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**The biology of the slug-killing
Tetanocera elata (Diptera: Sciomyzidae)
and its potential as a biological control agent
for pestiferous slugs**



A thesis submitted to the National University of Ireland, Galway for a
degree of Doctor of Philosophy

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Abstract

Laboratory experiments were carried out to determine the effects of controlled temperatures (14°C, 17°C, 20°C, 23°C, and 26°C) and ambient outdoor and laboratory temperatures on the development and predation capacity of *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae), potential biocontrol agent of pestiferous slugs. In addition, predatory behaviour of the third-instar larvae was observed and categorised using infrared digital recordings.

Results suggest that of the temperatures tested, the optimum for egg hatching is 14°C with a median duration of 13 days and a hatch rate of 52% (the highest hatch rate of all temperature conditions examined). The low hatch rate (5%) for eggs stored at 2-3°C indicates that storing eggs at low temperatures does not seem to be a viable option for *T. elata*.

The effects of temperature on the larval stages of *T. elata* were found to be similar to those for the egg stage. In general, as temperature increased, the duration decreased. However, percentage larval survival at constant high temperatures (26°C and 23°C) was poor (0% and 4% respectively). Nevertheless, larval survival rate at ambient outdoor temperatures (which rose to 30°C) was 52% suggesting that larvae can tolerate higher temperatures but not for prolonged periods. The recommended optimum temperature of those tested for rearing *T. elata* larvae is 20°C which had the greatest percentage larval survival (62%) with a median duration of 44 days. In addition, the sex of adult flies can be predicted prior to emergence using puparial weights which could prove useful in mass culture and biocontrol release programmes. No significant difference in the median number of slugs killed per larva at each temperature was detected with each larva killing 6 – 8 slugs.

An analysis of digital recordings of the behaviour of third instar *T. elata* larvae indicate three different strategies for prey finding: 1) searching and attacking; 2) searching and waiting; 3) waiting. Previous contact with a slug was not always required for a larva to actively search for a slug. Slugs were

immobilised by a larva with the head being the preferred site where feeding took place although the head was not always the site at which the initial attack took place.

The results of this study are discussed in the context of developing optimum conditions for the rearing of *T. elata* with the view of using it in biological control trials. Recommendations for future work are also discussed.

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Chapter 1
General Introduction

1.1 Slugs

1.1.1 Classification

Slugs can be generally described as a visceral mass with a broad flattened foot, a distinct head bearing two pairs of retractable tentacles and a mantle located towards the anterior end of the slug under which the slug can retract its head (South, 1992) (Figure 1.1). The posterior tentacles have an eye at each tip (ocular tentacles) while the anterior tentacles act as sensory tentacles. Slugs breathe through an opening called a pneumostome located along the right hand side edge of the mantle, the position of which is dependent on the family to which the slug belongs (South, 1992).

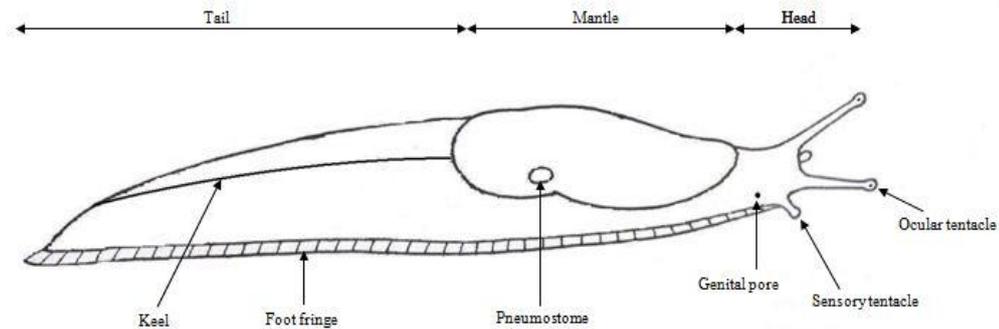


Figure 1.1: Diagram of slug adapted from Mc Donnell *et al.* (2009)

Slugs, along with snails, are members of the Gastropoda, a class within the phylum Mollusca, probably the third most successful animal group after the arthropods and vertebrates (South, 1992). Terrestrial slugs, of which there are at least 500 known species (Burton, 1982), are a heterogeneous group which has resulted in frequent changes to their taxonomy (South, 1992). South (1992) lists twelve families under the class Gastropoda which contain slugs but in general, only five of the families, all from the order Stylommatophora, are represented in Britain and/or Ireland i.e. Arionidae, Boettgerillidae, Milacidae, Limacidae and Testacellidae.

Cameron *et al.* (1983) illustrate certain characteristics of the Arionidae, Limacidae and Milacidae families based on the keel, the tail, the mantle and the location of the pneumostome (Table 1.1), while the species of the Family Testacellidae are recognised by the presence of a small external shell at the hind end of the body. South (1992) describes the Family Boettgerillidae as being narrow and worm-like.

Table 1.1: Identification of slug families adapted from Cameron *et al.* (1983).

Family	Keel	Tail	Mantle	Position of Pneumostome
Arionidae	No true keel ^a	Blunt tail with mucus gland	Granular, no concentric rings	In front half of mantle
Limacidae	Extends from tail but does not reach the mantle	Pointed	Pattern of fingerprint	In rear half of mantle
Milacidae	Extends from tail to mantle	Pointed	Varied texture but has pronounced grooves	In rear half of mantle

^a Some *Arion* species exhibit slightly larger & paler tubercles in the mid line known as a “false keel”.

Anderson (2008) listed 36 slug species found throughout Britain and/or Ireland. Following a recent study by Rowson *et al.*, (2014), eight species (some apparently not described) were found that were not recorded in Anderson’s (2008) list. These eight species are thought, by the authors, to be mainly introduced species, with several already widespread throughout the two countries. This finding brings the total number of slug fauna found throughout Britain and/or Ireland to 44 species.

1.1.2 Ecology

Terrestrial slugs have proved to be one of the most successful of all of the molluscan groups (South, 1992). The absence of an external shell as found on other members of the class (snails) has reduced the need for calcium salts, allowing the distribution of slugs to extend over a wide range of habitats including grasslands, forests and areas of high disturbance caused by humans. In addition, they occur throughout tropical and temperate regions (South, 1992; Thomas *et al.*, 2010). Slugs produce mucus which protects the skin by reducing damage caused by friction, locomotion and reproduction (South, 1992). Since slugs have a permeable cuticle, they are subject to high levels of body water loss under dry atmospheric conditions (Stephenson, 1968). To overcome this, slugs take shelter in the soil or in resting sites during the day, their worm-like body allowing them to squeeze through tiny crevices in soil for shelter (South, 1992). Hunter (1966) found that there were significant differences in the vertical distribution of different slug species in arable ground but that no slugs were found deeper than 1 ft (0.3m). They become active at night when conditions are favourable with activity being greatest under moist conditions although low temperatures and/or low moisture levels can reduce the surface activity (Port and Port, 1986). Slugs feed on an array of food varying from living plant matter to decaying organic matter. Under laboratory conditions, slugs are frequently fed and reared mostly on carrot, lettuce and potato while in their natural habitats they usually feed on herbaceous plants and grasses (Port and Port, 1986).

Slugs can have an annual life cycle e.g. *Deroceras reticulatum* or a biennial life cycle e.g. *Limax maximus* with the time necessary to reach sexual maturation varying between species (Thomas *et al.*, 2010). Although all slugs are hermaphrodite, they are also protandrous in that, once sexually mature, they function as males first and females later (Stephenson, 1968). Self fertilisation is possible in many species but cross fertilisation is most common (Port and Port, 1986). The reproductive cycle of terrestrial gastropods can be divided into the following phases: courtship; copulation; nest-building; egg-laying and embryonic development and hatching (Godan, 1983). Courtship normally begins by trail-

following (one slug follows the mucus trail of another slug) and generally leads to mating (Stephenson, 1968; Port and Port, 1986). Mating typically takes place at night on the surface of the soil (South, 1992). The period between mating and oviposition varies between species ranging from a few days to several weeks (Godan, 1983; Port and Port, 1986; South, 1992).

Eggs are normally laid in crevices or holes in the soil or on the surface underneath pieces of wood or stones (Godan, 1983; South, 1992). The largest batches of eggs are laid when conditions are most suitable, maximising the potential for development and survival of the offspring (Willis *et al.*, 2008). Most species have recognised egg laying peaks but these can vary depending on weather conditions (Port and Port, 1986; South, 1992). The quantity of egg batches laid and the number of eggs per batch vary between individuals and species (Port and Port, 1986; South, 1992). Similarly the duration of the egg stage can vary from 21 to 60 days depending on the species (South, 1992). After hatching, the young slugs feed on the egg capsules before searching for other sources of food (Godan, 1983). Quality and quantity of food dictates the rate at which young slugs reach maturity (Port and Port, 1986). Generally, young slugs grow relatively fast after hatching but after gonad maturation, growth rate, in terms of both size and weight, declines as they reach the adult stage (Godan, 1983).

1.1.3 Pestiferous slugs and damage caused

In nature, pest slugs cause damage to plants both above and below the ground (Howlett, 2012) and certain crops are more at risk than others e.g. oilseed rape seedlings, cereals and potatoes (Agriculture and Horticulture Development Board, 2013). Slug damage results in crop failures or a reduction in yield while the presence of slugs or slug faeces / mucus can devalue goods all of which contribute to considerable losses for the grower (South, 1992; Speiser *et al.*, 2001a; Howlett, 2012). Trade in food and horticultural produce has also aided the spread of pestiferous slugs (Howlett, 2012) and several of the pestiferous slugs of concern in Australia, New Zealand and North America are European species

(Barker, 1979; Micic *et al.*, 2007). On average, slug damage in Europe appears to be most severe and frequent in Ireland, Great Britain, France and the Netherlands which is predominantly due to the types of climate, soils and crops grown in these countries (Speiser *et al.*, 2001a). The families Arionidae, Limacidae and Milacidae contain the most important crop pests (Godan, 1983; Port and Port, 1986) of which *Deroceras reticulatum* (Müller), *Arion hortensis* aggregate of species and *Tandonia budapestensis* (Hazay) are listed as the most important pest slugs in Britain (Stephenson, 1968; Port and Port, 1986; South, 1992).

Approximately 140,000ha of potatoes are grown in the UK per annum and although the amount of damage caused by slugs can vary each year depending on weather, 37% of this area is affected by slugs with the potential loss of 37% yield if left untreated. Even with the use of slug pellets, slugs still cause losses of over £15 million per annum (Twinning *et al.*, 2009). It is estimated that, if no molluscicides were used, the cost to the UK industry in wheat and oilseed rape would be £43.5 million approximately (Nicholls, 2014). Without molluscicide treatment, an average of approximately 5% of yield for winter wheat would be lost to slugs which is equivalent to £25.5 million (Nicholls, 2014). Currently, 20-25% of the wheat crop area in the UK is treated annually using slug pellets. For oilseed rape, 59% of the crop area is affected by slugs and if left untreated, would result in a 2.4% reduction in production (Clarke *et al.*, 2009) which equates to £18 million per annum (Nicholls, 2014).

Currently, Wales and the south-west of England have the most favourable conditions for the pestiferous slug *D. reticulatum* throughout the UK. However, research shows that due to climate change leading to wetter but warmer winters and drier and hotter summers, it is suggested that by 2080, the north and west of Scotland will have the most suitable conditions for the species while the east of Scotland and the east of the UK will have the least suitable conditions (Willis *et al.*, 2006).

1.2 Current Control Options for Slugs

Current slug control methods may be divided into three categories, namely chemical, cultural and biological control. Each control has advantages as well as disadvantages as highlighted below.

1.2.1 Chemical

Chemical control in the form of baited pellets consisting mainly of carbamate compounds (e.g. methiocarb) or metaldehyde is the most popular method of slug control (Howlett, 2012). Both compounds affect the nervous system of slugs and cause paralysis and as a result lead to death by desiccation since the slug cannot move to seek shelter (Howlett, 2012). As the only form of delivery is by pellets, they only target surface-active slugs. However, these pellets are toxic to birds, mammals (Wilson *et al.*, 1993) and other non-target species (Schley and Bees, 2003; Howlett, 2012). Most recently in December 2013, the European Union Commission (Committee on the Food Chain and Animal Health) voted to revoke the use of slug pellets containing methiocarb due to the risk to grain-eating farm birds (European Commission, 2013; NFU, 2014; Nicholls, 2014). While it is still not clear when this ban will come into effect, it is thought that sales will be permitted until August 2014 after which usage will be allowed until August 2015 (Bayer Crop Science, 2014). There are also concerns with regard to metaldehyde entering public waterways (Howlett, 2012). A threshold of 0.1 parts per billion for metaldehyde residue is set by the European Environment Committee (98/83/EC) for drinking water standards and water companies in the UK currently find it difficult to reach these standards due the use of pesticides by farmers and domestic gardeners. Prevention of metaldehyde entering the water is key as current treatment processes cannot remove metaldehyde, unlike other pesticides, completely from the water (Water UK, 2014).

Due to their chemical makeup, slug pellets containing metaldehyde or carbamates are not suitable for use in organic agricultural systems (Jeong *et al.*, 2012). However, ferric phosphate is approved for use on organic farms (Horgan, 2006; Howlett, 2012) and is also an effective control for slugs (Speiser and

Kistler, 2002). It prevents the slug from feeding by disrupting calcium metabolism in the slug gut (Howlett, 2012). It is considered not to have any harmful effects on human health, non-target organisms or the environment (United States Environmental Protection Agency, 2014) and it is biodegradable during which process it releases phosphate and iron as plant nutrients (Howlett, 2012). However, findings have suggested that molluscicides containing ferric phosphate combined with EDTA (ethylene diamine tetracetic Acid) or EDDS (ethylene diamine succinic acid), both chelating agents, negatively affect the growth and activity of earthworms (Langan and Shaw, 2006; Edwards *et al.*, 2009).

1.2.2 Cultural

Making changes to the management of the land or how a crop is grown can help reduce the impact of slug damage. One such method is to reduce weed growth in planting beds, thereby decreasing sources of food and shelter for slugs. Repeated cultivations and firm consolidated seedbeds also increase mortality and reduce slug activity, respectively (MacDonald, 2009). In addition, the depth at which seeds are sown (Glen *et al.*, 2006) along with timing of irrigation (Speiser and Hochstrasser, 1998) aid in the prevention of slug damage. Planting less susceptible varieties e.g. Record or King Edward potatoes instead of the more susceptible Maris Pipers is another approach (Winfield *et al.*, 1967). However, some of these measures can prove expensive both in terms of time and labour (Wilkinson, 2010).

Other material suggested for slug control include beer, caffeine and tobacco (Jeong *et al.*, 2012). Hollingsworth *et al.* (2002) found that large slugs (*Veronicella cubensis*) could be killed when sprayed with a 1 or 2% solution of caffeine and that leaves sprayed with a 0.01% concentration of caffeine solution significantly reduced feeding by slugs while a mixture of 0.5% tobacco extract and 7% ethyl alcohol results in over 80% mortality of *Lehmannia valentiana* (Jeong *et al.*, 2012). Placing materials with rough surfaces such as broken egg shells, sand or ashes can create barriers between slugs and plants as they are unpleasant for slugs to move across (Symondson, 1990). Physical barriers such as

copper tape that inflict a tiny electric shock when the slug slides over it or spreading salt are commonly used but these are more home based remedies and are not suitable or adequate for large scale agriculture production.

1.2.3 Biological Control

Biological control is the use of living organisms such as parasitoids, predators or herbivorous arthropods to reduce pest numbers (Van Driesche and Abell, 2009). Stephenson and Knutson (1966) name 46 species of predators and parasites associated with 25 species and sub-species of slugs, with fourteen species of slug known to be killed by ten species of invertebrates. Of the 46 species, the most important enemies are protozoans, brachylaemid flatworms, lampyrid beetles, lung worms and the larva of some species of Sciomyzids (Stephenson and Knutson, 1966). The parasitic nematode *Phasmarhabditis hermaphrodita*, which is lethal to many slugs and snails, has been formulated into a biological molluscicide and sold under the trade name Nemaslug® (Rae *et al.*, 2007). Nematodes are currently the only biological control commercially available for slugs (Wilkinson, 2010). The larvae of *P. hermaphrodita* enter their prey under the mantle and upon developing into hermaphroditic adults produce young which leads to the mantle becoming swollen, a characteristic associated with nematode infection (Wilson *et al.*, 1993). Bacteria, released by the nematodes, are thought to kill the slug (Wilson *et al.*, 1993; Rae *et al.*, 2007). The nematode is capable of killing a wide range of slug species (Wilson *et al.*, 1993) but not all species (Grewal *et al.*, 2003) and larger sized slugs also appear to be less susceptible to infection by the nematode (Speiser *et al.*, 2001b). Although quite an effective control, the use of nematodes as a biological control is hampered by some disadvantages, namely high production costs, limited storage life and specific application requirements (Glen and Wilson, 1997; Grewal *et al.*, 2005; Speiser *et al.*, 2001b). It is also not currently available for use in the USA as *P. hermaphrodita* has not previously been found there (Rae *et al.*, 2007).

Slugs are also preyed on by birds, reptiles, mammals and insects (Schley and Bees, 2003). The beetle *Pterostichus melanarius* (Illiger) is a generalist predator and one of the most abundant and widespread large polyphagous

carabids in arable fields and other open field ecosystems (Symondson *et al.*, 1996; McKemey *et al.*, 2003). Based on both laboratory experiments and field investigations, it has the potential to reduce slug populations in the field (Symondson *et al.*, 1996; Oberholzer and Frank, 2002; McKemey *et al.*, 2003; Symondson *et al.*, 2006) but the presence of other prey on which it feeds can reduce its predation rate on slugs (Symondson *et al.*, 2006). Research has also been carried out on methods to increase numbers of predatory carabids in agricultural lands and it has been found that grass strips of land, also known as “beetle banks” can be managed in such a way as to increase the population of carabids on agricultural land (Thomas *et al.*, 1991; Lys and Nentwig, 1992; Symondson, 2004). Lys and Nentwig (1992) found that in a cereal field there were much higher densities of carabid beetles in the area of the field containing strip-managed areas compared to the rest of the field. A perennial grass mix containing species that form tussocks (e.g. *Dactylis glomerata*) is recommended for use on “beetle bank” strips while the strips should measure 2m wide x 0.4m high (Home Grown Cereals Authority, 2005)

The Family Sciomyzidae (Diptera), commonly known as marsh flies, are another highlighted predator of molluscs. Indeed, some species of the family have previously been linked with the biological control for the host snails of liver fluke disease (Gormally 1988a, Gormally 1988b; Reidenbach *et al.*, 1989). Research has also been carried out on some slug-killing species and in turn, they have been suggested as a potential biological control for pestiferous slugs (Knutson and Vala, 2011)

1.3 Family Sciomyzidae

The Family Sciomyzidae is one of the best known families of the order Diptera. Vala *et al.* (2012) lists four sub-families (Huttoniinae, Phaeomyiinae, Salticellinae and Sciomyzinae) with a total of 63 genera and 545 extant species worldwide (Figure 1.2). However, recent studies (Tóthova *et al.*, 2013) using DNA analysis indicate that only the Phaeomyiinae, Salticellinae and Sciomyzinae

should be included in the Family Sciomyzidae thereby reducing the number of genera to 41 (536 species) with Huttonininae assigned to the Family Huttoninidae.

The Phaeomyiinae contains two genera (*Akebono* Sueyoshi and *Pelidnoptera* Rondani) with five species while the Salticellinae contains just two species (*Salticella fasciata* Meigen and *S. stuckenbergi* Verbeke). The Sciomyzinae is made up of two tribes (Sciomyzini and Tetanocerini) with 13 and 45 genera, respectively. The two tribes are generally separated from each other by the presence (Sciomyzini) or absence (Tetanocerini) of a strong seta on the propleuron above the base of the fore coxa (Knutson and Vala, 2011) although there are exceptions i.e. the said seta is hair-like in *Atrichomelina*, short and weak in some *Colobaea* and absent in *Pseudomelina* while it is present in the Tetanocerini genera *Eutrichomelina*, *Perilimnia* and *Shannonia* (Knutson and Vala, 2011). The Huttonininae is also made up of two tribes (Prosochaetini and Huttoninini) but each with only one genus (*Prosochaeta* Malloch and *Huttonina* Tonnoir et Malloch) containing one and eight species, respectively (Figure 1.2).

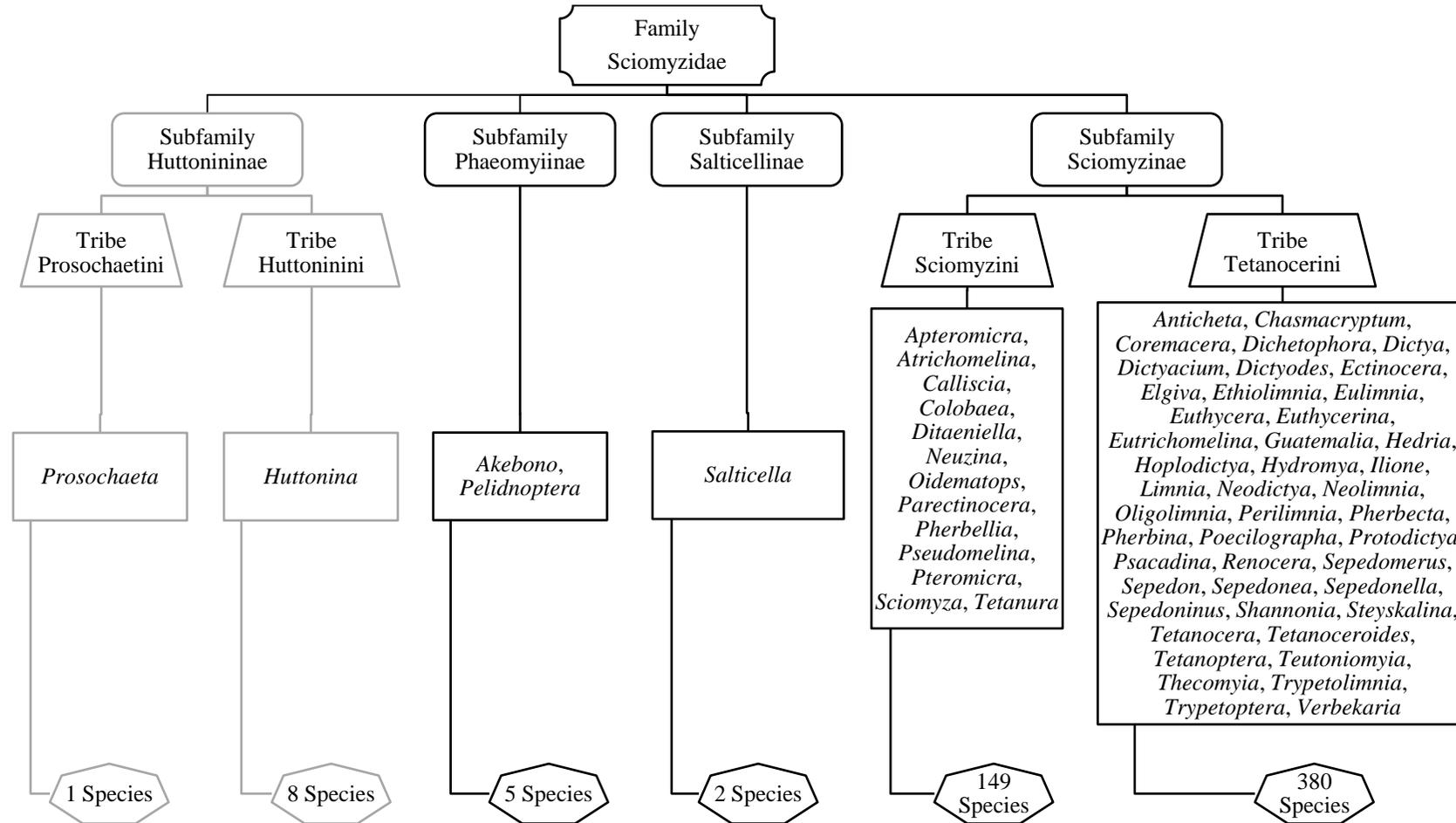


Figure 1.2: Hierarchy for the Family Sciomyzidae adapted from Vala *et al.*, (2012). The faded boxes indicate the subfamily excluded by Tóthova *et al.*, (2013).

Sciomyzids are distributed worldwide (Table 1.2) with the majority of species found in the Nearctic and Palaeartic regions (Knutson and Vala, 2011; Vala *et al.*, 2012). The most species rich genera are *Pherbellia* (tribe Sciomyzini) and *Sepedon* (tribe Tetanocerini) having 94 and 79 species, respectively (Vala *et al.*, 2012).

Table 1.2: Distribution of Sciomyzidae genera and species (adapted from Vala *et al.*, 2012).

Zoogeographic Region	Total Number of Genera	Total Number of Species
Palaeartic	29	157
Nearctic	23	172
Neotropical	23	91
Oriental	13	30
Afrotropical	12	65
Subantarctic	4	25
Australian	3	12
Oceanic	2	4

Sciomyzid adults range in size from 1.7mm to 13.0mm in length and occur in a range of colours including brown, yellow and black or a combination of all three. *Sepedon* species differ slightly in that many of them are dark blue with orange legs (Knutson and Vala, 2011). Adults are short distance, low fliers and can be found in habitats (generally undisturbed) ranging from wet to dry, mainly on emergent rushes or grasses (Berg and Knutson, 1978; Barker *et al.*, 2004; Knutson and Vala, 2011). They use their fore-tarsi to tap the substrate in front of them as they walk and while at rest they generally hold their head focussed downward, a characteristic of the family (Barker *et al.*, 2004; Knutson and Vala, 2011). There is no known association with specific plant species and they appear not to be attracted to flowers (Knutson and Vala, 2011) but vegetation height and disturbance caused by grazing can affect species composition (Mc Donnell and

Gormally, 2000; Williams *et al.*, 2009; Maher *et al.*, 2014). Adults have been reported to feed on snails (both alive and decaying), insect eggs, dead insects and flowers (Berg and Knutson, 1978) although it is not known for certain what the full dietary composition is in their natural habitat (Mc Donnell and Gormally, 2000). Many adults of numerous species have been successfully raised in the laboratory using a mixture of honey, brewer's yeast and dried milk (Knutson and Vala, 2011) with crushed snails added to increase fecundity (Berg and Knutson, 1978; Barker *et al.*, 2004; Knutson and Vala, 2011). Sciomyzids rarely enter buildings and are not attracted to humans, food or domestic animals (Berg and Knutson, 1978).

A complete sciomyzid life cycle (Figure 1.3) from egg to adult generally encompasses 3 larval instars, a pre-pupal stage, a pupal stage and pharate adult (in the puparium) stage (Knutson and Vala, 2011). Species can be univoltine or bivoltine but the majority of sciomyzids are multivoltine, producing more than one generation per year. Overwintering in the pupal stage is most common but overwintering can also occur in the egg, adult or larval stage depending on the species (Knutson and Vala, 2011).

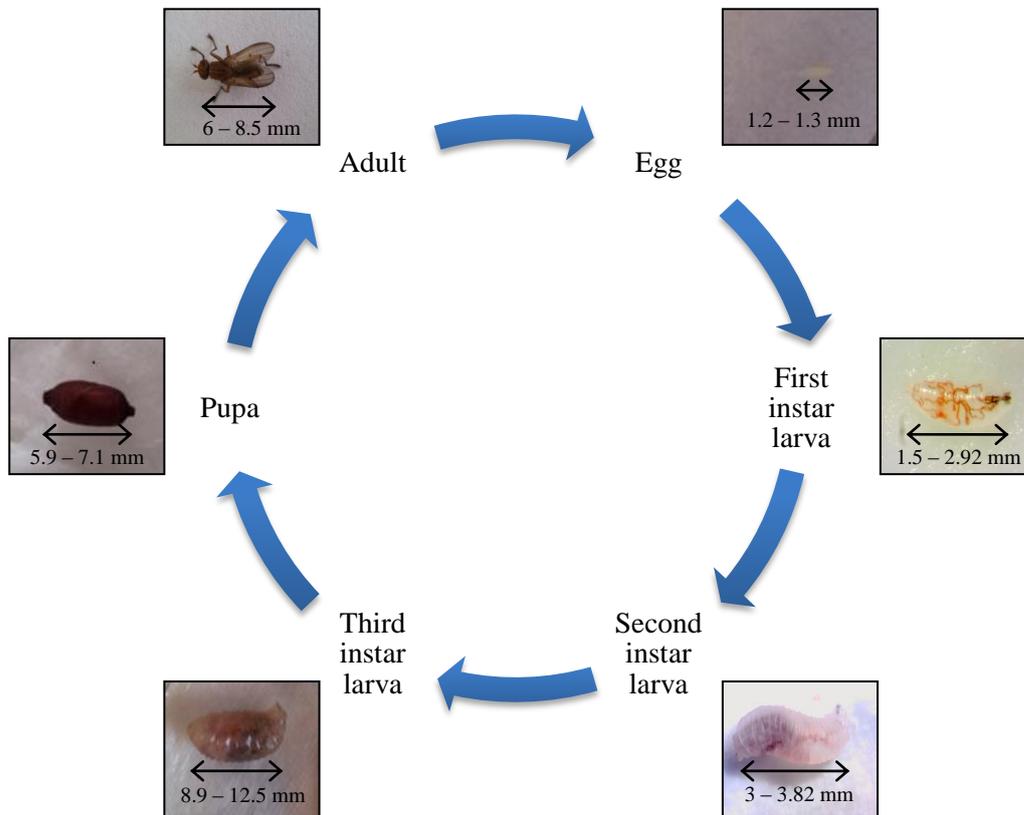


Figure 1.3: General life cycle of a sciomyzid using *Tetanocera elata* images as representation.

Berg *et al.* (1982) created five phenological groups based on the voltinism and overwintering habits of sciomyzids in the temperate zone (Table 1.3). Vala (1984) subsequently proposed the splitting of Group 5 into two (5a and 5b) while Barker *et al.* (2004) added a sixth group for species in warm temperate and tropical areas.

Table 1.3: Phenology groups as classified by Berg *et al.* (1982), Vala (1984)¹ and Barker *et al.* (2004)².

Group	Voltinism	Overwintering State	Example
1	Multivoltine	Pupa	<i>Tetanocera elata</i> Fabricius
2	Multivoltine	Adult	<i>Sepedon fuscipennis</i> Loew
3	Univoltine	Egg	<i>Tetanocera loewi</i> Steyskal
4	Univoltine	Larva	<i>Tetanocera vicina</i> Macquart
5(a/b) ¹	Univoltine	Pupa	<i>Antichaeta analis</i> (Meigen)
6 ²	Multivoltine	N/A	<i>Sepedon plumbella</i> Wiedemann

Group 1 species, with the exception of some species of the genera *Dictya* and the *Atrichomelina pubera* (Loew), are not found at any other stage in their life cycle other than pupa during winter although they can be collected during this stage at any time throughout the year. Adults emerge in spring and a series of overlapping summer generations occur. This widespread phenological group is found in both the Tetanocerini and Sciomyzini regardless of whether the species is aquatic or terrestrial (Berg *et al.*, 1982). The fact that larvae in these tribes are present in the autumn months when the larvae of other groups are absent ensures access to food without competition.

Although breeding continuously during the spring and summer months by Group 2 species results in the overlapping of generations similar to Group 1, Group 2 species hibernate as adults during the winter months. This characteristic allows larvae to be produced prior to those of Group 1 as there is no delay waiting for the emergence of the adult before breeding can occur in spring. On the counter side, this group more than likely suffers higher mortality rates due to the necessity to survive the winter months as adults (Berg *et al.*, 1982). All the species found in this group have quick-killing aquatic predator larvae with the exception of

Pherbellia schoenherri maculata (Cresson) which is a terrestrial parasitoid larvae of *Oxyloma* snails (Berg *et al.*, 1982).

Only one generation of each species in Group 3 is produced annually. This is as a result of a larval diapause which occurs while they are still encased in the egg membrane during the winter months coupled with an adult reproduction diapause during late spring and early summer (Berg *et al.*, 1982; Vala *et al.*, 2012). However, this trait allows for an even quicker start to a new generation than members of Groups 1 and 2. Univoltinism in this case ensures that larvae are only present at the same time their prey is available in vernal ponds and marshes (spring months).

Species in Group 4 are also univoltine and since they overwinter as larvae, they are generally found as adults throughout the summer. Their eggs hatch much more quickly compared to Group 3 and therefore larvae have started to develop prior to the arrival of the winter months. This gives them the advantage of being able to utilise ponds and marshes occurring in the autumn months for food without competition from other larvae that have already completed their larval stages. So similar are groups 3 and 4 that some species may be more characteristic of one of either group depending on specific winter conditions (availability of food and water) where they are located, e.g. *Ilione albiseta* (Scopoli) and *I. lineata* (Fallén) (Berg *et al.*, 1982). The eggs of these species will not hatch unless they are submerged in water (Berg *et al.*, 1982) while in warmer latitudes *I. lineata* has the characteristics that match Group 4 (Vala *et al.*, 2012).

Group 5 which includes representatives of both the Sciomyzini and the Tetanocerini live in both terrestrial and seasonally aquatic breeding sites. Some species do not oviposit until standing water has nearly cleared, allowing larvae to find food more easily in the drying ponds (Berg *et al.*, 1982). Vala (1984) proposed splitting the group based on geographic location (5a northern Palearctic/Nearctic; 5b southern Palearctic/Mediterranean) with those species in Group 5b having long pre-oviposition periods (Vala *et al.*, 2012).

Group 6 is based on both Sciomyzini and Tetanocerini freshwater and semi-terrestrial predators found in tropical zones. They breed all year round, resulting in numerous generations per year and show no indication of diapause at any stage (Barker *et al.*, 2004; Vala *et al.*, 2012). Species that have a north-south distribution e.g. *Dictya montana* Steyskal display characteristics belonging to both Group 1 and Group 6 depending on their geographical location. Populations in low elevation, warm areas are more characteristic of Group 6, while those at higher and cooler elevations are more representative of Group 1 (Vala *et al.*, 2012). From all the groups, multivoltinism with overwintering in the puparium (Group 1) is the most common behaviour for the Sciomyzidae (Berg *et al.*, 1982; Knutson and Vala, 2011).

Sciomyzid eggs range in size from 0.4mm to 1.9mm (length) and 0.1mm to 0.6mm (width). They are white or yellowish in colour when laid first and change to a greyish colour in line with the maturing of the eggs (Knutson and Vala, 2011). Eggs are generally laid in the microhabitat of the larvae. Generally, eggs (as observed from laboratory rearing) are laid either singly or in groups (organised or scattered) on damp to dry substances e.g. vegetation, damp cotton wool, sides of rearing jars (Knutson and Vala, 2011). Five species are known to lay their eggs only on the shell of the host snail, one species (*Tetanura pallidiventris*) on the flesh of the host while another five species of *Anticheta* lay their eggs on the eggs of their hosts.

Puparia can range in length from 2.6mm to 12.0mm and 1.0mm to 4.2mm in width. While some puparia of terrestrial and semi-terrestrial Sciomyzini species are formed within the shell of their host, most are generally formed in water, in the litter or on the shoreline. Aquatic species can form pupae both in and out of the water and are adapted for floating (Knutson and Vala, 2011).

Although every detail is not known about each of the 545 species, data relating to larval behaviour is currently available for 204 sciomyzid species (Murphy, 2014). Similar to the formation of phenology groups for sciomyzid life-

cycles, behavioural groups have also been formed for the larvae. The most recent classification by Vala *et al.* (2012), with 16 groups (Table 1.4), is based on information regarding the kind of food eaten by the larva, the manner in which it kills or feeds, and its microhabitat. The groups are ordered according to feeding (from least to most specialised) and microhabitat (damp to terrestrial to aquatic) (Knutson and Vala, 2011)

As the behavioural groups illustrate (Table 1.4) most sciomyzid larvae are obligate mollusc killers and feeders, generally restricted to non-operculate freshwater, semi-terrestrial, or terrestrial snails while other species attack operculate snails, bivalve Mollusca, brackish-water-loving snails, snail eggs and slugs (Knutson and Vala 2011; Murphy *et al.* 2012). Their habitats can range from strictly terrestrial to strictly freshwater while their feeding behaviour can range from saprophagous to predaceous to parasitoid with some species having the ability to adjust or mix behaviours if necessary (Knutson and Vala, 2011). Most of the species of the tribe Tetanocerini have a freshwater, predatory behaviour with each larva killing numerous snails while the Sciomyzini species tend mainly to feed on terrestrial, semi-terrestrial or exposed freshwater snails as parasitoids. Species with slug-killing, snail-egg feeding or clam killing larvae, along with *Sepedonella nana* and *Sepedon knutsoni* (freshwater oligochaete predators) tend to be more host specific and have a more limited food choice.

Table 1.4: Behavioural groups of Sciomyzidae larvae (adapted from Vala *et al.*, 2012)

Group	Feeding behaviour	Prey Type	Example
1	Opportunistic Predator/Parasitoid/ Saprophage	Dead/moribund/living snails	<i>Salticella fasciata</i> Meigen
2	Predator/Saprophage	Pulmonate, exposed freshwater snails	<i>Pherbina coryleti</i> (Scopoli)
3	Parasitoid Parasitoid/Predator	Pulmonate, exposed freshwater snails	<i>Colobaea</i> <i>bifasciella</i> (Fallén)
4	Parasitoid Parasitoid/Predator	Hygrophilous, semi- terrestrial Succineidae snails	<i>Sciomyza aristalis</i> (Coquillett)
5	Obligate Parasitoid/Predator (early larval stages) Predator	Exposed snail eggs of freshwater Lymnaeidae or <i>Aplexa hypnorum</i> or Succineidae snails (early larval stages); Juvenile to mature snails in damp situations	<i>Anticheta analis</i> (Meigen)
6	Parasitoid	Pulmonate, terrestrial snails	<i>Pherbellia dubia</i> (Fallén)
7	Predator/Saprophage	Pulmonate terrestrial snails	<i>Pherbellia cinerella</i> (Fallén)
8	Opportunistic Predator/Saprophage	Terrestrial snails and slugs	<i>Euthycera cribrata</i> (Rondani)
9	Obligate ectoparasitoids*/predators	Slugs	<i>Tetanocera elata</i> (Fabricius)
10	Obligate mesoparasitoid*	Slugs	<i>Euthycera</i> <i>chaerophylli</i> (Fabricius)

Table 1.4 contd.: Behavioural groups of Sciomyzidae larvae (adapted from Vala *et al.*, 2012)

Group	Feeding behaviour	Prey Type	Example
11	Predator	Pulmonate snails at or just below water surface	<i>Dictya adjuncta</i> Valley
12	Predator Predator/Parasitoid	Exposed and neustonic operculate aquatic snails	<i>Holodictya setosa</i> (Coquillett)
13	Predator	Pulmonate snails under the water surface	<i>Ilione albiseta</i> (Scopoli)
14	Predator/Parasitoid	Clams	<i>Ilione lineata</i> (Fallén)
15	Predator	Freshwater oligochaetes	<i>Sepedonella nana</i> (Verbeke)
16	Internal parasitoids	Millipedes	<i>Pelidnoptera nigripennis</i> (Fabricius)

* Ectoparasitoid slug feeders keep posterior spiracles exposed to ambient air. Mesoparasitoids live entirely within the slug.

Since Berg (1953) first published information on the ability of the Family Sciomyzidae to feed on molluscs, much research has been undertaken investigating the potential of this family to be used as a biological control for snail hosts of trematode diseases of livestock (fascioliasis), humans (schistosomiasis) and pestiferous slugs (Knutson and Vala, 2011). For example, the species *I. albiseta* (Scopoli) and *Sepedon spinipes* (Scopoli) have been investigated as two possible control agents for *Galba truncatula* (Müller), the intermediate snail host of *Fasciola hepatica*, by Gormally (1985, 1987, 1988a, 1988b) and Mc Donnell *et al.*, (2005), respectively.

1.3.1 Slug-Killing Sciomyzidae

Of all the known sciomyzids, only nine species are documented slug killers. The basic life-cycles of seven (*Tetanocera clara* Loew, *T. plebeja* (Loew), *T. valida* (Loew), *T. elata* (Fabricius), *Euthycera cribrata* (Rondani), *E. stichospila* (Czerny) and *Limnia unguicornis* (Scopoli)) have been previously described and published (Knutson and Vala, 2011; Trelka and Foote, 1970). Limited information only regarding *E. arcuata* (Loew) and *E. chaerophylli* (Fabricius) has also been published by Knutson and Vala (2011).

Tetanocera elata and *T. plebeja* are widespread in various habitats across Europe and North America, respectively, while *T. valida* and *T. clara*, also distributed in North America, prefer low-lying forests and mesic woodlands (Knutson and Vala, 2011). *Euthycera stichospila* and *E. cribrata* have a primarily Mediterranean distribution where they are adapted to the long dry seasons (Knutson and Vala, 2011). *Euthycera chaerophylli* is commonly found in mesic woods throughout much of Europe (Knutson and Vala, 2011).

The diets of the larval stages of *T. elata*, *T. valida* and *E. chaerophylli* are completely restricted to slugs unlike the other six species which can feed on snails (Knutson and Vala, 2011). Host specificity during the first instar larval period appears to apply to the *Tetanocera* species and *E. chaerophylli* (Knutson and Vala, 2011). *Tetanocera* larvae are parasitoid, and remain under the mantle, in the “mouth” or in the eye tentacle of their host during their early life until they become predatory during the third-instar. They have the capability of killing up to 9 slugs throughout their 20 – 48 days of larval development (Knutson and Vala, 2011). Similarly, *E. cribrata* and *E. stichospila* are parasitoidal for their early life stages but become predaceous / saprophagous in the later larval stages, consuming between 15 – 25 slugs over the course of the 60 – 90 days of development (Knutson and Vala, 2011). *Limnia unguicornis* larvae, observed feeding on one or two slugs, are immersed in the decaying tissues for up to 30 days (Knutson and Vala, 2011). *Euthycera arcuata* has been seen to feed on the slugs *Pallifera* and *Philomycus* and although *E. chaerophylli* has not been raised past second-instar, it

is known to be an internal endoparasitoid of *Deroceras* spp up until that time (Knutson and Vala, 2011). While *Euthycera cribrata*, *E. stichospila* and *L. unguicornis* are univoltine unlike the multivoltine *Tetanocera* species, they all overwinter as pupae (Knutson and Vala, 2011).

It has been suggested that due to their slug killing ability, both *T. elata* and *T. plebeja*, have the potential to be used as a biocontrol of pestiferous slugs (Reidenbach *et al.*, 1989) particularly in greenhouses or semi-natural situations (Knutson and Vala, 2011). However, optimum rearing conditions must first be determined and host/prey tests undertaken to further evaluate this. *Tetanocera elata* was selected for this study due to its availability in Ireland and its first and second instar host specificity to *D. reticulatum* and *D. laeve* (Müller), two commonly occurring pestiferous slugs.

1.3.1.1 *Tetanocera elata*:

Tetanocera elata has previously been found in a variety of habitats throughout Europe such as improved and unimproved grasslands, field margins in humid grasslands, fen / marsh / calcareous flushes in both blanket and raised bog, wet woodland of *Alnus* and *Salix* and wetland/open ground (Speight and Kuntson, 2012). The flight period is thought to be continuous during warm months and is listed as early May to September in Britain, although records of the species have been confused with *T. phyllophora* and so this is questionable (Knutson *et al.*, 1965). It has previously been caught in Ireland by sweep netting in mid-June (Williams *et al.*, 2007).

Knutson *et al.* (1965) captured and reared larvae of *T. elata* to study the life cycle. Adults were kept in breeding jars and fed on a mixture of dried milk, honey and brewer's yeast plus crushed snails. Vegetation (*Iris*, *Phragmites* and *Typha*) was used to provide oviposition and resting sites. Copulation, which was common during the first two weeks after field collection, occurred less frequently thereafter. Repeated copulation is not necessary for the oviposition of fertile eggs (Knutson *et al.*, 1965). During the months of June to September, 2,382 eggs were

laid by 23 females over periods ranging from two to forty-two days with daily batches varying from one egg to twenty-two eggs. Eggs were laid singly or in groups of two to five on vegetation or were stuck to the sides of the breeding jars. In nature it is probable that eggs are laid in the micro-habitat of the host slugs.

Unlike most newly hatched sciomyzid larvae that move to search for a host, *T. elata* neonates remained motionless after hatching until touched by a passing host resulting in larval deaths due to starvation if the host had not made contact with the larva. When contact was first made with a passing slug, the larvae raised its anterior end swaying from side to side and after firm contact was made, crawled quickly on to the host (Knutson *et al.*, 1965). First instar larvae were host specific to *Deroceras reticulatum* and *D. laeve* and while numerous snail species have been tried as a food source, the larvae did not feed or attack any of these species (Knutson *et al.*, 1965). Bar the production of some chalky-white mucus after penetration by larval mouth hooks, the slugs appeared to behave normally when attacked by a larva. The most common site of penetration was beneath the edge of the mantle of the slug in close proximity to the respiratory pore but subsequently the larva could move freely under the whole mantle.

Mucus is the main source of food for the parasitoid first instar larva and early part of the second instar during which time the larva rapidly increases in size while remaining under the mantle of the slug (Knutson *et al.*, 1965). The slug stays alive until a few days before the larva reaches third instar after which the larva will feed on the dead tissue of the slug. Third instar larvae feed as predators and immobilise their prey before feeding for a period of time. Knutson *et al.* (1965) considered that the larva achieved this immobilisation by lacerating the nervous tissue in the head of the slug to prevent its escape as the prey does not die immediately when attacked. However, Trelka and Berg (1977) subsequently identified that immobilisation of the slug was due to the injection of a toxin by the predator before feeding began. The larva rests after feeding before attacking a new host. Between four and nine slugs can be killed by one larva during its larval development. Slugs can harbour more than one larva at a time but as a result the

larvae do not grow as quickly and only one larva may reach second instar before the death of the slug. However, these larvae will feed on the dead host before attacking another host. *T. elata* loses its host specificity on reaching third instar and has been known to feed on some species of the *Arion*, *Limax* and *Milax* genera (Knutson *et al.*, 1965).

Puparia are formed on or just beneath the surface of the soil. Four to five days prior to pupation, larvae cease feeding. Duration of the pupal period ranges from less than 20 days to 52-82 days which suggests the presence of a facultative quiescent period in the late autumn generation (Knutson *et al.*, 1965). Subjecting pupae formed in the late autumn generation to a number of weeks at -6°C before raising them back to laboratory temperature has been found to be effective in shortening this quiescent period. The species is multivoltine and during an active five month period two generations may be produced with a possible third overwintering throughout the pupal stage (Knutson *et al.*, 1965).

The total duration for the complete life cycle of a larva at a temperature range of 19 to 24°C ranges from 61 to 90 days – this entails a pre-oviposition duration of 13 days; an egg incubation period of 7 to 9 days; a first instar duration of 4 to 10 days; a second instar duration of 8 to 15 days; a third instar duration of 15 to 23 days; and for non-overwintering / non-quiescent pupae, a pupal period of 19-20 days (Knutson *et al.*, 1965).

1.4 Aims and Objectives

In order to determine the viability of a species for the biological control of pestiferous slugs, an understanding of its development under controlled conditions must first be achieved (McDonnell and Gormally, 2007). Barker *et al.*, (2004) lists seven requirements that must be met for a successful biological control agent:

- 1) Laboratory rearing
- 2) Long-distance transport
- 3) Favourable recipient environment
- 4) Pest suppression
- 5) Stability in permanent crops versus opportunism in temporary crops
- 6) Freedom from natural enemies
- 7) Minimal adverse effects on biodiversity.

These requirements will be discussed in more detail in relation to *T. elata* and the results found in this study in the general discussion.

In this context, the aims of the study which are to:

- Investigate the effect of different temperature regimes on the egg hatch rate and larval / pupal development of *T. elata* to determine the optimum conditions for purposes of mass rearing.
- Determine the fecundity and adult longevity of *T. elata* with a view to determining the optimum egg-laying periods of adult females.
- Quantify the predatory capacity of *T. elata* on slugs during its larval stages.
- Describe the behaviour and interaction of the third instar larva and its prey (*D. reticulatum*) to gain a better understanding of the methods used by *T. elata* to find its host.

1.5 Structure of the thesis

This thesis is presented as three self-contained chapters, all of which have been published in peer-reviewed journals (Chapters 2 – 4). Each chapter consists of an abstract, introduction, methods and materials, results and discussion. Chapter 2 examines the oviposition, adult longevity and temperature effects on the eggs of *T. elata* while Chapter 3 deals with the effects of temperature on the larval stages of *T. elata*. Chapter 4 describes the larval feeding behaviour of *T. elata* and the methods it employs to source its food. Chapter 5 is a general discussion of the results of Chapters 2 – 4 along with conclusions and recommendations for future research. Due to the same species being used throughout the research, there may be some repetition and overlap between chapters. Although the work presented here is primarily laboratory based, details of where the *T. elata* adults were captured are given Appendix I along with an additional co-authored publication on *T. elata* (Appendix II) and another sciomyzid (*Ilione albiseta*) in Appendix III which were additional outcomes to this research.

1.6 References

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Chapter 2

Oviposition, adult longevity and temperature effects on the eggs of *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae): a potential biocontrol agent for slugs

2. Oviposition, adult longevity and temperature effects on the eggs of *Tetanocera elata* (Fabricius) (Diptera: Sciomyzidae): a potential biocontrol agent for slugs

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

The article as referenced above is available in its published form at <http://onlinelibrary.wiley.com/doi/10.1111/jen.12120/abstract>

Chapter 3

Effect of temperature on the larval stage of *Tetanocera elata* (Diptera: Sciomyzidae) - Potential biological control agent of pestiferous slugs

3. Effect of temperature on the larval stage of *Tetanocera elata* (Diptera: Sciomyzidae) - Potential biological control agent of pestiferous slugs

Hynes T., Mc Donnell, R.J., Kirsh, A., Dillion, R., O'Hara, R., Gormally, M.J., 2014. Effect of temperature on the larval stage of Tetanocera elata (Diptera: Sciomyzidae) - potential biological control agent of pestiferous slugs. Biological Control. 74, 45-51.

The article as referenced above is available in its published form at DOI: 10.1016/j.biocontrol.2014.03.005 with kind permission from Elsevier.

Chapter 4

Larval feeding behaviour of *Tetanocera elata* (Diptera: Sciomyzidae): potential biological control agent of pestiferous slugs.

4. Larval feeding behaviour of *Tetanocera elata* (Diptera: Sciomyzidae): potential biological control agent of pestiferous slugs

*Hynes, T., Giordani, I., Larkin, M., Mc Donnell, R.J., Gormally, M.J., 2014. Larval feeding behaviour of *Tetanocera elata* (Diptera: Sciomyzidae): potential biocontrol agent of pestiferous slugs. *Biocontrol Science and Technology*, 24 9, 1077-1082.*

The article as referenced above is available online in its published form at <http://www.tandfonline.com/10.1080/09583157.2014.912259>.

Chapter 5
General Discussion

5.1 Discussion

Knutson and Vala (2011) suggest that *T. elata*, given its wide distribution and specialist slug feeding habits, could potentially be used as a biological control agent, particularly in greenhouses which are essentially closed systems. This provided the incentive for the first part of this project whereby, for the first time, research under controlled temperature conditions was undertaken to determine optimum rearing conditions for mass culturing of the species. The results of this part of the study will support the development of future mass cultures of the species, an essential requirement for an effective biological control agent (Barker *et al.*, 2004; Mc Donnell and Gormally, 2007). The second part of the project examines the effects of different temperatures on the number of prey killed by *T. elata* in addition to observing its behaviour, both of which are required to fully understand predation, which is critical for selecting safe and effective biological control agents. It is important to note that for the third chapter of this thesis, the number of larvae used for the trials was limited. Only eight third-instar larvae were available for trials at the time they took place and therefore only 20 trials were run in total. This is due to the difficulty in locating high numbers of adults of the species *T. elata* in the field during the experimental stage. The third chapter is intended to be a descriptive article, describing the range of prey-searching patterns exhibited by these third instar larvae.

With respect to longevity, the maximum adult life duration of 115 days found for one of the laboratory reared males (24 days greater than the longest surviving field caught adult), gives an indication of the potential adult life expectancy for the species. Nevertheless, it would appear that egg-laying by females ceases some time prior to natural mortality. Although the last eggs of 15 field-caught females were laid on Day 40 post-capture, two individuals lived for 73 and 95 days after capture in the field. This information has cost-saving implications for large scale cultures regarding the duration of care of adult females post egg-laying. Females laid a mean (SD) of 164 (\pm 68.8) eggs each with one female laying a maximum of 285 eggs. That Knutson *et al.* (1965) also recorded a single female laying 373 eggs, indicates the potential for producing

large numbers of larvae for biological control purposes. Females are also capable of producing fertile eggs while not in continuous contact with a male. Seventy-three (44%) of 166 eggs laid over 14 days by a field-collected female hatched without the presence of a male from five days before oviposition began in the laboratory. This suggests that should males be in short supply in a mass rearing situation, they could be rotated between female colonies to enhance fertile egg numbers. Regardless of temperature, most eggs hatched over a short period of time (5 – 15 days) after oviposition. This coupled with a short pre-oviposition period (median of 8 days) allows for the potential production of a large number of eggs over a relatively short time period.

The egg hatch rate was low across all temperatures ($\leq 52\%$) compared to Beaver (1973) who achieved a hatch rate of 19 – 86% at a temperature range of 20 – 25°C. Beaver (1973) transferred the eggs, from the jars in which the flies were held, on to wet filter paper in a Petri dish while Knutson *et al.* (1965) transferred the vegetation that bore the eggs on to moist filter paper. Knutson *et al.* (1965), does not give any details of hatch rates. The lower hatch rate in this study may be due to the environmental conditions at which the eggs were maintained. It is possible that the level of wetness may have affected the hatch rate found in this study. Knutson and Vala (2011) state that the requirement for the hatching of sciomyzid eggs may not necessarily represent the habitat in which the species is found, e.g. terrestrial or aquatic, but may be an adaptation to ensure emergence of the larvae at the proper time when prey are available. Perhaps the fact that the eggs were in constant contact with water was not favourable for hatching and subjecting them to a higher humidity would instead be more suitable. Slugs are most active on the surface in moist conditions (Port and Port, 1986) and should larvae emerge from eggs in these conditions, their prey would more than likely be present in higher numbers on the surface than if they emerged in drier conditions. Further research however, is required to determine this.

In general as the temperature increased, the median duration required for the eggs to hatch decreased, with the median duration at lower constant

temperatures (14°C and 17°C) taking significantly longer than that of the higher ones (20°C, 23°C, 26°C). This finding is similar to those of Gormally (1985) and Mc Donnell and Gormally (2007) who also carried out experiments on the effect of constant temperatures on the duration of the egg stage of sciomyzid species. Both found that as the temperature increased, the duration for egg stage decreased. In this study, the most successful hatch rate (52%) occurred at the lowest temperature (14°C), with the highest constant temperature (26°C) producing the lowest hatch rate (20%). Since median duration for egg stage at 14°C (13 days) is only a maximum of 6.5 days longer than the shortest median duration at 26°C (6.5 days), opting for 14°C makes most sense given that hatch rate is more than doubled at this temperature.

The potential for storing the eggs at low temperatures with respect to delaying their hatching was investigated. This could be important especially if the species is to be mass produced since delaying egg hatching could allow any stage of the life cycle to be available year round. Gormally (1985) found that eggs from the univoltine species *I. albiseta* that were stored for more than 200 days at a temperature of 2 – 3°C, hatched successfully. In this study, the majority of eggs held at 2 – 3°C for 7 – 41 days failed to hatch when returned to laboratory temperatures with only six out of 199 eggs hatching successfully. Of the six eggs that hatched, five of them were held at 2 – 3°C for 11 days and one for 10 days. Five of these eggs hatched seven days after being returned to laboratory temperature while the sixth took 18 days. Although only a small number of eggs hatched, the median duration for the eggs to hatch once returned to room temperature was 7 days which is similar to the median duration found at laboratory temperature in this study. These preliminary results suggest that subjecting eggs to a low temperature for a long period of time will result in extremely low hatch rates. A similar study using *Sepedon spinipes* eggs also resulted in the failure of eggs to hatch after being held at 4°C for 300 days (Mc Donnell and Gormally, 2007). Mc Donnell and Gormally (2007) suggest that perhaps the phenology of the species plays a part in this. The univoltine species *I. albiseta* overwinters as larvae and the eggs do not hatch until the colder

autumn/winter months. In contrast, *S. spinipes*, a multivoltine species, overwinters as an adult and completes its oviposition period before the onset of the colder winter months. As a result, the eggs of *I. albisetia* are probably more adapted to hatching at lower temperatures than those of *S. spinipes*. The same theory could apply to *T. elata* in that it overwinters as a pupa with eggs having hatched prior to the commencement of the cooler winter season.

The effect of temperature on larval development was similar to the effect on the egg stage. As temperature increased, overall median larval duration generally decreased, with development at 23°C being the fastest of the controlled temperatures with a median duration of 37.5 days. These findings again mirror temperature experiments carried out by Gormally (1987) and Mc Donnell *et al.* (2005) on *I. albisetia* and *S. spinipes*. The survival rate for *T. elata* larvae, however, was poor at the higher constant temperatures with no larvae reaching third-instar at 26°C and only 4% larval survival at 23°C. This suggests that the larvae may not be tolerant of constant high temperatures. Given the survival rate of larvae reared at ambient outdoor temperatures (52%) (which saw the temperature reach a maximum of 30°C), it is likely that larvae can tolerate high temperatures but not when exposed to them constantly (Krebs and Loeschke, 1995; Hoffmann *et al.*, 2003). Taking both the median duration (44 days) and the percentage survival of the larvae (62%) at the constant temperatures into consideration, 20°C is recommended as being the optimum temperature at which to rear larvae for mass production purposes.

Although 65 pupae were formed during this study, only 10 adult flies successfully eclosed. A facultative quiescent period is thought to exist during this stage for *T. elata* and Knutson *et al.* (1965) suggests that subjecting pupae to low temperatures for several weeks before returning them to laboratory temperature could break this diapause. In this study there were not enough pupae to undertake such investigations. However, five of the ten adults that emerged from pupae in this study, were accidentally subjected to a drop in temperature from 17°C to 2°C

for at least eight hours before being returned to room temperature. These five adults all emerged between 3 – 27 days after this temperature drop.

Manguin (1989) determined the sex of adult *Tetanocera ferruginea* prior to their emergence with 80.4% accuracy based on the length of the puparia. She also found that females of the species (length and width) were significantly larger than males. Following from this, Mc Donnell *et al.* (2005) found that the sex of *S. spinipes* could be determined with a 95% accuracy using the weight of the puparia. Preliminary results from this study also indicate the possibility of predicting the sex of the *T. elata* adult based on the weight of the pupa before emergence. An air stream separator could be used as an automated method of separating the adults prior to emergence, thereby reducing the need for manual labour and consequently lowering the costs of a mass-rearing system. Such separators use an air stream (generated by an adjustable fan) to lift lighter puparia (males) to a collecting vessel at the top of the machine, while heavier puparia (females) sink to a lower chamber (Bautista *et al.*, 1999, Jackson *et al.*, 1996). The possibility of predicting the sex of an adult before its eclosion is beneficial in that pupae are easier to manipulate than motile adults. This would, therefore, be useful both in terms of facilitating mass cultures and also in terms of biocontrol release programmes should future studies indicate that the pupa is the best stage to release *T. elata*.

Data regarding the number of slugs killed by a potential biocontrol agent is essential in determining its efficacy. From this study it would appear that temperature is not an important factor governing predation capacity. A median of six, seven and eight individuals of *D. reticulatum* were killed by each larva at laboratory/outdoor temperatures, 17°C and 14°C/20°C/23°C respectively, which is important information for determining adequate numbers of individuals required for release to control pest slug populations in the target area. That the majority of these killings occur after the larva exhibits predatory behaviour, shortly before the third-instar larval stage, has important implications for deciding the most suitable stage for release in the field. Releasing third-instar larva would ensure that slug

killing would begin quickly and a number of slugs could be targeted over a shorter time compared to the earlier parasitoid instar stages. In saying this however, transportation and handling of third-instar larvae may not be feasible due to the fragile nature of the larval stages. The releasing of pupae may, therefore, be the most appropriate option in terms of maximising survival of *T. elata* for the biological control of pestiferous slugs. It would also ensure that eggs are laid in locations where larvae, upon hatching would be in close proximity to their host. However, timing of release would be of utmost importance to ensure that third-instar larvae resulting from the pupae are synchronised with pestiferous slug populations. Further research is required to determine the optimum stage (in terms of efficacy and transportation limitations) at which to release *T. elata* as a biological control agent.

The ability of a predator to locate its prey is key to the success of a good biological control agent. *Tetanocera elata* larvae, however, do not appear to exhibit a single prey location mechanism. Trail following has been noted for species such as *I. albiseta* (Dillon *et al.*, 2014) (Appendix III) and *Dictya montana* (Mc Donnell *et al.*, 2007) with the possibility of influencing, to some extent, the food preferences of larvae for particular snail species (Dillon *et al.*, 2014). Trelka and Berg (1977) who undertook experiments to investigate the response of *Tetanocera plebeja* to slug mucus trails revealed that *T. plebeja* followed fresh mucus trails only after recent contact had been made with the prey species. Taking into account the limited numbers of larvae used, this study identified that *T. elata* third-instar larvae do not appear to require recent contact with a slug before searching for their prey. They did not appear to follow the mucus trails left behind by the slugs although an increase in larval activity was noted in the presence of these trails. Three different types of prey searching strategies used by the larvae were identified: 1) searching and attacking (SA); 2) searching and waiting (SW); and 3) waiting (W), each of which resulted in an attack on a slug. What determines the strategy used by the larva is not clear. It is possible that tracking of volatile cues released by the slug or present in the mucus (which is a recorded

prey location mechanism in the malacophagous larvae of the carabid beetle *P. melanarius* (Thomas *et al.*, 2008)), plays a role in their active search behaviour.

The searching behaviour strategy could also be dictated by other factors such as level of hunger or proximity (in time) to pupa formation. Dillon *et al.*, (2014), found a significantly stronger response by *I. albiseti* larvae to snail mucus trails after four days without feeding compared to one, two and three days without feeding. Between feeding, third-instar *T. elata* rested for long periods and ceased feeding 4 – 5 days before pupation occurred (Knutson *et al.*, 1965). Before the commencement of trials in this study, larvae which subsequently displayed different searching behaviours, were left without food for at least four days so it does not appear that level of hunger dictated the searching strategy of the larvae. From the seven trials that did not result in feeding, four larvae pupated one or two days later or subsequently died. Further investigation is required to determine confidently the manner by which slug prey is located by *T. elata* larvae and the factors that may affect the strategy chosen. Dillon *et al.*, (2014) showed that the first meal choice of *Ilione albiseti* can influence subsequent prey selection and following from this it is important to bear in mind that early behaviour may influence subsequent behaviour of the larva. Having said this, perhaps having different strategies is an evolutionary adaption to maximise the likelihood of third-instar larvae finding prey. This adaption would have implications regarding the biocontrol potential of the species in respect of higher numbers of larvae surviving and in turn increased numbers of slugs being killed.

Knutson and Vala (2011) suggest that slug-killing sciomyzids would be suitable for biological control in greenhouses where temperature and humidity can be regulated to suit particular crop types. Summer crops grow best at 23°C to 30°C during the day while cool weather crops e.g. lettuce and broccoli grow best at 15°C to 18°C (Organic Gardening, 2014). Based on the results of this study, it appears that larvae can survive these temperatures although not when exposed to a constant high temperature of 26°C. As temperatures fluctuate in a greenhouse both during the day and at night, it is unlikely that larvae would be exposed to constant

high temperatures at least in temperate climates. Greenhouses may, therefore, be a good place to run preliminary release trials with the species before the commencement of controlled field release trials in agricultural settings.

Barker *et al.* (2004) lists requirements necessary for a successful biological control agent and the following requirements are discussed in relation to *T. elata* and its potential as a biological control agent.

1) Laboratory rearing:

The success of a biological control agent requires the capability to release a large number of the agent in the field (Barker *et al.*, 2004). In this study, 14°C was established as the best of the temperatures tested to return the highest egg hatch rate for *T. elata*, while subjecting the three larval stages to a constant temperature of 20°C allows for the best return in terms of both larval survival and shortest duration. With this information it is possible to mass produce the species to pupal stage under laboratory conditions. Considering the low number of adults that emerged from pupae formed under laboratory rearing conditions (10 from 65), further research, as mentioned below under Recommendations for Future Research is recommended to improve or ensure the successful emergence of adults.

2) Long-distance transport

If a biocontrol agent is to be mass produced, it is important that the agent is in a form that allows it to be easily transported from the site of production to the site where the target pest is found. Biological control agents which are currently commercially available are sold in different forms. *Adalia bipunctata*, for example, which is a ladybird used for aphid control is sold in both its larval and egg stage. Other control agents, however, such as *Eretmocerus eremicus*, a parasitic wasp used to control white fly, is sold as loose pupae (www.biobest.be). Although not tested in this study, it seems fair to suggest that like other Sciomyzidae, *T. elata* is robust in its egg, pupal and adult stages (Barker *et al.*, 2004). The adult stage, while still transportable, is likely to be the most

susceptible to damage and mortality and therefore the pupal form is likely to be the more appropriate form to use for transport and handling.

3) Favourable recipient environment

The individuals collected in the field for this study were captured in either uncultivated field margins or ungrazed grassland. Plant species identified throughout the field margins were *Trifolium pratense*, *Urtica dioica* and *Rumex* spp. (Appendix I, Figure 1), while the dominantly occurring species in the ungrazed grasslands were *Holcus lanatus*, *Anthoxanthum odoratum* and *Rubus fruticosus*, with *Dactylis glomerata*, *T. pratense* and *Cirsium vulgare* occurring frequently (Appendix I, Figure 2). The creation of a habitat which aids the establishment of *T.elata* populations adjacent to vulnerable crops could be incorporated within “beetle banks” which provide suitable habitats for carabid predators (e.g. *Pterostichus melanarius* (Illiger)) of crop pests (Thomas *et al.*, 1991; Lys and Nentwig, 1992; Symondson, 2004). In a study carried out by Thomas *et al.* (1991), grass strips were created within crops in an attempt to improve beetle overwintering sites and in turn, increase densities of invertebrate predators. It was found that the plant species *D. glomerata* and *H. lanatus* provided the most appropriate overwintering habitat which returned high densities of predatory Carabidae. Given that both of these plant species were present in the habitat where *T. elata* was captured, it is fair to suggest that beetle banks could be explored as a potential suitable habitat for *T. elata*. It may also be necessary to provide some of these plant species (carefully controlled to prevent spreading into the crop) in greenhouses to provide a suitable habitat for the maintenance of a *T. elata* population.

4) Pest suppression

As found for this study, the median number of *D. reticulatum* individuals (between 6 and 8) killed by one *T. elata* individual, permits a rough estimation of the quantity of individuals needed to reduce the number of slugs in a known population. However, because slug activity is dependent on weather conditions

and time of year, it is difficult to determine the absolute densities of slugs per m² in arable land. A project by Glen *et al.*, (2002) which investigated different methods for sampling slug populations, indicated a population high of 191 slugs per m² (using bulked core samples). However, this is the highest figure during its peak hatching time and populations will be lower than this at different times of the year. As a result, the timing of the release of the agent is also important so that high populations of *D. reticulatum* can be targeted. Models for predicating populations of *D. reticulatum* have been examined (Choi *et al.*, 2006) and could be used to help determine the best time for release of the agent.

5) Stability in permanent crops versus opportunism in temporary crops

Permanent stability of enemy-prey interactions and pest suppression is required in permanent crop habitats, while in temporary crop habitats it is only required temporarily. A functional response can provide a means for regulating prey density in both of these habitats. This is the ability of an individual predator to kill more pests as pest density increases. Whether or not *T. elata* is capable of this response was not examined in this study. Only one live slug was given to each larva at a time and a new live slug was added only when the larva had stopped feeding on the dead slug. However, some sciomyzid species have been found to have a functional response e.g. Mc Donnell (2004) found that *S. spinipes* fed on more individuals of *Radix baltica* when the snail species was present in excess.

6) Freedom from natural enemies

According to Knutson and Vala (2011), there are no natural enemies of Sciomyzidae that would significantly limit the use of these species as biological control agents. Nevertheless, they do suggest the parasitoid Hymenoptera as a potential threat. *Trichopria atrichomelinae* Muesebeck and *T. popei* (Muesebeck) (Hymenoptera: Diapriidae) are two known species that are internal parasitoids of some species of aquatic and terrestrial Sciomyzidae including *T. plebeja*, *S. fuscipennis* and *Dictya* sp. (O'Neill, 1973). However, further research would be required to investigate whether this order would have a detrimental effect on *T. elata* populations. Knutson and Vala (2011) suggest placing larvae reared in the

laboratory in suitable containers in the field and returning them to the laboratory to await emergence of possible parasitoids from pupae. Egg parasitoids could also be assessed using this method by leaving eggs in the field and subsequently returning them to the laboratory.

7) Minimal adverse effects on biodiversity

In classical biological control, imported natural enemies of an invasive species are released into an affected area (Van Driesche and Abell, 2009). This could apply in countries such as the USA where European slug species are now serious pests (Mc Donnell *et al.*, 2009) and where *T. elata* could be potentially introduced as a biocontrol agent. However, the implications of introducing a non-native sciomyzid as a classical biocontrol agent would require many years of research to determine its possible effects on non-pest endemic slug species. The discovery that *T. elata* can attack and kill the EU protected species *Geomalacus maculosus* (Gastropoda: Arionidae) (Giordani *et al.*, 2014) (Appendix II) is a reminder that while *T. elata* could be a useful biological agent of pestiferous slugs, it also has the potential of killing other non-pest species. *G. maculosus* is currently only found in western Ireland and northern Portugal and Spain (Kearney, 2010; Mc Donnell *et al.*, 2013) in habitats such as blanket bog, *Quercus* dominated deciduous woodland, commercial forestry plantations and wet grasslands (Mc Donnell *et al.*, 2013; Reich *et al.*, 2012). Compared to the habitat in which *T. elata* is found as discussed previously, it is unlikely that these two species would occur in the same habitat e.g. Tillage crops are generally located on better soils in Ireland and would not generally overlap with the habitats mentioned above. However, it highlights the importance and need for considering potential non-target species prior to the release of this species as a control agent.

Based on the results gathered from this research both in terms of the ability to raise the species in the laboratory and their ability to locate prey, there appears to be potential for the use of *T. elata* as a biological control agent whether it be augmentative control or conservation biological control in both greenhouses and in the field.

- Augmentative control where the species is commercially reared and sold at a stage ready for releasing (Van Driesche and Abell 2009) could possibly be applied to *T. elata* by rearing the larvae under the conditions described above and releasing pupae in affected areas. Augmentative control provides pest suppression for a limited time period since the eventual decline of the target pest population often results in less food for the predator.
- Conservation biological control which focuses on enhancing the survival rate of the species by managing the habitat in which it is found (Barbosa, 1998) is also a possibility. Due to the occurrence of *T. elata* in arable field margins (Speight, 2004), these could be maintained in such a way (yet to be determined) to increase the population of the species. This habitat enhancement could possibly be incorporated with beetle banks which provide suitable habitats for carabid predators of crop pests e.g. *P. melanarius* (Illiger) (McKemey *et al.*, 2003).

Further research, some of which is mentioned below under Recommendations for Future Research, is required before the true potential of this species as a biological control agent can be determined.

5.2 General Conclusions

With respect to this study, the following general conclusions apply:

- From the controlled temperatures used in this study, the optimum temperature at which to rear the species appears to be 14°C for egg stage and 20°C for larval development giving a median duration from egg stage to pupal stage of approximately 57 days (13 days: egg hatch; 44 days: larval development).

- Each larva has the potential of killing a median of 6 – 8 individuals of *D. reticulatum* over a range of temperatures.
- The sex of adults can be determined by pupal weight prior to pupal eclosion. Pupae can be divided by weight, the heavier pupae indicating the presence of a female.
- While larvae can actively search for a slug without having made previous contact with the slug, other predatory behaviours were observed including searching and attacking; searching and waiting; and waiting.

5.2 Recommendations for Future Research

The results of this study indicate a number of future research pathways to fully realise the potential of *T. elata* as a biological control agent. Examples of further research could include:

- As the hatch rates of *T. elata* eggs were low compared to other species of sciomyzids, rearing eggs at temperatures below 14°C and possibly across a range of humidity levels could be examined in an effort to improve hatch rates. As *T. elata* is a terrestrial species, placing its eggs on moist filter paper in direct contact with a permanently wet surface, may not have been the most appropriate conditions for egg development. Instead a saturated salt solution or silica gel could be used to regulate humidity (Piechota, 1992) in chambers in which the eggs are placed. In addition, an individual slug could be maintained with each pair of adult flies to investigate whether an adult female would lay eggs on the slug or slug mucus or whether oviposition rates increase in the presence of suitable prey. Some species of sciomyzids lay their eggs directly on their hosts but there is no record, to date, of this for *T. elata*.

- Investigations could be undertaken to determine whether the larva would return to a previously killed *D. reticulatum* using choice experiments with live and dead individuals coupled with exposing the third-instar larva to more than one slug of the same species as well as different pest slug species. If given multiple *D. reticulatum* to feed on, will the larva feed for a shorter duration before moving to its next host or will it remain on one slug for the same length of time as those fed individual *D. reticulatum* slugs and in turn will this affect larval duration and percentage survival? Prey preference trials using different pest slug species also need to be carried out to indicate the potential of *T. elata* to control these pest species should it also be required.
- Further explore the possibility of breaking the overwintering diapause by subjecting the pupae to periods at low temperatures. Knutson *et al.* (1965) successfully subjected pupae to low temperatures for several weeks to induce the emergence of adults on their return to laboratory temperature. Trelka and Foote (1970) found similar outcomes for *T. plebeja* (6 – 10 weeks at 5 – 10°C) and *T. valida* (2 – 3 months at 5 – 10°C) but further investigation based on these findings needs to be undertaken on *T. elata* to confirm the least amount of time required at low temperature to induce emergence.
- Recording more detailed *T. elata* habitat preferences / phenology to determine appropriate field conditions for biocontrol release programmes. If the ideal habitat including preferred plant species and structure was identified for *T. elata*, it could be incorporated into a); field margins to encourage an increase in population or b) “beetle banks” which are created to increase the population of beetles which also prey on slugs. Williams *et al.*, 2010 carried out a mark-recapture study in a turlough (seasonal lake) located in the west of Ireland which suggests that Sciomyzidae have limited movement throughout their habitat. Mark-recapture experiments were carried out on zones (10m x 10m) which were 23 meters apart and

differed in respect of dominant vegetation and structure. From the 47 recaptured individuals (e.g. *I. albiseta*, *Pherbina coryleti*, *S. spinipes*, *Tetanocera* spp. *Hydromya dorsalis*) all were subsequently recaptured in their original capture zone. This indicates that Sciomyzidae do not travel far (at least not greater than 23m) throughout their chosen habitat. This is also supported by Knutson and Vala (2011). Although not captured in the study by Williams *et al.* (2010), should the same phenomenon be found for *T. elata* it would be encouraging for biological control in that once released and established, *T. elata* would remain within its released habitat.

- Determining how larvae find their prey and the reasons for the three searching approaches used by larvae in this study. Sensilla have previously been identified using light microscopy and stereoscan studies for some species of Sciomyzidae, including the slug-killing *E. cribrata* (Knutson and Vala, 2011). Based on this, investigations could be undertaken to establish whether sensilla are used by *T. elata* larvae for prey location. In studies of other invertebrates, Thomas *et al.* (2008) examined the ability of *P. melanarius* larvae (Carabidae) to detect slug (*D. reticulatum*) odours using behavioural bioassays. Similar methods could be employed for *T. elata* to determine whether it responds to specific odours or prey items.

5.4 References

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Appendices

Appendix I

APPENDIX I: Site, grid reference and date of collection for field-collected *Tetanocera elata*

Site	Hare Island, Lough Ree, River Shannon		Kilcolgan, Co. Galway		Green Earth Organics, Corrandulla, Co. Galway		Beechlawn Organic Farm, Ballinasloe, Co. Galway		Cummer, Tuam, Co. Galway	
Description	Abandoned grassland surrounded by hedgerow		Ungrazed grassland alongside hedgerows		Uncultivated field margin of tilled organic field		Uncultivated field margin of tilled organic field		Uncultivated margin of large potato field	
Grid Reference	N 044 470		M 416 183		M 353 383		M 848 298		M 488 360	
Date Collected	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
17/08/2011	0	0	0	0	1	1	0	0	0	1
30/08/2011	0	0	0	0	3	1	0	0	0	0
31/08/2011	0	0	0	0	3	2	0	0	0	0
06/07/2012	0	0	0	0	1	0	2	2	0	0
11/07/2012	0	0	0	0	2	2	0	0	0	0
20/07/2012	0	0	0	0	0	0	3	0	0	0
27/07/2012	0	0	0	0	0	0	4	2	0	0
19/07/2013	0	0	0	0	0	1	0	0	0	0
25/07/2013	0	0	3	2	0	0	0	0	0	0
14/08/2013	2	2	0	0	0	0	0	0	0	0
16/08/2013	2	2	0	0	0	0	0	0	0	0
Total	4	4	3	2	10	7	9	4	0	1



Figure 1: Uncultivated field margin habitat of field-collected *Tetanocera elata*



Figure 2: Abandoned grassland habitat of field-collected *Tetanocera elata*

Appendix II

Appendix II: Short Communication

***Tetanocera elata* (Diptera: Sciomyzidae) Larvae Feed on Protected Slug Species *Geomalacus maculosus* (Gastropoda: Arionidae): First Record of Predation**

Irene Giordani, Tracy Hynes, Inga Reich, Rory J. Mc Donnell, Michael J. Gormally

Giordani, I., Hynes, T., Reich, I., Mc Donnell, R.J., Gormally, M.J., 2014. Tetanocera elata (Diptera: Sciomyzidae) larvae feed on protected slug species Geomalacus maculosus (Gastropoda: Arionidae): first record of predation. Journal of Insect Behavior. 27 5 652-656.

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Appendix III

Appendix III: Influence of snail mucus trails and first snail meal on the behaviour of malacophagous sciomyzid larvae.

Robert J. Dillon, Tracy M. Hynes, Rory J. Mc Donnell, Christopher D. Williams, Michael J. Gormally

Dillion, R.J., Hynes, T.M., Mc Donnell, R.J., Williams, C.D., Gormally, M.J., 2014. Influence of snail mucus trails and first snail meal on the behaviour of malacophagous sciomyzid larvae. Biological Control. 74, 6-12.

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