-	-	0.4 ± 0.2	-	-	-	-	0.6 ± 0.3	-	0	0	0	0	0	3

Species	Mean Cover (%) ± SE										Median Abundance (DAFOR)					Frequency (%)
	12 mo.					Flight Period										
	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	
Cirsium vulgare	0.8 ± 0.4	0.1 ± 0	-	-	-	1.6 ± 0.7	0.1 ± 0	-	-	-	0	0	0	0	0	10
Cynosurus cristatus	-	-	-	0.1 ± 0	-	-	0.1 ± 0	-	0.1 ± 0	-	0	0	0	0	0	8
Cyperaceae	-	-	-	0.3 ± 0.1	-	-	-	-	0.6 ± 0.3	-	0	0	0	0	0	5
Dactylorhiza fuchsii	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	3
Festuca arundinacea	-	-	7.3 ± 3.0	-	-	-	-	2.0 ± 0.6	-	-	0	0	0	0	0	5
Ficaria verna	0.1 ± 0	0.5 ± 0.2	-	0.1 ± 0.1	-	0.1 ± 0	-	-	-	0.1 ± 0	0	0	0	0	0	13
Filipendula ulmaria	-	-	-	3.1 ± 1.4	-	-	-	-	4.8 ± 2.1	-	0	0	0	0	0	8

Species	Mean Cover (%) ± SE										Median Abundance (DAFOR)					Frequency (%)
	12 mo.					Flight Period										
	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	
Galium verum	1.5 ± 0.4	1.2 ± 0.4	1.6 ± 0.4	0.2 ± 0.1	0.7 ± 0.3	2.2 ± 0.6	1.8 ± 0.6	2.1 ± 0.5	-	1.1 ± 0.4	0	0	0	0	0	33
Hedera helix	0.1 ± 0	0.5 ± 0.2	-	-	-	-	0.5 ± 0.2	-	-	-	0	0	0	0	0	5
Holcus lanatus	3.4 ± 3.1	2.6 ± 0.5	0.7 ± 0.2	4.0 ± 1.1	2.2 ± 0.6	4.3 ± 1.9	1.4 ± 0.4	-	4.3 ± 1.3	0.7 ± 0.3	0	0	0	0	0	56
Juncus effuscus	-	1.8 ± 0.8	-	9.4 ± 2.9	-	-	1.6 ± 0.7	-	8.3 ± 2.4	-	0	0	0	0	0	8
Lathyrus pratensis	0.9 ± 0.2	2.1 ± 0.5	5.7 ± 1.2	5.0 ± 1.0	1.8 ± 0.4	1.3 ± 0.2	3.4 ± 0.8	9.1 ± 1.9	6.9 ± 1.8	4.0 ± 1.2	0	0	2	0	0	59
Lichen/moss	-	0.1 ± 0	-	-	-	-	-	-	-	-	0	0	0	0	0	5
Lotus corniculatus	2.2 ± 0.6	3.5 ± 1.3	0.5 ± 0.2	0.9 ± 0.4	1.6 ± 0.4	3.5 ± 1.0	5.5 ± 2.1	0.8 ± 0.4	1.4 ± 0.6	3.2 ± 0.8	0	0	0	0	0	38

Species	Mean Cover (%) ± SE										Median Abundance (DAFOR)					Frequency (%)
	12 mo.					Flight Period										
	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	
Phleum pratense	0.2 ± 0.1	-	-	-	0.1 ± 0.1	0.3 ± 0.1	-	-	-	0.2 ± 0.1	0	0	0	0	0	3
Poa trivialis	-	0.5 ± 0.2	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	-	0.8 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	1.2 ± 0.5	0	0	0	0	0	18
Potentilla anserina	-	-	-	-	-	-	-	-	-	0.2 ± 0.1	0	0	0	0	0	3
Pteridium aquilinum	0.2 ± 0.1	1.5 ± 0.7	-	-	0.2 ± 0.1	0.3 ± 0.1	2.5 ± 1.2	-	-	0.8 ± 0.4	0	0	0	0	0	8
Ranunculus acris	-	0.5 ± 0.2	-	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0	0.6 ± 0.2	-	0.2 ± 0.1	0.4 ± 0.2	0	0	0	0	0	15
Rubus fruticosus	4.2 ± 1.2	2.5 ± 0.8	-	-	0.6 ± 0.2	4.2 ± 1.3	1.2 ± 0.4	-	-	1.2 ± 0.7	0	0	0	0	0	23
Rumex acetosa	-	-	0.2 ± 0.1	2.9 ± 0.7	0.2 ± 0.1	0.1 ± 0	-	0.3 ± 0.1	3.8 ± 1.0	-	0	0	0	0	0	18

Species	Mean Cover (%) ± SE										Median Abundance (DAFOR)					Frequency (%)
	12 mo.					Flight Period										
	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	E/N	U/N	E/D	U/D	TE	
Rumex crispus	-	-	-	-	0.1 ± 0	-	-	-	-	-	0	0	0	0	0	5
Taraxacum spp.	0.3 ± 0.1	-	-	0.2 ± 0.1	0.2 ± 0.1	-	-	-	0.3 ± 0.1	-	0	0	0	0	0	15
Veronica persica	0.4 ± 0.2	0.7 ± 0.2	-	-	0.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	-	-	-	0	0	0	0	0	13
Vicia sepium	2.7 ± 1.1	-	0.4 ± 0.1	-	0.3 ± 0.1	3.2 ± 1.2	-	0.6 ± 0.2	-	0.5 ± 0.2	0	0	0	0	0	15

Plot category abbreviations: E/N = expected/near; U/N = unexpected/near; E/D = expected/distant; U/D = unexpected/distant; TE = plot where *T. elata* was recovered

Appendix III.4

Results of comparisons made in the percentage cover between plot categories in the study site for the most abundantly occurring species. Analyses were conducted using Kruskal-Wallis tests with Chi-square distribution (df = 4). Statistical significance is indicated with asterisks.

Species	P	χ^2
Dead vegetation	0.0017*	17.263
Poa pratensis	0.0002**	23.467
Dactylis glomerata	< 0.0001***	110.93
Festuca pratensis	0.0314*	10.607
Centaurea nigra	0.0013*	17.844
Plantago lanceolata	0.0001***	34.79

* $P \leq 0.05$; ** $P \leq 0.001$; *** $P \leq 0.001$

Appendix III.5

Pairwise differences resulting from *post-hoc* Dunn tests with Bonferroni adjustment for multiple comparisons following significant Kruskal-Wallis tests for the percentage cover of each of the five most abundant plant species and dead vegetation. Asterisks indicate pairs that differ significantly within each vegetation type.

	Expected/ Near	**Unexpected/ Near**	**Expected/ Distant**	**Unexpected/ Distant**
Dead vegetation				
Unexpected/Near	0.3241			
Expected/Distant	0.0944	1.0000		
Unexpected/Distant	0.0394	1.0000	1.0000	
TE	1.0000	0.0786	0.0161*	0.0055*
Poa pratensis				
Unexpected/Near	0.0001*			
Expected/Distant	0.5535	0.0262		
Unexpected/Distant	0.0016*	1.0000	0.2106	
TE	0.1908	0.0715	1.0000	0.4796
Dactylis glomerata				
Unexpected/Near	0.0000*			
Expected/Distant	0.0306	0.0000*		
Unexpected/Distant	0.0000*	1.0000	0.0000*	
TE	0.0091	0.1510	0.0000*	0.0026*
Festuca pratensis				
Unexpected/Near	1.0000			
Expected/Distant	0.3365	1.0000		
Unexpected/Distant	0.0931	0.9091	1.0000	
TE	1.0000	1.0000	0.1600	0.0355
Centaurea nigra				
Unexpected/Near	0.3729			
Expected/Distant	1.0000	0.1589		
Unexpected/Distant	0.0183*	1.0000	0.0052*	
TE	1.0000	0.1375	1.0000	0.0036*
Plantago lanceolata				
Unexpected/Near	0.3376			
Expected/Distant	0.8478	1.0000		
Unexpected/Distant	0.0000*	0.0152*	0.0026*	
TE	0.0000*	0.0683	0.0121*	1.0000