Better Use of Molluscicide Pellets for Improved Management of Slugs

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For my grandmother, Eleanor

General Abstract

The grey field slug, *Deroceras reticulatum*, is one of the most important pests of agriculture, with an estimated 59% and 22% of total planted oil seed rape and wheat respectively affected by slugs in the UK annually. It is estimated that without molluscicides, the cost to the UK's agricultural industry from loss of crop yield due to slug damage could be in excess of £100 million per year. Metaldehyde is the most commonly used molluscicide in UK agriculture however it presents a large water pollution risk and threat to non-target organisms, in addition, control of slugs by metaldehyde is often inefficient.

Understanding how *Deroceras reticulatum's* behaviour is affected by the presence of slug pellets with different molluscicide concentrations, and how these pellets affect locomotion, feeding and the health of a slug once a pellet is consumed will help develop more effective slug pellets. The pest-pellet interaction is a key process in successful slug control and can only be effective if slugs accept and consume a pellet when encountered. This research indicated that the concentration of metaldehyde in pellets does not impact on *D. reticulatum's* foraging activity, however the presence of a molluscicide may impact on foraging behaviour compared to non-molluscicide pellets. *D. reticulatum* was more likely to accept and feed on a pellet of lower concentration (1%) compared to higher concentrations (3% and 5%).

Paralysis due to the effects of molluscicide is likely to be a key factor in successful slug control. Slugs poisoned with 1% or 3% metaldehyde were more likely to be paralysed after exposure to pellets when compared to higher concentrations, however, were more likely to recover after exposure to 1% pellets.

This research has demonstrated that slugs are highly sensitive to metaldehyde. *D. reticulatum* can detect metaldehyde that has been incorporated using inorganic Controlled Release Technology, with as little as 0.28% metaldehyde content. Controlled release technology involving molluscicides is still in its infancy, however, has potential as a future focus for improved pellet design.

Declaration of Authorship

I declare that this research herein is my own intellectual property. Dr Gordon Port, Dr. Roy A. Sanderson and Prof. Stephen P. Rushton and Dr. Neil Audsley made contributions to each chapter. Additional contributions to the thesis have been acknowledged appropriately.

Inorganic controlled release formulations trialled in chapter 5 were manufactured and provided by Lucideon LTD. Lonza Axcela® metaldehyde and control grain pellets were manufactured and provided by Lonza.

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Chapter 1: General Introduction

1.1 Introduction to the Research

This research provides behavioural insight into how *Deroceras reticulatum,* the grey field slug, interacts and is affected by metaldehyde-based slug pellets. Metaldehyde pellets are the most frequently employed form of slug control across the globe, and without such control slug damage could cause in excess of £100 million to the U.K. agricultural industry annually (Nicholls 2014). This research focuses on the interaction between pest and pellet, and how the locomotive and foraging activity *D. reticulatum* is affected by the presence and consumption of metaldehyde. This research also provides information into the initial testing of a novel pellet formulation, using inorganic Controlled Release Technology for metaldehyde, which has the potential to reduce the damaging environmental risks that application of metaldehyde pellets present, and increase slug control efficiency in the field.

1.2 The Grey Field Slug

Deroceras reticulatum, or the grey field slug, is the most economically important gastropod pest of agriculture and horticulture in the UK and many temperate regions of the world (Shirley et al. 2001). A particular threat to oilseed rape (OSR) and winter wheat, the slug presents a threat to the success of a plant at each life stage. Slugs cause damage under the soil surface by seed hollowing and grazing of germinated seeds, and above the soil surface through root and shoot shredding and feeding. It is estimated that without control in the U.K. damage to crops from slugs could cost the agricultural industry in excess of £43.5 million annually for OSR and winter wheat alone, adding to a total in excess of £100 million for the entire agricultural sector (AHDB 2015).

The grey field slug (Class: Gastropoda, Family: Agriolimacidae) is a small (3-5cm in length) slug that varies from a milky white to grey/ brown in colour, which is often dappled. The body of the grey field slug follows a similar plan of other terrestrial gastropod molluscs. The body is divided into four main sections: The head which features the mouthparts sensory tentacles and redundant eyes, the foot with the pedal gland at the front of the animal, the mantle which is a fold of skin of the respiratory cavity on the dorsal part of the slug and the visceral mass underneath the mantle that contains the inner organs (Baker 2001)

The skin of the *D. reticulatum* is extremely thin and is protected with a layer of mucus which cover the whole slug body. Slug mucus has extremely high water content of between 80- 99% water (Davis and Hawkins 1988) and is produced by the pedal gland on the front of the foot of the slug which further regulates body water content but more importantly facilitates movement and locomotion (South 1992). In addition to the pedal gland, there are glands that cover the body of the slug that secrete mucus which also regulate the slugs water content. *D. reticulatum* has a body water content of 80% and can rehydrate from dermal contact with moisture such as damp soil.

The mouthparts of terrestrial molluscs are unique compared to their aquatic relatives. The mouth consists of a circular opening called the radula which contains layers of chitinous teeth arranged around the tongue of the buccal cavity (Wright and Huddart 1999). Slugs rasp on their food source as opposed to taking bites, grazing and shredding their food. Once consumed, food enters the oesophagus and a crop which contains digestive enzymes (Bowen 1970). Partially digested food then passes into the stomach followed by the intestines and is eventually expelled via the anus.

Due to their high body water content, *D. reticulatum* is frequently associated in areas of high soil moisture. The ability to remain hydrated impacts on all parts the slug’s life from locomotion, to feeding to reproduction, and therefore slugs are more commonly active at night-time when the ambient temperature is lower, and moisture (atmospheric and soil) is higher. This endogenous rhythmical activity is seen across most terrestrial molluscs and shows seasonality following changes in light levels (Morton 1979, Sokolove et al. 1977). Dependency on moisture is also likely to influence movement of populations and individuals of slugs. Slug activity is heavily dependent on meteorological conditions including precipitation and temperature. Studies have shown that in lower temperatures (less than 10°C *D. reticulatum* travel significantly less distance than compared to temperatures of around 15°C (Godan 1983).

Damage to crops by *D. reticulatum* coincides with maximal hatching of young and the population increase because of this. Breeding of *D. reticulatum* can occur throughout the year, however peaks in autumn and spring (Howlett et al. 2005, Hunter 2006) Forecasting of slug damage is possible and used in order to judge when to apply molluscicide pellets. Slug forecasting considers weather conditions soil type, stage of plant growth, season of sowing and proximity to areas of refuge such as meadows (Glen et al. 1993).

1.3 Control of slugs

The protection of crops from pest predation is imperative in order to feed a human population that is expected to reach over 9 billion people by 2050 (Godfray et al. 2010). Control of pest populations of slugs can be divided into three categories: physical, biological and chemical control. A combination of methods is advised in order to obtain optimal slug control as part of an integrated pest management scheme (Glen 2000 and Leake 2003), which aims to reduce the amount of synthetic pesticide applied to arable land.

Physical (mechanical) control or prevention of slug damage includes ploughing, removal of weeds from field margins and rolling after seed drilling in order to create a firm, compacted seed bed in order to reduce slug access (Get Pelletwise 2020). These mechanical practices are not preventative in terms of slug control but can significantly reduce slug damage later in the season (Douglas and Tooker 2012).

Biological control of slugs encompasses both naturally occurring predators and artificially supplemented parasites on the field. The application of nematodes, notably *Phasmarhabditis hermaphrodita,* has become increasingly popular in the control of slugs over the past 10-20 years. A facultative parasite, *P. hermaphrodita,* can be applied to soil during watering and infect slugs upon contact, usually in an area below the mantle and cause swelling of the mantle, leading to death 7-21 days after infection (Rae et al. 2008). There are several issues with the use of this biological agent which make large scale agricultural use impractical. Firstly, the cost of applications of nematodes is significantly higher compared to metaldehyde pellets (£14 for Nemaslug® to treat 0.004 ha vs £10- £15 for metaldehyde to treat 1 ha). Nematodes also have a short range, and therefore application must be precise in order to avoid further increased costs, as well as being sensitive to temperature and moisture. For these reasons’ nematodes remain a viable option for slug control for gardeners and allotment owners, but application in an agricultural setting is not practical.

Slugs have several natural predators, birds, hedgehogs and ground beetles (Mair and Port 2002) often share similar habitats to where pest populations of slugs are found, though control by natural predators is likely to be less successful in controlling pest slug populations compared to synthetic pesticides (Ayre 1995).

1.4 Synthetic Pesticides

Whilst the employment of pesticides has long been documented as early as 2500 BC (Flint and van den Bosch 1981), synthetic pesticides have only gained momentum in their development and usage in the second half of the 20th century (Haines 2000), predominantly due to advances in technologies surrounding synthetic chemistry.

Probably the most infamous synthetic pesticide to come of the 20th century was the insecticide DDT, or dichlorodiphenyltrichloroethane. Though initially described as a “wonder chemical” (North America Pesticide Action Network 2020) and used as an insecticide worldwide having proven to be effective in controlling both insect borne diseases such as malaria and agricultural pest infestations, it was not until the latter half of the 20th century that the negative effects of DDT on human health and the environment were drawn to the attention of both scientists and the general public. Though the effects of DDT on the environment had been noted previously by scientists, it was not until the documentation of these effects in Rachel Carson’s Silent Spring (Carson 1962) that the devastating impact of such a chemical on the environment and human health was made visible to the world. The detrimental effects on human health and undeniable evidence of the devastation caused by the chemical to wildlife created a huge backlash from the public on chemical companies and manufactures of DDT and scientists and governing bodies that promoted its usage. Silent Spring is regarded as one of the “founding texts of environmentalism” for this reason (Dunlap 2008) and marks a significant shift of the attitudes of not only the general public towards pesticides, but of also the way in which pesticides are created, applied and monitored in the environment. Risks and issues from pesticides produced in the latter half of the 20th century echo Carson’s warning of the impact such chemicals in the environment, and to this day pesticides still present a range of serious environmental and public health issues such as non-target poisonings, pesticide resistance, bioaccumulation in both animals and vegetation, leaching and water pollution and human health impacts such as respiratory issues (Aktar et al. 2009), the reduction of which are now the focus of development for an effective but safe pesticide.

Chemical pesticides vary greatly in their formulation and application, which is typically dependent on the target species. Application of pesticides can range from wettable powders, water dispersible granules, seed treatments, spray mists and dusts and pellet granules and rates of application not only depended on the formulation type, but a range of biotic and abiotic factors that can make pesticide application requirements highly variable from one season to the next (Seaman 1990). The primary focus in developing a new pesticide formulation is the biological effect and selectivity for the desired target species (Sparks 2013). The demand for species-specific chemical pesticides has increased greatly as a result of the shift in agricultural practices to an integrated pest management (IPM) approach, which aims to minimise the amount of chemical pesticide applied by greater management and inclusion of physical, biological and cultural (as well as chemical) control into farming practices (Stenberg 2017). Apart from being species specific, the effect of the pesticide once in the environment must be considered. Pesticides should have minimal impact on the environment, be safe to the user and be applied at the lowest possible dosage (Knowles 2008) as well as ideally being biodegradable, non-persistent and inexpensive to both the manufacturer and user.

Control of slugs with synthetic pesticides is by far the most common practice in agriculture. Cheap, easy to apply and requiring no maintenance, pellets can be applied both before and during the growing season. Pellets are often incorporated into the seed bed during drilling as a first line of defence, but additional pellets are broadcast over the surface of the soil, particularly during the early stages of plant growth. Two molluscicides dominate the market: metaldehyde and ferric phosphate (iron III phosphate). Metaldehyde is by far the most commonly used molluscicide globally, employed by 23 of the 27 EU member states (EU Pesticides Database 2021), the USA and Australia. The use of ferric phosphate is becoming increasingly common. Sold as an organic pesticide because the active ingredient is not synthetic, and at a slightly greater cost compared to metaldehyde (£10-15/ha for metaldehyde and between £4-5 more expensive per hectare for ferric phosphate), (DEFRA 2010) and, the use of ferric phosphate is likely to be increasing due to a pressure to move away from metaldehyde and as public demand increases for organic produce.

Metaldehyde is the cyclic tetramer of acetaldehyde and hydrolyses quickly upon slug consumption to acetaldehyde which leads to increased mucus production from the slug and rapid dehydration (Castle et al. 2017). The molluscicide is incorporated into a grain-based pellet which often contains a dye in order to discourage consumption by non-target organisms, and anti-fungal agents. Metaldehyde slug pellets are sold commercially at concentrations between 1-5%, however pellets up to 10% concentration have been documented (Gupta 2007). These pellets irritate slug mucus glands, causing excessive mucus secretion. Slug mucus is extremely high in water content (Davis and Hawkins 1998), and so excessive mucus production can rapidly dehydrate a slug. At higher consumption levels, metaldehyde is a nerve poison, and can paralyse the muscle mass of an animal if consumed in high enough quantities. Dehydration due to excessive mucus loss immobilises the slug shortly after consumption of metaldehyde on the soil surface, eventually leading to complete desiccation and death. After poisoning, slugs typically remain on the soil surface immobilised, due to the lack of ability to produce mucus to move, and it is at this point where either desiccation or predation from naturally occurring predators is likely to occur. Dehydration is further increased by sunlight and an increased ambient temperature after dawn, which may lead to quicker death. Predation by ground beetles, birds and hedgehogs is likely to be at an increased rate when slugs are paralysed on the soil surface (Langan 2004), which will contribute to control by metaldehyde pellets.

1.5 Problems with Metaldehyde

Over the past 20 years several problems with the use of metaldehyde on agricultural land have arisen. The effectiveness of control by metaldehyde pellets has been questioned. Though farmers are routinely applying pellets throughout the season, there is not a corresponding decrease in the amount of crop damage due to slugs after harvest. There is also concern with the poisoning of non-target species with metaldehyde pellets (Grubisic et al. 2018), particularly hedgehogs, though poisonings are usually anecdotal, this concern is enough to generate a negative perception of the use of such pellets among the general public, which in turn generates pressure onto growers to switch to alternative control means. There are also cases of non-target poisonings in pets, particularly dogs, however this is likely to be due to improper use or storage of pellets in households (LD50 for a 4.5kg dog is 28g of 3%

metaldehyde pellets, which is less than half the maximum recommended weight of application for a garden/ allotment of $10m^2$).

The most impactful issue surrounding the use of metaldehyde pellets is the pollution risk it presents to drinking water systems. Metaldehyde is highly mobile in water and is readily leached out of pellets on damp soil surfaces into surface water runoff, where it contaminates watercourses such as rivers and streams that run nearby the farmland. Spikes of metaldehyde levels in rivers and reservoirs have been documented over the past 10 years surpassing the EU drinking water directive of $0.1\ \mu g\ L^{-1}$ for any pesticide (Castle et al. 2017). Currently, metaldehyde is extremely difficult and expensive to remove from contaminated water systems because the chemical does not respond to traditional water remediation methods due to its high polarity (World Health Organisation 1996). Metaldehyde removal methods are a major focus of research in the water industry (Doria et al. 2013, Rolph et al. 2018) in combination with the “Get Pelletwise” scheme by the Metaldehyde Stewardship Group (2020) aimed to provide best practice information for the responsible use of metaldehyde, particularly in sensitive water catchment areas.

1.6 Slugs and Pellets: Interactions

Pellets are currently the most feasible method of slug control, due to the biology and behaviour of slugs in the field. Slug activity is highly dependent on temperature and humidity, and therefore it is possible to predict the likelihood of increased slug activity but locating precisely where patches of slugs are in the field is more challenging (Archard et al. 2005, Mueller -Warrant et al. 2014, Petrovskaya et al.2018, Petrovskaya et al. 2020). Predicting or locating the whereabouts of a group of slugs in the field would allow for precise, targeted and much more efficient slug control (Choi et al. 2004, Willis et al. 2006, Forbes 2020). The pest-pellet interaction is a key component for successful slug control, and control relies predominantly on the consumption of a lethal dose of molluscicide from the pellet. It has been speculated that inefficiencies in control are due to consumption of metaldehyde based pellets that may cause paralysis and immobilisation of the radula, rendering the slug unable to feed for long on a pellet (Bowen and Antoine 1995), however it is more likely that early detection of metaldehyde by the slug, before or during feeding is likely to be a key factor in the unsuccessful delivery of a lethal dose. Evidence suggests that slugs forage at random (Bailey 1989, Howling 1991), and so broadcast spreading of pellets over the soil surface is likely to remain the optimal method of pellet application however it is important to consider the impact that the presences of molluscicide pellets has on the slugs feeding and foraging behaviour before, during and after an encounter with a pellet.

1.7 Gastropod Locomotion

Locomotion is an energetically costly but necessary behaviour for the majority of animals. In terrestrial gastropods, the energetic cost of locomotion is increased greatly by the requirement to produce mucus in order to crawl over surfaces. Feeding, mating, predator avoidance and hydration are likely to be among the main factors controlling locomotion in terrestrial slugs and snails. The study of the locomotive behaviour of pest species of slugs and snails is vital for understanding pest population dynamics and allowing decisions to be made on appropriate and effective control of pest populations as well as providing accurate predictions of pest invasions or outbreaks. The application of molluscicidal pellets is the most utilised form of pest control for slugs and snails in agriculture and better understanding of the locomotive behaviour of gastropods once subject to these molluscicides may provide information on how to improve slug and snail control in the field.

The study of animal movement and its associated behaviours informs many areas of ecological research. Aside from satiating scientific curiosity, behavioural research can contribute towards understanding the dynamics of animal populations through space and time, which in turn allows scientists and policy makers to make informed decisions on the conservation or control of animal populations. The exact definition of “behaviour” is one that is often argued and contested amongst ethologists, and descriptions range from more simplistic terms stating that behaviour is merely any recognisable movement of an individual animal, to definitions acknowledging that behaviour is often elicited by a combination of both internal and external cues that trigger a response from one or several organisms (Colgan 1990, Levitis et al. 2009, Tinbergen 1963). Exactly what causes an animal or group of organisms to behave in a certain manner involves a complex and in depth understanding of the individual’s environment, ecology, evolution and neurology. These categories of factors controlling behaviour are scarcely studied at once, however studies of individual behavioural stimuli can be combined and modelled in order to allow predictions of behaviour to be made (Figure 1.1).

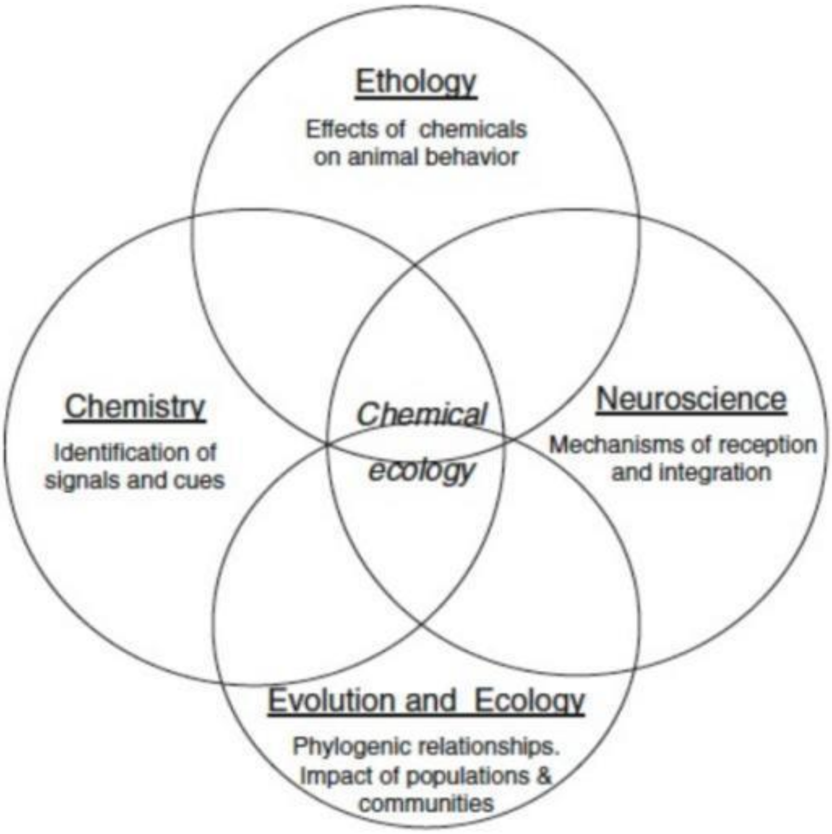

Figure 1.1 Disciplines contributing to the study of animal behaviour. Venn diagram by Derby and Sorensen (2008).

It should be noted that for the purpose of this research, the term "behaviour" will be used with regard of any internal or external recognisable movement made by an animal or group of animals, and behaviours are assumed to be generated from internal and/ or external stimuli. Internal stimuli may include hormonal or chemical changes within an organism, whereas external stimuli refer to the physics and chemistry of the animal's environment, as well as interactions with other organisms. Chemical or physical changes to an environment can have a significant impact on animal behaviour. Stimuli can generate behavioural responses by detection from a range of sensory receptors on an animal such as mechanoreceptors, chemoreceptors, electromagnetic receptors, thermoreceptors and nociceptors. An organism may only possess one or a few kinds of receptor, and response to stimuli may depend on the age or maturation of an organism, meaning sensitivity to stimuli varies both within and between species. Environmental stimuli may include long and short-term changes to an organism's surroundings such as daylight and temperature changes, varying seasons, presence of other organisms and human interference such as the application of pesticides or the removal or change of habitat. More long-term changes to environment may affect behaviour at an animal population or eventually a species level, causing an adaptive evolutionary change, a form of behavioural change that allows a population of organisms to better survive in their environment (Magurran et al. 1993).

Over time the study of the behaviour of insects and other invertebrates has gained increasing momentum, largely due to the pest status of many species of invertebrates and therefore the need to understand specifically what makes these animals successful pests in order to implement effective control methods (Nair 1985). The study of invertebrate behaviour is a vast area of research and includes quantification of actions such as movement, feeding, mating and species interactions. Information on the aforementioned behaviours can allow scientists to better understand the population dynamics of pest insects, enabling appropriate control methods to be implemented when necessary, as well as aiding in the prediction of pest outbreaks which can significantly reduce damage to crops, livestock or forestry. Therefore, better understanding of pest insect behaviour contributes directly to a more accurate integrated pest management approach to control.

Field based behavioural studies of terrestrial pest invertebrates including insects and gastropods can be time consuming and laborious, often with relatively low success due to the impracticalities involved with studying small invertebrates in a large field setting (Dent 2000). Many invertebrate pests such as species of coleopteran, lepidoptera and gastropod molluscs are more active at night (Ma and Ma et al. 2012), which can also present further difficulties for observing behaviour in a field setting. It is therefore common for behavioural assays to take place in a laboratory-based setting by which various conditions such as light, temperature, humidity and soil type can be altered to best reflect the reality of a field-based setting, but also controlled so that results acquired from research are representative and reliable. It is often the case that research questions are more easily obtained in one setting than another, for example it is easier to measure the response to stimuli in a highly controlled laboratory setting as opposed to in the field. Though it is not possible to recreate an entirely realistic field-based setting in a laboratory, and it is inevitable that certain factors that may influence behaviour that cannot be controlled such as weather and the presence of predators (Lewis and Gower 1980), laboratory studies are at least an appropriate starting point for a great deal of invertebrate behavioural research.

One of the main interests to many invertebrate behavioural scientists is the locomotive behaviour of the animal. The movement of animals can be studied at both an individual or population scale level and, in the case of pest invertebrate studies, can provide essential data on pest invasions. Locomotion is an energetically costly behaviour for most animals (Tucker 1970) and can be used to infer more complex behaviours such as foraging or mating. In the case of gastropod molluscs, the mechanism of locomotion has been studied in great detail. Identifying the factors controlling the behavioural response of gastropod mollusc movement is a vast area of research with many factors yet to be explored. Many species of terrestrial slugs and snails are significant pests in agriculture across the world. A number of these species are introduced (Cameron 2016) and can cause significant losses to agricultural and horticultural yield if not appropriately controlled. Understanding the locomotive behaviour of pest slugs and snails is therefore fundamental in developing successful pest population control techniques. Due to the limbless and adhesive nature of locomotion by slugs and snails, locomotion is not only of interest to those from a pest control perspective, but to a broad range of researchers.

1.8 The Biology of Slug and Snail Movement

Limbless crawling of animals is one of the most primitive forms of directional movement and is observed in a range of exothermic species including snakes, earthworms and molluscs (Tanaka et al. 2012). The locomotive mechanism of terrestrial gastropods has long been of interest to both biologists and biophysicists, attention from the latter due largely to the potential for advances in robotics gained from insight into gastropod adhesive locomotion (Lai et al. 2010). Biologically, the mechanism of adhesive movement by use of a single, large muscular foot has been studied extensively in both aquatic and terrestrial gastropods. In aquatic gastropods, gliding movement is facilitated by waves produced by ciliated epithelial cells along the foot (Pavlova 2010). In contrast, terrestrial gastropods are propelled forward by the contraction and relaxation of muscles that run centrally down the ventral surface of the foot (Figure 1.2) and a thin layer of mucus acts as the adhesive which affixes the foot to the substrate the animal is moving on (Pavlova 2019). Known as pedal waves, these muscular contractions create thrust and coupled with a mucus layer allow the animal to move forwards with adhesive motion (Lai et al. 2010).

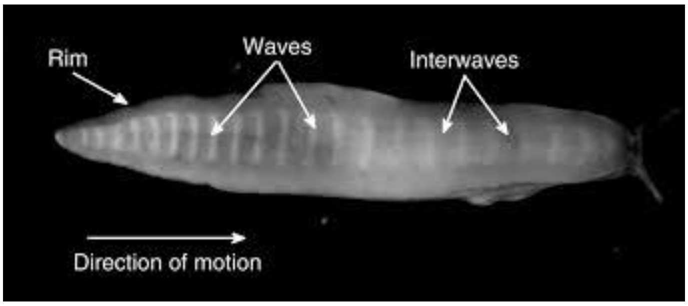

Figure 1.2 Pedal waves and interwaves on the ventral surface of the foot of the Banana slug (*Ariolimax californicus*) moving along a glass surface. Taken from Lai et al. 2010.

Slugs and snails are heavily reliant on water availability and are very susceptible to dehydration. In order to reduce the risk of desiccation, the animals are coated in a layer of viscous, clear mucus that aids the retention of water. Mucus is secreted from glands on the epidermis of the skin and is made up of a mixture of large molecules including glycoproteins, polysaccharides, lectins and hemocyanin (Deyrup- Oslen and Martin 1982). Mucus is also produced from the pedal gland at the front of the foot in gastropods that coats the whole foot in a thin layer of mucus, which allows the animal to then glide across surfaces (Godan 1983). The energetic cost of mucus production is extremely high and accounts for approximately one third of a slug's energy budget, meaning that crawling by slugs is one of the most energetically costly forms of locomotion known in the animal kingdom (Lauga 2006).

1.9 Factors Controlling Slug Locomotion

1.9.1 Water availability

It is estimated that gastropod mucus comprises of between 80-99% water (Davies and Hawkins 1998) and therefore, in order to maintain levels of mucus required to keep the animal hydrated and to facilitate locomotion, slugs and snails are heavily reliant on water or moisture availability in order to avoid desiccation and death. The majority of terrestrial slug and snail species are therefore more active at night and display a nocturnal circadian rhythm in order to avoid increased risk of dehydration during warmer daytime hours. Dehydrated slugs will gravitate to areas with where water is available, such as in damp soil (Prior 1984) in order to reabsorb water through the epidermis through contact rehydration. Some species of slugs such as *Limax maximus* have been shown to demonstrate a huddling behaviour whereby slugs will aggregate together in dry conditions in order to reduce overall body moisture loss (Prior et al. 1983). It is likely that aggregating reduces the overall surface area of a slug population and therefore reduces water loss (South 1965), it is also likely that aggregating provides a form of predator defence (Taylor 1977) and potentially thermoregulatory benefits (Richter 1976)

1.9.2 Foraging

Slugs and snails are generalist herbivores, and largely tend to forage at random and graze on food sources they encounter (Bailey 1989). The grey field slug, *Deroceras reticulatum*, has been shown to increase locomotive activity when starved (Arias and Crowell 1963, Airey 1987), whilst *Milax budapestensis* reduced its locomotive activity when subject to the same conditions as *D. reticulatum.* The superficial behavioural response to hunger appears vary between slug species (Hamilton and Wellington 1981), and it is speculated that this could be due to a range of factors including differences in the energetic cost of locomotion, the natural habitat of the slug as well as behavioural responses to stress caused to the animals due to being subject to laboratory conditions. Though it was expected that locomotive behaviour when foraging is be random and non-directional, the grey field slug, *D. reticulatum* has demonstrated preferential grazing towards acyanogenic morphs of white clover and will select more desirable feeding choices (Burgess and Ennos 1987), and therefore there may be some degree of selectivity when foraging. The presence of predators may alter the foraging behaviour of slugs, as it has been shown that *Deroceras reticulatum* may be able to detect chemical cues from the predatory ground beetle *Carabus nemoralis* (Bursztyka et al. 2013 and 2016) and avoid areas the beetle has been detected. Predator avoidance behaviour may trigger locomotion of a slug or snail, which may in turn alter the foraging success of the gastropod.

1.9.3 Trail following

In certain gastropod species, chemoreception and the olfactory system heavily influence the movement and behaviour of the animal (Croll 1983). Olfactory systems are more redundant in some gastropod species than others, and are more studied in aquatic molluscs, however species of the large terrestrial slug, *Limax*, have long been used as biological models for the olfactory system processing and learning (Matsuo et al. 2018). Behavioural responses to plant volatiles have been observed from *Deroceras reticulatum*, which would follow aqueous trails containing volatiles from food sources such as lettuce, although it is difficult to distinguish whether the animal was responding to olfactory or gustatory cues (Pickett and Stephenson 1980). Mucus trail following is a behaviour observed in many gastropod species (Terence et al. 2013) and appears to have several functions. Trail following may aid in homing behaviour of gastropods, whereby a population or individual returns to a specific resting area after feeding (Cook 1992, Cook 1979). Following a pathway may be energetically beneficial in homing species of gastropod with regards to locomotive output due to the certain directional nature of following mucus trails.

Trail following is also heavily relied upon for slug and snail mating, including locating and selecting correct species of mate. Snails show preference to trails of their own species, particularly when of sexual maturity (Chase et al. 1978). Selectivity for mucus trails has also been demonstrated by the slug *Deroceras reticulatum* (Wareing 1986), *Limax grossui* Lupu (Cook 1977) and by comparison during laboratory experiments *L. grossui* would only follow trails of the same species, and not trails of *Milax budapestiensis* or *Deroceras reticulatum.*

1.10 Chemical Control of Pest Gastropods and locomotion

The application of chemical molluscicide pellets is the most heavily utilised form of pest slug control in agriculture. Cheap and relatively simple to apply, molluscicide pellets are either scattered on the soil surface or incorporated into the seed bed when seeds are drilled into the ground. In principle, slugs encounter these pellets when foraging and graze on them upon encounter, allowing a fatal dose of molluscicide to be consumed to the animal however, slugs and snails often recover from the toxic effects of these molluscicides most likely due to consuming a non-lethal dosage from the pellet grazed. There is no published evidence to suggest that slugs can detect olfactory cues from molluscicide pellets and therefore there is a need to study the behaviour of slug upon encountering the pellet. In locomotive terms, the molluscicide metaldehyde inhibits movement of the slug by stimulating excess mucus production causing them to release an excess of mucus, dehydrating the animal and by paralysis of the muscle mass (Wedgewood and Bailey 1988). Both track length and locomotive activity is shortened after contact with novel molluscicides (Schuder et al. 2004) and this could impact upon the success of feeding behaviour of the animal, reducing predation of crop.

Terrestrial gastropod locomotion is an energetically costly and complicated form of directional movement controlled by waves of pedal contractions controlled by numerous neuronal patterns (Chase 2002). It is impossible to directly infer the motivations for movement; however, studies provide strong evidence that water regulation, food requirements, breeding behaviour and predator avoidance are all likely to elicit locomotion in gastropods. Due to its costly nature, the reward gained by movement must outweigh the energetic output of the animal upon sensing a chemical or physical change in the environment. Slugs can recover from the toxic effects of molluscicide pellets; however, it is not fully understood what impact a non-lethal dosage of molluscicide may have on the locomotor and feeding behaviours of pest gastropods. Understanding the impact molluscicide pellets have on the locomotive behaviour of slugs will allow inferences to be made regarding the likely success of a pest population consuming a significant proportion of crop and therefore also help aid in improved pellet design.

1.11 Thesis Rationale

The application of metaldehyde baits is a contentious topic, due to the risk presented to both drinking water systems and wildlife. Nevertheless, the application of synthetic pellet-based pesticides is likely to remain the control method for farmers due to slug population pest dynamics, cost and ease of application. Over 170 tonnes of metaldehyde and ferric phosphate was applied to UK arable crops in 2018 (Garthwaite et al. 2019) and it is therefore essential that research focuses on enhancing the efficiency as well as the environmental stability of these pellets in order to create effective pellets that minimise risk to the wider environment. Considering pellets work by consumption by the slug, studying the behaviour of the slug before, during and after interactions with a pellet may provide insight into the pest-pellet interaction and how improvements to the pellet could be made to increase consumption of the molluscicide by the slug.

The research aims to provide behavioural insight into the pest-pellet interaction between *Deroceras reticulatum* and molluscicide bait pellets. The thesis is divided into three main sections:

1) The foraging and feeding behaviour of *Deroceras reticulatum* in the presence of molluscicides.

This chapter describes the impact different concentrations of metaldehyde pellets have on the foraging and feeding behaviour of *D. reticulatum.* The impact on foraging before encountering a pellet is assessed by assessing the difference in time taken for a slug to locate pellets of various concentrations, which challenges the hypothesis that metaldehyde pellets may attract or repel slugs. Time spent feeding on a pellet is monitored, and this is considered in terms of delivery of a lethal dose and the effects that spending more time at a pellet are likely to have on overall slug health. Foraging time between pellets is measured by way of determining whether metaldehyde consumption has an effect on foraging behaviour. Pellet acceptance and acceptance of subsequent pellets is analysed as it is predicted that reacceptance of pellets in the field may be important in order to ensure successful control of slugs if a lethal amount of pellet is not consumed on the first pellet encounter.

2) The fate of *Deroceras reticulatum* following molluscicide poisoning.

This section explores the effect of metaldehyde on the health of the slug, mimicking pellet exposure overnight in the field and moving poisoned slugs to an area of ideal conditions where the slug may rehydrate. This chapter explores the effect of changing metaldehyde concentration on the likelihood and severity of paralysis, recovery or death.

3) The potential uses for inorganic Controlled Release Technology (iCRT) for metaldehyde based molluscicides

Though metaldehyde presents risks to the environment, it is nevertheless one of the few successful molluscicides available. Advances in technology mean that manufactures are now able to produce pesticides and herbicides that are more stable in their intended environment, are less polluting and are targeted in their delivery. Microencapsulation of metaldehyde with controlled release technology is explored in this chapter, with acceptability and mortality being the key areas of focus. Controlling the release of metaldehyde could reduce the water pollution and wildlife risk and ensure more effective slug control by concealing metaldehyde in grain-based pellets where it is only released under certain conditions once inside the slug. The application of ferric phosphate base molluscicides has increased gradually in recent years (Garthwaite et al. 2019) and is likely to continue increasing with the forthcoming removal of metaldehyde as an approved pesticide for UK outdoor use. The use of controlled release technology for ferric phosphate molluscicides may reduce the risk of the pellets to non-target organisms, as well as the potential risk to the environment of increased application of chelating agents, which are often included in ferric phosphate based molluscicides (Edwards et al. 2009).

This research explores both the pest-pellet interaction and the fate of slugs following consumption of molluscicide. It challenges the recommendation of 3% metaldehyde industry standard pellets and provides insight into the sensitivities that *Deroceras reticulatum* has to metaldehyde. By exploring novel inorganic controlled release metaldehyde pellets, this research offers a potential future avenue of research into new formulations of slug pellets that may be more environmentally friendly, while providing higher levels of slug control.

Chapter 2: The foraging and feeding behaviour *of Deroceras reticulatum* in the presence of molluscicides

2.1 Abstract

The global drive to reduce the amount of pesticides applied to agricultural land to improve biodiversity and promote sustainable agricultural practices includes the use of metaldehyde, the primary molluscicide for control of the *D. reticulatum* across the world. The effect of metaldehyde on the foraging and feeding behaviour of *Deroceras reticulatum* was tested in relation to changing the concentration of the molluscicide in bait pellets. Metaldehyde of a range of concentrations (1, 3 and 5%) and a commercial pellet was presented to small groups of *D. reticulatum* and the foraging behaviour before and between pellet encounters was recorded, as well as the time spent feeding on a pellet. *D. reticulatum* fed for a significantly longer time on 1% metaldehyde compared to higher concentrations and was also more than twice as likely to accept a second pellet compared to other tested pellets. The concentration of metaldehyde did not have an effect on the foraging behaviour of *D. reticulatum*, however time spent between the first and subsequent pellet encounters was low for all concentrations, indicating metaldehyde reduced foraging activity compared to before the first pellet encounter.

2.2 Introduction

2.2.1 Deroceras reticulatum and U.K Agriculture

The grey field slug, *Deroceras reticulatum,* is considered one of the most economically damaging crop pests of U.K. agriculture and is of particular threat to oilseed rape and winter wheat. In 2016, over 25% of wheat and 61% of oilseed rape grown in the U.K. was treated with a form of molluscicide or repellent, equating to 131,588 tonnes of active substance utilised for the growth of oilseed rape and wheat alone (Garthwaite et al. 2019). Molluscicide usage for the aforementioned crops therefore contributed 82% of the total weight of molluscicide applied to all crops in the U.K. It is estimated that without control of terrestrial gastropods in oilseed rape and wheat crops, losses of £43.5 million of yield could been seen annually (Nicholls 2014).

Like many terrestrial gastropods, *Deroceras reticulatum* is a generalist herbivore, grazing on a range of living and dead plant matter. Damage to crops may occur before germination and under the soil by seed hollowing, and later by reducing plant biomass in developed seedlings and plants from leaf and shoot grazing, which can have significant impacts on crop yield (Rodriguez and Brown 1998). Though considered a generalist, *D. reticulatum* may demonstrate preferential feeding behaviour when offered a choice between cultivars or morphs of living plant matter, as demonstrated in choice feeding studies of both crop and weed variants (Barlow et al. 2013, Burgess and Ennos 1987, Cook et al. 1996, Hanley et al. 1995). Slug grazing preferences are a large area of research; with studies focused on the physical factors determining plant palatability (plant size/age, leaf toughness and cuticle structure) as well as research orientated towards the chemical components and volatiles in plants making certain species or breeds less susceptible or even resistant to herbivory by slugs (Dizro 1980). Research thus far is inconclusive as to whether particular cultivars of wheat are more or less susceptible to slug herbivory (Cook et al 1996, Spaull and Eldon 1990). In any case, reduced plant susceptibility to slug damage may not equate to significantly higher crop yield because there is likely to be some level of predation on even the more resistant cultivars, and therefore the need for molluscicides is still likely to be required on these crops.

2.2.2 Control of Slug Pests

Broadly, control of slugs is divided into three categories: physical (soil management, seedbed preparation), biological (natural predators, nematode application) and chemical (molluscicides). These management techniques are employed at different stages of the growing season as part of an integrated pest management approach to slug control in order to ensure maximum control success. Chemical control by the application of molluscicide pellets is the most common form of terrestrial gastropod control and is available to both commercial and non-commercial growers. Molluscicide pellets are small (usually no larger than 5mm in length), grain pellets containing a small percentage of active ingredient which is the molluscicidal agent. Pellets are intended to be consumed by slugs and snails upon encounter on the soil surface so that a lethal dose of molluscicide can be delivered to the target slug. There are two active ingredients that dominate the molluscicide market: metaldehyde and ferric phosphate (Iron (III) phosphate). Both molluscicides are either broadcast on the soil surface or drilled into the seedbed at the time of seed drilling, though their modes of action are very different. Metaldehyde is the cyclic tetramer of acetaldehyde, and it can be found in commercially sold pellets ranging from 1 – 5% metaldehyde, and its molluscicidal effect exploits slugs' high dependency on high body water content. Slugs can experience the effects of metaldehyde by either dermal contact or ingestion. Initially, metaldehyde acts as an irritant to the mucus glands, causing the slug to produce an increase of mucus, which in turn leads to dehydration and death on the soil surface. At higher doses, metaldehyde may also cause immobilisation of the muscle mass, which may also lead to desiccation and death on the soil surface and increases the risk of predation by natural predators.

Ferric phosphate pellets often contain a chelating agent such as EDTA (Ethylenediaminetetraacetic acid), and are recognised as an organic form of chemical control. Ferric phosphate pellets rely solely on consumption by slugs for their molluscicidal effects to take place, and act as a stomach poison to the slug. Ferric phosphate disrupts slugs' calcium metabolism, which eventually leads to changes in the animal's crop and digestive tract (Horgan 2006). Slugs poisoned by ferric phosphate usually retreat under the soil surface where death occurs after a number of days (Horgan et al. 2006). For this reason, it is difficult to quantify the success of slug control when using ferric phosphate due to the lack of evidence of slug death, and therefore ferric phosphate efficiency is usually estimated by observations of slug damage to crops (Speiser and Kistler 2002) when compared to the increase in mucus and slug cadavers on the soil surface when using metaldehyde.

In 2016, metaldehyde was employed by over 86% of farmers that applied some form of molluscicide or repellent to their crops and contrastingly only 13% utilised ferric phosphate (Garthwaite et al. 2019). Between 2014 and 2016, the area treated, and the weight applied of metaldehyde increased by 33% and 21% respectively. This increase in molluscicide use demonstrates a sustained need for effective slug control in U.K. agriculture.

Though widely used, metaldehyde presents serious risks to the environment. When pellets are dampened by rain or soil moisture, metaldehyde leaches into the soil where it contaminates surface water, leading to the contamination of drinking water, causing pesticide limits in drinking water to exceed the EU drinking water directive concentration limit of pesticides of $0.1\ \mu g\ L^{-1}$. In 2015, there were 102 compliance risks regarding metaldehyde pollution across the U.K. water companies (Anthony et al. 2015). Furthermore, due to its high polarity, metaldehyde is extremely difficult to remove from contaminated water using traditional drinking water treatment processes, and for this reason costs the water industry millions of pounds annually to rectify (Castle et al. 2017). Both metaldehyde and ferric phosphate pellets are a poisoning risk to non-target organisms and are usually stained blue to discourage consumption by non-target animals.

2.2.3 Inefficiencies with Slug Control

In addition to the environmental risks presented when using a molluscicide, there is also little evidence to suggest strong successes of either metaldehyde or ferric phosphate based slug control in U.K. agriculture, with pellets repeatedly applied throughout the growing season in order to control slug populations and slug damage reflected in loss of yield (Storr et al. 2019). The cost of application of molluscicide far outweights the potential loss of yield annually in the U.K, with average U.K. annual applications of molluscicide applied to wheat costing between £5.8-£8.7 million, and between £3.2-£4.9 million for oil seed rape (DEFRA 2010), and potentially losses estimated to be up to £100 million for all of U.K. agriculture annually without slug control. There is therefore an ever increasing need to develop molluscicides that are effective and economically viable in order to reduce levels of crop damage and economic losses to the agricultural industry. There are numerous reasons why pellets may not be as effective as predicted. The structural integrity of the pellet may deteriorate over time, especially when exposed to water in the form of rainfall or soil moisture, this may cause the pellet to become inedible by the slug. In addition, a dampened pellet may cause metaldehyde to leach into the soil surrounding a pellet, which may be detected by an approaching slug and lead to a repellent effect from the molluscicide. Slugs may recover from the dehydrating effects of metaldehyde poisoning if exposed to sufficient moisture and have not consumed a lethal dose of active ingredient. For these reasons, metaldehyde pellets are less effective during periods of rainfall which is conversely when slugs may be more active (Bourne et al. 1990). On a population level, it is very difficult to predict where slugs are in a field and it could be that refuge areas for slugs are not being targeted (for example, field margins) meaning later populations of slugs are missed for control when applying molluscicide. Methods for predicting slug activity/ density have been known for a long time (Yong et al. 1993), but logistical constraints mean they are not widely used. Recent research has explored the possibility to detect patches of slugs in the field (Forbes et al. 2020), which has the potential to allow for more targeted application of molluscicides in the future.

2.2.4 Slug Behaviour and Control

Information regarding the consequences for slugs after encountering a molluscicide pellet is often conveyed in terms of the effectiveness of a molluscicide and is seldom about the effect a molluscicide has on the movement and feeding behaviour of the slug after pellet contact, and how the concentration of active ingredient (metaldehyde or ferric phosphate) alters these behaviours. Due to the nocturnal behaviour of slugs, it is extremely difficult to observe slug populations (and even more so, individual slugs) in the field and therefore laboratory based behavioural assays tend to be preferred initially over field studies. Technological advances in time-lapse video recording and infra-red lighting allow laboratory-based studies to observe behaviour over many hours and at night, so to not disrupt the slug's circadian rhythm (Bailey 1989).

Data regarding the pest - pellet interaction between slugs and molluscicide pellets may offer insight into what extent inefficiencies in slug control by pellets is caused by alterations to slug locomotion and feeding behaviour when exposed to different molluscicide pellets. This research aims to quantify the foraging and feeding behaviour of *Deroceras reticulatum* before, during and after encounter with either a metaldehyde (1, 3 or 5%) or ferric phosphate (3%) pellet with the aim to provide insight into the effect that both different molluscicide active ingredients and increasing molluscicide concentration has on slug behaviour.

2.3 Aims and Objectives

The aim of this research was to provide insight into the movement and foraging behaviour of *Deroceras reticulatum* around varying concentrations of metaldehyde slug pellets and a commercial ferric phosphate pellet. The specific objectives were:

1) Investigate the rate and time taken for *D. reticulatum* to encounter slug pellets and whether the formulation of a slug pellet influences the likelihood of encounter. This research is proposed to test the hypothesis that some formulations of slug pellet are attractive to slugs.
2) Compare time spent feeding on a pellet with the likelihood of reacceptance of a subsequent slug pellet and whether concentration of the molluscicide has an impact on this. It is hypothesised that after feeding on a pellet of higher AI concentration, the slug will reject subsequent pellets.
3) Quantify foraging behaviour of *D. reticulatum* after pellet encounter in terms of mobility and time spent moving. It is hypothesised that slug locomotion will be reduced after contact with a slug pellet, and reduction will be greater with increased metaldehyde concentration.
4) Evaluate the impact of time spent feeding on a slug pellet with the health status of the slug after exposure and predict the likelihood of a slug reencountering a pellet and mortality after one night’s exposure to molluscicide. It is predicted that the longer a slug feeds on a pellet, the more likely it will succumb to poisoning by metaldehyde and that a longer initial encounter with a pellet will reduce the likelihood of reencountering a subsequent pellet.

2.4 Methods

2.4.1 Deroceras reticulatum

Slugs (*Deroceras reticulatum*) were collected from the edges of amenity grassland in Northumberland, UK, 24 hours before the beginning of each trial. The site selected for slug collection was on the edge of regularly mown grassland where there was an unmanaged verge of scrub and tall ruderal species, dominated by bramble (*Rubus fruticosus*) and stinging nettle (*Urtica dioica*). There was no sign of dieback on the plants from herbicide spray and the site was over 500m from the nearest garden and over 1km from any agricultural field and so the risk of pesticide application/ contamination was extremely low. Slugs were collected from the soil surface and on the under canopy of the verge, usually found on clover and dock species. Slugs were selected irrespective of size however only slugs that appeared “healthy” were collected. Healthy slugs were those that reacted to touch, showed no sign of excess mucus loss (such as being surrounded by a white mucus discharge) and had no abnormal deformities. Between collection and inclusion in trials, slugs were stored in a refrigerated environment 3-5°C on wet filter paper in a sealed container with small air holes. Slugs collected were used in trials within 24 hours of collection. Once in the laboratory, slugs were checked again to confirm the correct species, and any slugs wrongly originally identified during collection were taken back to the collection site and released. Slugs were acclimated to the controlled temperature room where trials took place for 24 hours prior to the start of each trial. Slugs acclimatising were placed in a Perspex box with air holes with a 5cm layer clay loam soil and no food. In order to maintain a suitably moist environment for the slugs to remain hydrated, the soil was watered with 750ml of tap water prior to the introduction of slugs which was checked twice during the acclimation period, and re-watered if the soil had started to dry out.

2.4.2 Slug Pellets

Pellets used in the trial consisted of both laboratory made and commercially available pellets. In their most basic form, commercial slug pellets are made from flour and an active molluscicidal substance (Bailey 2002, Lonza 2019). Laboratory made pellets were made using 99.9% pure metaldehyde powder mixed with a measured amount of plain flour in order to create pellets of concentrations 1, 3 and 5% metaldehyde. The dry mixture was then combined with olive oil in order to produce a malleable dough. Pellets were then formed from the dough in 1g rolled portions. Pellets were stored in refrigerated environment until use. Commercial slug pellets used were Lonza Axcela® 3% metaldehyde and Certis Sluxx® HP 3% ferric phosphate. A non-toxic control pellet containing the same ingredients as the Axcela® 3% pellet only without metaldehyde was provided by Lonza and was used as the control.

2.4.3 Experimental Set Up

Experiments were held in a controlled temperature room (15 $\underline{+}1^{o}$C) that was lit with white light from a 400-watt halogen lamp between 0600-1800 and by a 96 blub LED infra-red light between 1800-0600 in order to encourage natural day/night slug activity. Both sets of lights were suspended 1.5m above the arena. Infra-red light does not appear to affect slug behaviour but allows recordings to be made at night (Howling 1990). Trials took place in soil filled arenas (43x56x26cm), filled with 8cm of clay loam soil obtained from agricultural land, that had not been exposed to any pesticide or herbicide. The soil was wetted with 1 litre of water and treatment pellets were then placed onto the soil surface at 9 baiting points (Appendix 1) Commercial pellets (Lonza Axcela® metaldehyde, Certis Sluxx® ferric phosphate) were placed onto wetted soil during the slug acclimation period, in order to soften pellets before the beginning of trial. Laboratory made pellets were placed onto wetted soil less than 30 minutes before the introduction of slugs to the arena, as these pellets did not require softening.

In order to avoid age bias (determined by slug weight) between experiments, the average weight of groups of 6 slugs was taken before introduction into the arena. The average weight of slugs used in the trial was 0.5g $\underline{+}$0.2g. Slugs were placed into the soil filled arenas in groups of six, distributed equally around the 9 pellet baiting points, between the pellets and the sides of the arena. Slugs were then left to move freely around the arena over a 14-hour period (18:00-08:00). Slug behaviour was recorded using Brinno BLC200 HDR Laboratory-Cam Time Lapse recording equipment (time lapse frame rate 10 fps) held on gantries 40cm above the arena.

After 14 hours of exposure to pellets, an assessment and recording of the health of each slug was made. Each trial ended after 14 hours of pellet exposure after which time slug behaviour was not recorded. A total of 5 trials per treatment pellet took place in order to record the behaviour of 30 individual slugs. Details of the following behaviours and movements were manually recorded from observations of the time lapse video recording in each trial:

- Time spent foraging from the start of the trial to first pellet encounter.
    - Pellet encounter was confirmed when a slug was observed to make physical contact with a pellet at the anterior end of the slug.
- Time spent feeding on the first pellet encountered.
    - Feeding on pellets was measured when the anterior end of the slug was in physical contact with a pellet and the pellet could be seen to be moving slightly as the slug grazed.
- Time between first and second (and subsequent) pellet encounters and feeding times.
    - Time measured between anterior contact with pellets.
- Acceptance of pellet and acceptance of subsequent pellets.
    - “Subsequent pellet” includes returning to the first pellet to feed.

Slugs that did not encounter a pellet at all throughout the trial were included in data analysis in order to imitate a field like situation as best possible.

2.4.4 Statistical Analysis

All data was initially analysed to confirm normality and homoscedasticity. The time taken for *D. reticulatum* to encounter the first pellet in the trial and the total time spent feeding were analysed using Analysis of Variance (ANOVA) in R (R Core Team 2020). Post Hoc analysis using Tukeys HSD (honestly significant difference) was applied where a significant result was indicated by the ANOVA. Analysis of Variance was also used to determine differences in pellet reacceptance and the time taken between pellet encounters.

2.5 Results

Mortality rates of *D. reticulatum* across all treatments was extremely low (Table 2.1), all but two slugs survived during trials. From the video recording analysis, at the end of each trial, it was observed that all slugs that had encountered a pellet at least once, however a 13% of slugs were not observed to feed on an Axcela® pellet once encountered, and 26% of slugs did not feed on the ferric phosphate treatment once encountered and were observed to turn away almost immediately after the head of the slug touched the pellet. A high proportion (93%) of slugs encountered and fed on at least one pellet during trials for each treatment, while fewer slugs fed on a subsequent pellet. Slugs showing signs of poisoning (increased mucus and/or paralysis on the soil surface) was high for all metaldehyde containing treatments and low the ferric phosphate treatment which was expected due to the contrasting modes of action of the two molluscicides. There were no slugs showing signs of poisoning for the control group.

Table 2.1 Number of *Deroceras reticulatum* per treatment that encountered a pellet and fed at least once, the number of slugs that then encountered subsequent pellets and fed more than once, and the number of slugs that were alive at the end of the 14 hour exposure period out of the 30 slugs included in each trial. Slugs considered poisoned showed signs of metaldehyde poisoning such as increased mucus and paralysis on the soil surface.

	No. of slugs fed once	No. of slugs fed on subsequent pellets	No. of slugs alive after trial	No. of slugs poisoned after trial
1% metaldehyde	30	14	30	25
3% metaldehyde	30	6	30	28
5% metaldehyde	30	6	30	20
3% Axcela® metaldehyde	26	5	29	27
3% Sluxx ferric phosphate	22	18	29	5
Non toxic pellet	30	21	30	0

2.5.1 Initial pellet encounter

From the start of each trial, *Deroceras reticulatum* made contact with non-toxic grain pellets and ferric phosphate pellets significantly more quickly when compared to the metaldehyde containing treatment pellets (F(5, 174)= 14.45, $p < 0.001$). Similar average encounter times were observed for both commercial and laboratory made 3% pellets (approximately 4.5 hours) Results of Tukeys HSD test showed that ferric phosphate pellets were encountered significantly quicker by slugs when compared to both Axcela® and 1% metaldehyde pellets ($p<0.001$ for both treatments). On average, the control grain pellets were encountered within 45 minutes from *D. reticulatum'*s introduction to the trial (Figure 2.1), whereas those slugs introduced to trials containing metaldehyde pellets took between three to five hours to make contact with a pellet (averages for 5% and 1% metaldehyde respectively). There was no significant difference in the time taken for a slug to encounter a metaldehyde pellet of any concentration.

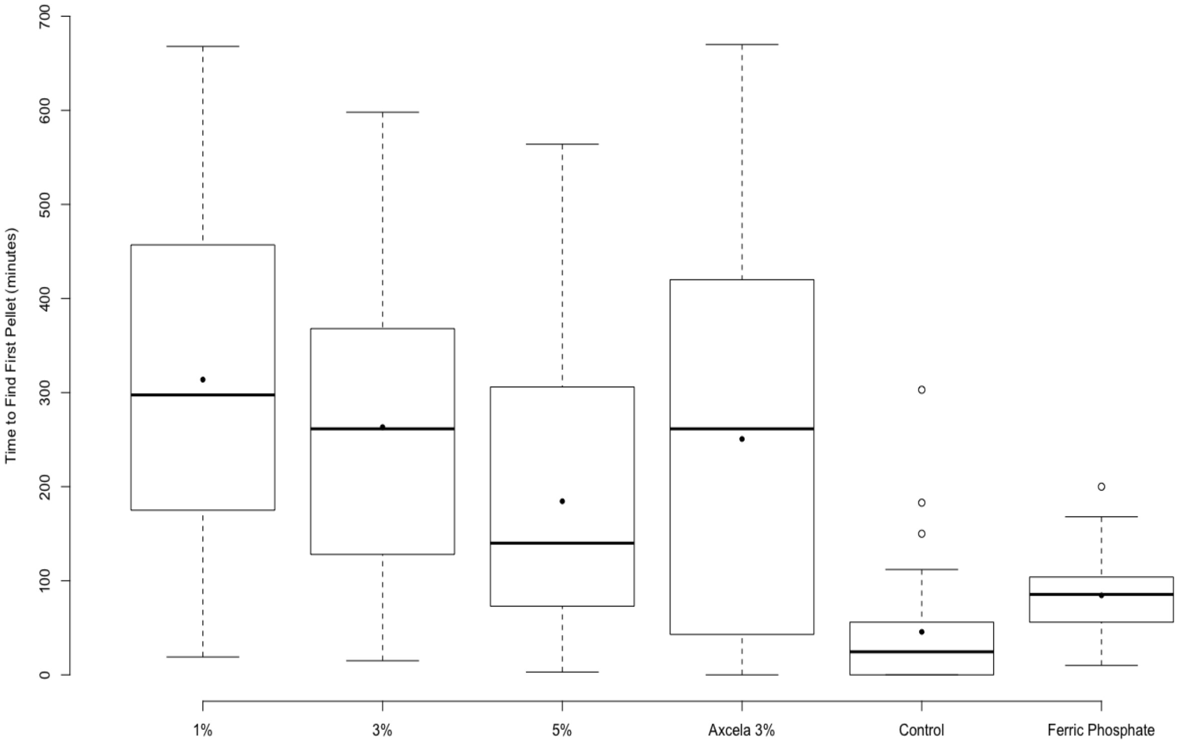

Figure 2.1 Median time taken for *Deroceras reticulatum* to contact the first pellet in a 14-hour trial (n =30 per treatment). Boxes represent upper and lower quartile ranges while

whiskers represent minimum and maximum times and white circles indicate outlying data. Black circles indicate the average time taken in minutes to find a pellet and contact a pellet.

2.5.2 Total Pellet Feeding Time

The total time *D. reticulatum* spent feeding on pellets varied significantly between some treatments (F(5,174)=14.45, p<0.001). *D. reticulatum* spent a significantly higher amount of time feeding on 1% metaldehyde pellets compared to 5% metaldehyde, Axcela® and the control pellet (p<0.001). *D. reticulatum* fed on pellets containing 3% metaldehyde for a significantly longer amount of time than slugs subject to 5% metaldehyde pellets (p<0.001). On average, slugs spent approximately 45 minutes in total feeding on 1% metaldehyde pellets throughout the entire 14-hour trials, compared to only 15 minutes of feeding on 5% metaldehyde pellets (Figure 2.2). Slugs spent a significantly higher average time feeding on ferric phosphate pellets compared to 5% and Axcela® pellets (p<0.001). Average pellet feeding time for non toxic control pellet was low (28 minutes), however it should be noted that approximately 1/3 of slugs subject to this treatment did not encounter a pellet at all.

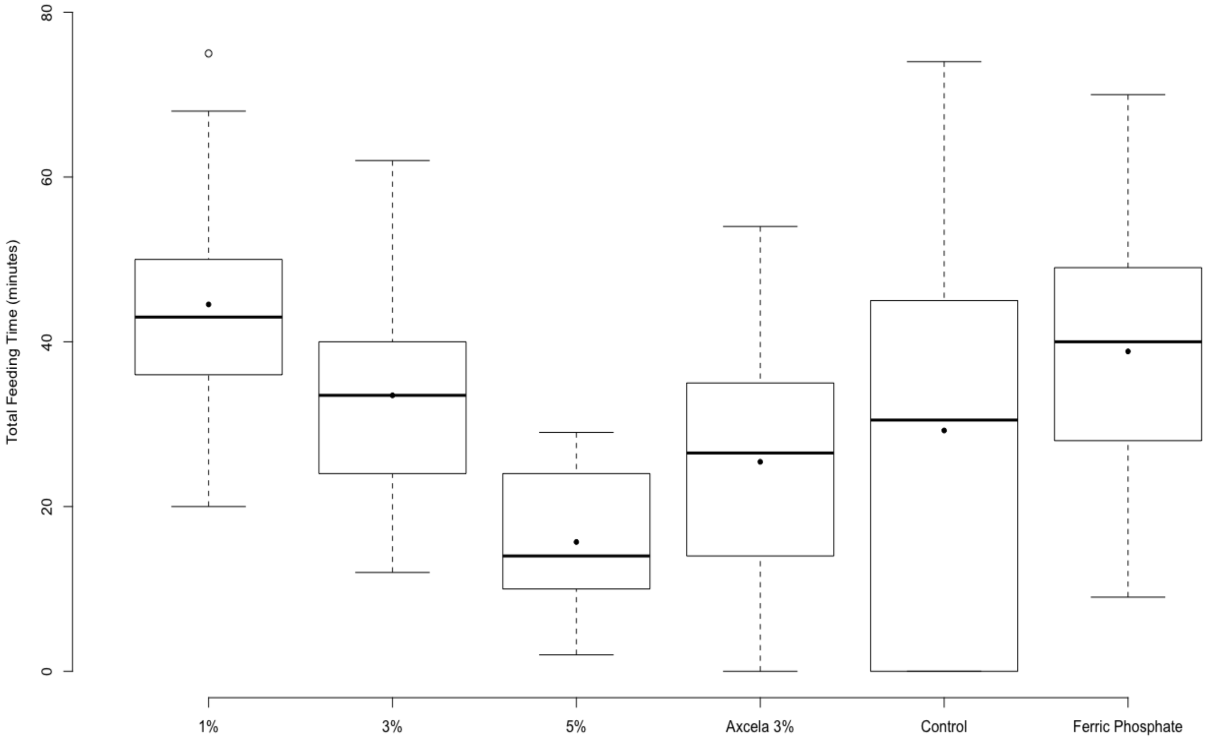

Figure 2.2 Total time *Deroceras reticulatum* spent feeding on slug pellets throughout a 14 hour trial (n=30 slugs per treatment pellet). Boxes represent upper and lower quartile ranges while whiskers represent minimum and maximum times and white circles indicate outlying data. Black circles indicate the average time *Deroceras reticulatum* spent feeding on all pellets over 14 hours.

2.5.3 Subsequent Pellet Encounters

Foraging time was classed as time spent encountering initial and subsequent pellets. Between the first and subsequent pellet encounter for *D. reticulatum*, there was no statistically significant difference between time taken to encounter a pellet of any concentration/ type (F(5,174)= 0.707, p=0.619). Average time between pellet encounters was low for all treatments, however for all treatments there were individual slugs whose time foraging varied greatly from the mean (Figure 2.3).

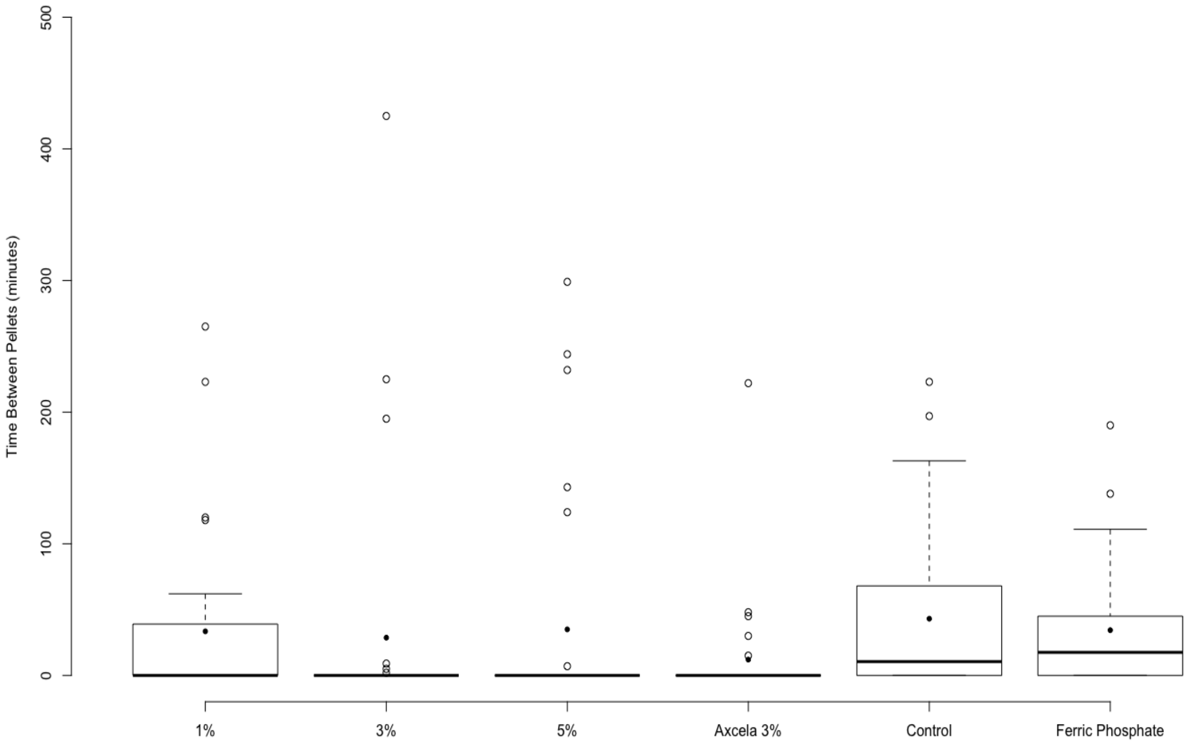

Figure 2.3 Median time between first and second pellet encounter for Deroceras reticulatum when exposed to pellets over a 14 hour trial (n=30 per treatment pellet). Boxes represent upper and lower quartile ranges while whiskers represent minimum and maximum times and white circles indicate outlying data. Black circles indicate the average time between contact with the first and second pellet.

2.5.4 Reacceptance of Pellets

All slugs in trials containing laboratory made metaldehyde pellets or ferric phosphate encountered and fed on a pellet at least once during the trial (Figure 2.4). A small proportion of slugs in the Axcela® 3% treatment did not encounter a pellet at all, and approximately a quarter of slugs in the control group did not encounter a pellet. Approximately 50% of slugs subject to 1% metaldehyde pellets were likely to reaccept a subsequent pellet when encountered when compared to approximately 20% of slugs in 3%, 5% and 3% Lonza Axcela® treatments. Of slugs that encountered a control pellet initially, 60% of slugs accepted a subsequent pellet (Figure 2.5). Reacceptance of ferric phosphate pellets was significantly higher than 3%, 5% metaldehyde and Axcela® pellets (F(, 174)= 14.45, p<0.001). There was no statistically significant difference between the reacceptance rates between any metaldehyde pellets (F(5, 174), p=0.642).

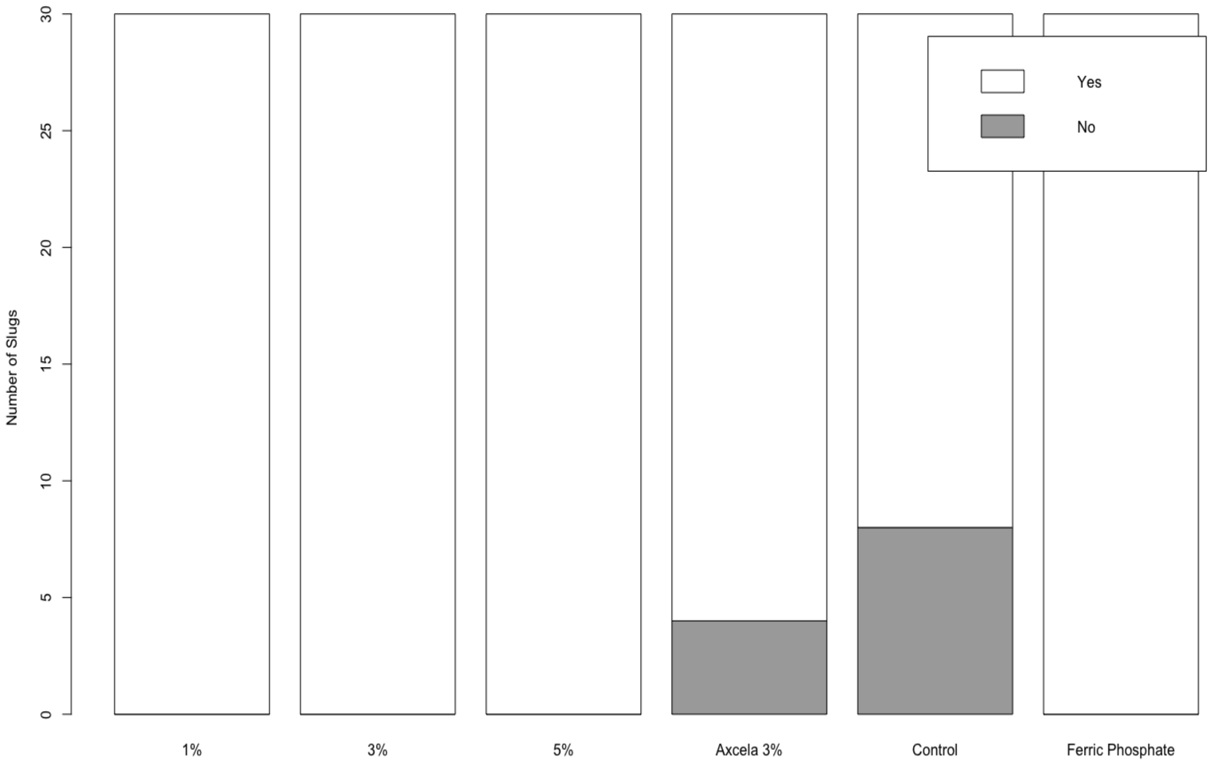

Figure 2.4 Number of *Deroceras reticulatum* that fed on a pellet when encountered for the first time in a 14 hour trial (n= 30 per treatment). White bars (Yes) indicate that the slug accepted and fed on a pellet, and grey bars (No) indicate that a slug either did not encounter or rejected a pellet once encountered and did not feed.

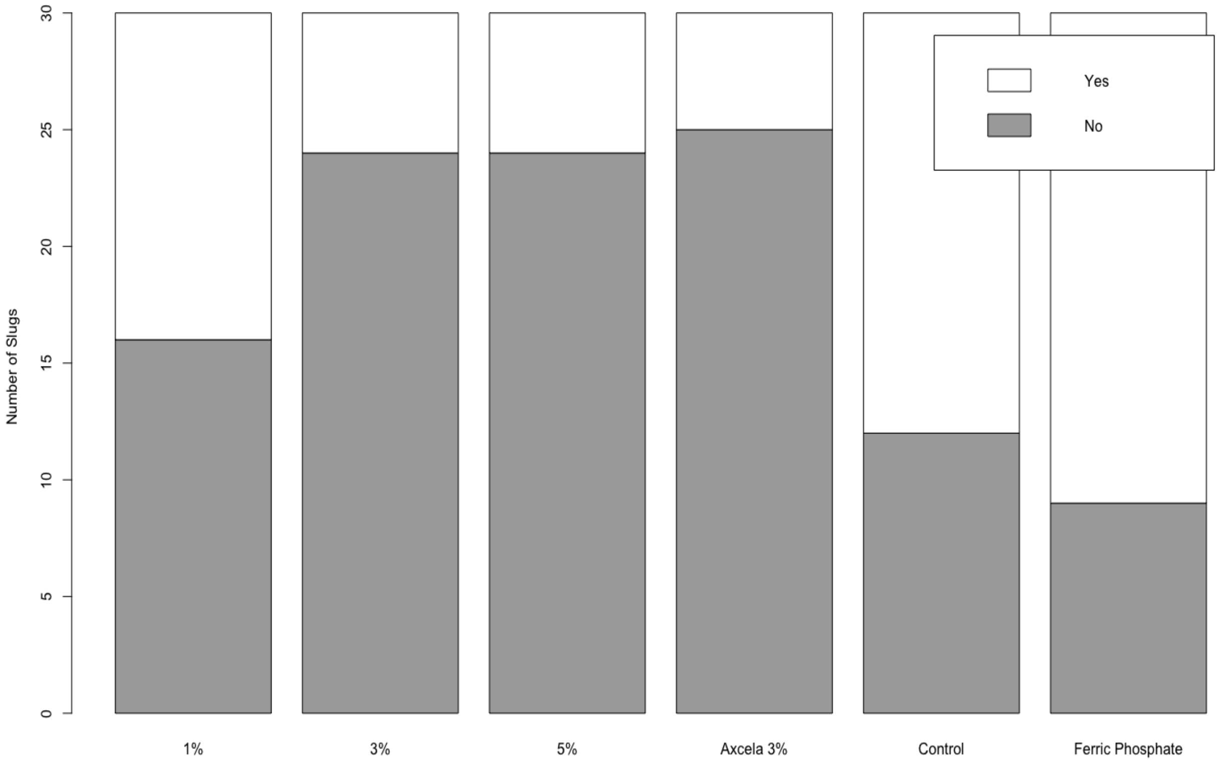

Figure 2.5 Number of *Deroceras reticulatum* that encountered a secondary pellet and fed on it having already encountered a pellet in a 14 hour trial (n= 30 per treatment pellet). White bars (Yes) indicate that a slug reaccepted a secondary pellet and grey bars (No) indicate that a slug either encountered a secondary pellet but rejected it as a food source or did not encounter another pellet during the trial.

2.6 Discussion

In this research, slugs were exposed to metaldehyde or ferric phosphate pellets over a 14 hour time period, in order to approximate the time course and conditions of an overnight field exposure. For metaldehyde containing trials, there was no difference in the time it took for an individual slug to locate and feed in the first encountered pellet, which provides evidence that metaldehyde concentration does not influence a slug’s ability to locate a pellet, however, compared to the control pellet, the presence of metaldehyde in any concentration, impacts on the time taken for a slug to encounter and feed on a pellet. This result is surprising, and contradicts previous studies suggesting that *D. reticulatum* forages at random (Howling 1990). *D. reticulatum* located and fed on the first grain or ferric phosphate pellet significantly faster when compared to metaldehyde treatments, which may indicate that *D. reticulatum* can detect the presence of metaldehyde pellet before encounter. *D. reticulatum* spent significantly more time feeding on 1% metaldehyde pellets when compared to all other treatment pellets, including the control grain pellet (although this is likely due to the low amount of pellet encountered in the control). At higher metaldehyde concentrations (5%), *D. reticulatum* fed on pellets for almost quarter of the time compared to the lower concentrations. Time spent foraging between pellet encounters was low for all treatments, it could be that after the initial pellet consumption slug locomotive ability is reduced and so foraging is restricted to a smaller area, and therefore slugs are more likely to reencounter a pellet. 93% of slugs encountered a pellet and fed on it at least once throughout all treatments, however acceptance of subsequent pellets was reduced to 25% of slugs for 3%, 5% and Axcela® pellets, and 50% of slugs for 1% metaldehyde pellets. Slugs exposed to ferric phosphate pellets were significantly more likely to reaccept a pellet when compared to 3%, 5% metaldehyde and Axcela® pellets. This could indicate that differences in the mode of action of ferric phosphate and metaldehyde impact on detection of the active ingredient upon consumption (Horgan 2006) It is less likely that ferric phosphate is detected by the slug when grazing on a pellet, because ferric phosphate works as a poison of the slug digestive system, and so more likely that the molluscicidal effects are experienced after consumption, which would account for the higher rate of reacceptance in this research compared to metaldehyde pellets.

Molluscicide pellets rely on contact and feeding by their target organism in order to be effective. While pesticide delivered as sprays or powders can be relatively easily broadcast over infested crops, with a high likelihood that the target pest will be reached, slug pellets must be located and fed upon by slugs for them to be effective. The pest - pellet interaction is therefore a crucial aspect of successful slug control, as it depends on the pellet to be an edible yet toxic food source for the slug while maintaining integrity on the soil surface in changing weather conditions. Successful interaction relies heavily on the slug's behavioural response to the pellet, from locating the pellet to acceptance and feeding on the pellet.

2..6.1 Initial pellet encounter

Slugs located grain pellets containing no metaldehyde and ferric phosphate pellets at a significantly higher rate when compared to metaldehyde treatments. This contradicts evidence that slugs forage at random (Howling 1991). Howling's research did indicate that random foraging was disturbed in the presence of methiocarb but not metaldehyde, and so there is potential for different metaldehyde formulations to impact on random foraging. Slugs may be able to detect metaldehyde pellets in this trial before contact was made with a pellet (Bourne et al. 1988). "Encounter" was defined as when a slug was observed to touch the pellet, and this could be seen clearly from video recordings from each trial. "Encounter" did not include slugs that approached a pellet and changed direction within extremely proximity (>1cm), as it was hypothesised that pellet detection from metaldehyde leached into the surrounding soil would be low. There was no difference in the amount of time taken for a slug to encounter a 1, 3, or 5% metaldehyde pellet, and this could imply that the concentration of the pellet has no effect on potential detection before contact has been made, however could also imply that slugs are extremely sensitive to even extremely low (1%) concentrations of metaldehyde.

Ferric phosphate pellets were encountered more quickly compared to metaldehyde pellets, and it could be that ferric phosphate is less likely to be detected by slugs in the field. If the molluscicidal effects of ferric phosphate are not detected immediately upon encounter by a slug, it could mean that there is a higher chance that a lethal dosage of ferric phosphate will be consumed compared to metaldehyde pellets. Were this the case, it would be advantageous for growers to use ferric phosphate instead of metaldehyde pellets, however it is much more difficult to quantify slug death from ferric phosphate due to its mode of action (Rae et al. 2009).

2.6.2 Pellet Feeding and Between Pellet Foraging

D. reticulatum spent significantly longer feeding on 1% metaldehyde pellets when compared to 5% metaldehyde, Axcela® and the grain control pellet. Regarding metaldehyde pellets, the higher rate of acceptance and duration of feeding on a lower concentration of metaldehyde could imply that a successful pest-pellet interaction whereby the slug feeds on a pellet and perishes is likely to happen at a lower metaldehyde concentration. Presuming that metaldehyde poisoning is not instant but still relatively soon after pellet encounter, the length of time spent feeding is more likely to deliver a lethal dose of metaldehyde to the slug in order to arrest the slug for a period of time while the molluscicide takes effect. Spending more time at a pellet also means that the slug is less likely to move to areas of soil that may allow for the slug to rehydrate from the initial effects of metaldehyde poisoning, provided the paralysing effects of the molluscicide ensure the slug cannot move away from the pellet to areas where rehydration may occur. In trials, it was observed that after a pellet feeding event the slug would remain close to the pellet, writhing on the soil surface. This writhing behaviour is observed after metaldehyde poisoning, and it could be that the animal attempts to increase body surface area contact with the soil in order to dermally rehydrate (Cragg and Vincent 1952, Glen et al. 1986, Wedgewood and Bailey 1988). A slug that has spent less time feeding on a pellet (such as those feeding on 5% metaldehyde), may be more likely to be capable to move to damper areas to hydrate, assuming a non-lethal dosage of metaldehyde has been consumed (Wilson et al. 2014). It is likely that in order to deliver a lethal dose of metaldehyde, time spent feeding is relative to the concentration of the pellet i.e., a higher concentration pellet requires a shorter period of feeding in order to deliver a lethal dose. It should be considered however, that a higher concentration of metaldehyde may be more easily detected by a slug, and therefore a short encounter with a high concentration pellet may be enough for the slug to recognise the pellet as an unsuitable food source, whereas metaldehyde in low concentrations may be harder to detect initially (Henderson et al. 1992). *D. reticulatum* spent a significantly longer amount of time feeding on ferric phosphate pellets when compared to 5% metaldehyde and Axcela® pellets, and it is again likely that higher concentrations of metaldehyde are more easily detected upon feeding, and that variations in formulation between commercial pellets, and the contrasting mode of action between these pellets could mean that ferric phosphate based pellets are more palatable compared to metaldehyde pellets. Between contacting pellets, slugs there was no difference in the amount of time spent foraging between pellets of any concentration, which means that after a pellet encounter slug foraging behaviour was impacted upon equally, regardless of concentration. Pellet feeding time on grain pellets was unusually low, however approximately a third of slugs did not encounter a pellet at all, and therefore feeding time is likely to have been distorted. It was decided to include slugs that did not feed on pellets in order to imitate field like conditions as best possible.

2.6.3 Pellet Reacceptance

If the first encounter and feeding event is not successful in hindering slug activity, it is essential that slugs reaccept subsequent pellets within a short period of time in order for metaldehyde take effect. *D. reticulatum* was 50% likely to reaccept a subsequent pellet after the initial encounter, whether this be the same pellet or a different pellet after a period of foraging from video recordings of the trials, it was observed over 80% of slugs that reaccepted a subsequent pellet returned to the same pellet that they had initially fed on, likely due to the slug remaining close to the first pellet due to being temporarily incapacitated by metaldehyde. The likelihood of reacceptance at 1% metaldehyde is 25% higher compared to only 20% reacceptance at 3 and 5% metaldehyde. The difference in reacceptance rates could be due to several reasons. After 3 and 5% metaldehyde consumption, it could be that slugs are disabled on the soil surface and unlikely to encounter a subsequent pellet due to the inhibitory effects of metaldehyde on locomotion (Bailey and Wedgewood 1991). It is unlikely that a slug has “learned” to avoid feeding on pellets of 3 and 5%, especially over a short period of time, however it is not implausible to suggest that these higher concentrations of pellets may have leached metaldehyde onto the soil surface in higher measures compared to 1% metaldehyde pellets, and so the slug can detect these pellets and avoid them more so than a lower concertation pellet. Reacceptance of ferric phosphate pellets was significantly higher compared to 3%, 5% metaldehyde and Axcela® pellets and this may be due to low detection levels of a poisonous substance during the consumption of ferric phosphate due to the delay between ingestion and the molluscicidal impact (Horgan et al. 2006).

2.7 Conclusion

The concentration of metaldehyde does not appear to impact upon the foraging behaviour of *Deroceras reticulatum*, however the presence of metaldehyde in any amount (when compared to ferric phosphate and non molluscicidal pellets) affects foraging behaviour whereby *D. reticulatum* encounters metaldehyde pellets at a slower rate. Observations from this research provide evidence that *D. reticulatum* is extremely sensitive to metaldehyde and may be able to detect the molluscicide before encountering a pellet. The feeding behaviour of *D. reticulatum* and the likelihood of accepting subsequent encountered pellets is also adversely affected by metaldehyde. In terms of foraging, it is likely that after feeding on a metaldehyde pellet the slug quickly suffers from the immobilising effects of the molluscicide, and its locomotive activity is reduced greatly due to increase in mucus and dehydration referring the slug at least partially paralysed. Feeding time is shorter with increase in metaldehyde concentration and may be because of faster detection by the slug due to increased metaldehyde presence and therefore early recognition by the animal of an unsuitable food source, or due to quicker slug poisoning due to delivery of a higher dosage. Slugs are more likely to reject a pellet of high metaldehyde concentration after consumption of an initial pellet, and but approximately half of slugs tested will reaccept a pellet of 1% metaldehyde. This research suggests that slugs are more likely to reaccept a pellet of a lower concentration that is too low to lead to morbidity after the first consumption event. Lower metaldehyde concentrations are likely to lose potency in the field more quicky compared to higher concentrated pellets if leaching occurs after rainfall. If low pellet concentrations are used in the field, it could be that reacceptance by slugs is essential in order to increase the chances of morbidity from consumption of slug pellets, as it is less likely a slug will consume a lethal dosage of metaldehyde in one consumption event compared to a higher concentration pellet. Reacceptance is important in order to ensure successful control in the field, particularly with current metaldehyde formulations that can leach metaldehyde when wetted.

Chapter 3: The Fate of *Deroceras reticulatum* Following Metaldehyde Poisoning

3.1 Abstract

The concentration of a pesticide used in agriculture not only has implications for effectiveness of pest control but may also have significant wider environmental consequences. This research explores the acceptability of metaldehyde slug pellets at different concentrations by *Deroceras reticulatum*, and the changes in the health status of the slug when allowed to recover. The highest metaldehyde concentration (5%) yielded the highest slug mortality, however also produced the highest proportion of not poisoned slugs, suggesting the highest level of pellet rejection. 1% metaldehyde pellets were as effective as 3% pellets in paralysing a significant proportion of the population after initial pellet exposure, however more slugs were able to recover from metaldehyde poisoning at 1% metaldehyde compared to 3%. There was no statistically significant difference between the mortality rate of slugs regardless of metaldehyde concentration, suggesting a lower concentration of metaldehyde may be as effective as a higher concentration.

3.2 Introduction

3.2.1 The Molluscicide Metaldehyde

The primary control of pest slug populations in agriculture is by chemical means with employment of synthetic pesticides. The most commonly used molluscicides are created as a bait to be ingestible, grain-based pellets that contain the active molluscicidal agent (wettable powders and sprays are also manufactured for slug control, albeit less frequently). Metaldehyde is widely used across the world as the active ingredient in slug pellets and is typically present in the pellet at concentrations of less than 5%.

Metaldehyde is primarily a dermal irritant and when ingested by a mollusc which exploits a slug's requirement for high body water content (Castle et al. 2017). It is likely that the irritant effect of the pellet is that which the mollusc will first encounter in the field, whereby, upon physical contact with the pellet, the molluscs' mucus glands become irritated which leads to an increase of mucus production and subsequently dehydration and desiccation (Triebskorn et al. 1988). Upon pellet ingestion by a mollusc, metaldehyde rapidly hydrolyses to acetaldehyde in the gut which elicits further production of excess mucus from the mucus glands and again leads to the mollusc becoming dehydrated and desiccating (Gupta 2007). At high concentrations, it is likely that metaldehyde is a nerve poison in molluscs, which may lead to immobilisation on the soil surface and consequently secondary mortality.

3.2.2 Secondary Mortality

Metaldehyde is successful as a molluscicide as it is able to cause slugs to lose large amounts of body water via increasing the amount of mucus produced. It is possible that dehydration from metaldehyde ingestion is not sufficient to kill a slug outright, and therefore it is likely that secondary mortality factors play a large role in slug control with molluscicides.

Once a slug has been poisoned by metaldehyde, it is likely to remain on the surface of the soil surrounded by its own mucus, writhing or completely unable to move due to a lack of body water content rendering it unable to produce further mucus to propel itself in any direction. The mucus produced by terrestrial gastropods is up to 98% water (Godan 1983), and therefore body water loss due to slug locomotion must be compensated by water uptake from the animals' immediate environment. Gastropod activity has been shown to decrease when body water content drops below 17% of the animal's normal value of 80% water content (Dainton 1954), however lack of movement after metaldehyde poisoning may also be due to paralysis of the muscle mass (Bailey 1989, Godan 1983). It is at this point, once paralysed after pellet contact, that a slug is likely to succumb to predation by birds, hedgehogs or ground beetles. Slugs are generally nocturnal and therefore it is likely that dehydration from encounters with metaldehyde pellets occur during the cooler, damper night-time, and therefore if a slug is still present and paralysed on the soil surface by the morning the increase in temperature may cause the extra dehydration required to kill a slug.

3.2.3 Recovery from Metaldehyde

One of the largest issues with control of slugs using metaldehyde based pellets is that although pellets may be successful in dehydrating a slug, they may recover. It is likely that, there may be sufficient soil moisture allowing the slug to rehydrate and recover from metaldehyde poisoning. Similarly, if a rainfall event occurs rehydration may be facilitated then. In previous feeding experiments, it has been suggested that 70% of slugs may recover from consuming metaldehyde (Wedgwood and Bailey 1986) when placed in ideal conditions.

3.2.4 The Need to Reduce Metaldehyde Application

Metaldehyde is a large water pollution risk due to its high mobility in soil once leached from slug pellets on agricultural land and was first detected in surface water in the UK in 2007 (Davey et al. 2014). Spikes in drinking water pollution levels cost the UK water industry millions of pounds each year and removal of metaldehyde from drinking water is extremely challenging. Metaldehyde is classed as "semi- persistent" in the aquatic environment and does not respond to conventional drinking water treatment processes (Castle 2017). There are a number of developing treatment methods for the remediation of metaldehyde from drinking water systems (Autin et al. 2013, Doria at al. 2013 and Rolph et al. 2014), however these processes are expensive to both manufacture and implement on a large scale. The pollution risk, coupled with the likelihood that metaldehyde may be less efficient at controlling slug populations due to slug recovery, means that it is imperative that further research into the short-term effects of metaldehyde on slug health immediately after the pest- pellet interaction have occurred are essential in order to gain insight into the rates of both slug morbidity and paralysis after exposure to metaldehyde. There may be the possibility to advise to manufactures to reduce the concentration of metaldehyde in slug pellets if mortality is seen at lower concentrations as well as higher concentrations of metaldehyde, while remaining aware that secondary mortality as a consequence of soil surface slug paralysis is likely to play a large role in slug control with metaldehyde.

4.3 Aims and Objectives

This research aimed to assess the likelihood of recovery by *Deroceras reticulatum* when placed in ideal artificial conditions allowing for rehydration after exposure to metaldehyde pellets, and how the health and recovery status a *D. reticulatum* differed after exposure to various concentrations of metaldehyde.

Specifically, the research objectives were to:

1) Assess mortality rate of *Deroceras reticulatum* after 14 hours exposure and after 72 hours to a metaldehyde pellet of either 1, 3 or 5% concentration, a commercial metaldehyde pellet and a non-toxic control pellet. It is hypothesised that higher pellet concentrations will result in higher slug mortality levels.

2) Compare the levels of slug paralysis after exposure and during recovery from metaldehyde pellets of various concentrations. It is hypothesised that slugs are more likely to be paralysed (as opposed to dead) after exposure to lower metaldehyde concentrations (1%) when compared to higher concentrations (5%) due to the prediction that slugs will reject higher concentrations of pellets soon after the initial test bite. It is also hypothesised that recovery will be more likely in slugs that have been exposed to lower concentrations of metaldehyde.

3.4 Methodology

3.4.1 Deroceras reticulatum

Grey Field Slugs (*Deroceras reticulatum*) were collected at random the edges of amenity grassland in Northumberland, UK, and selected, stored and acclimated to the laboratory as described in section 3.4.1. Slugs were selected at random, however only slugs regarded as healthy were used in the trial. A healthy slug showed no signs of white/ excess mucus production, responsive movement/ skin rippling to light touch and no obvious deformations. Slug collection took place on the evening before the start of the trial and collected slugs were stored in damp, lidded containers in a refrigerated environment (3-5ºC) until transfer into the laboratory. Slugs were starved and acclimatised for 24 hours to a controlled temperature room where all trials took place.

Immediately before transfer into each Petri dish, slugs were weighed in order to ensure an even distribution of sizes across treatments. Average slug weight was 0.5 $\underline{+}$0.2g.

3.4.2 Pellet Creation

Trials involved manufactured Lonza Axcela® 3% metaldehyde and Lonza Axcela® non-toxic pellets as well as laboratory made pellets. Laboratory made pellets were made with a base of plain flour mixed with 99.9% pure metaldehyde powder in order to form 1, 3 and 5% concentrations made to 20g in weight. Olive oil was then added to the dry mix by 1ml pipette drops (up to 15 ml dependent on metaldehyde concentration) in order to form a firm but malleable dough. The molluscicidal dough was then cut into 1g segments and rolled in order to form a pellet.

Commercial slug pellets are dry pressed as part of the manufacturing process and tend to be lighter in weight than the 1g laboratory pellets used in the trial, however the laboratory made pellets were of similar size compared to the Axcela® pellets also used in this research and therefore it was considered appropriate. Pellets created for use in trials were made within 24 hours of use in the trial and stored in a sealed container in a refrigerated environment until use.

3.4.3 Pellet Exposure

Deroceras reticulatum were first exposed to a pellet type for 14 hours, and each slug was only exposed once to one type of treatment pellet. Acclimated slugs were divided into groups of 6 of random weight and introduced into an open topped plastic arena (56x 43x23cm) on a layer of damped agricultural clay loam soil approximately 10cm thick. This soil was obtained from Cockle Park farm, Northumberland, and had not been exposed to pesticides or herbicides. Soil in the area was raked in order to produce a fine, even tilth in order to reduce the number of soil clods and shelters for slugs. Around the edge of the top of the arena a layer of polytetrafluorethylene (Fluon) was painted in order to prevent slugs leaving the arena. Fluon was observed to have no apparent impct on slug behaviour, other than upon encounter with the painted layer around the outside of the arena, a slug would turn away from the layer and continue to move around the arena. During video analysis, slugs were numbered, and their behaviour recorded throughout the trial in order to identify and record whether a slug encountered a pellet during the exposure phase. Treatment pellets (Table 3.1) were placed at 9 baiting points evenly distributed around the arena (Appendix 1). Slugs were then placed at equidistant intervals around the pellets (a maximum of 6 slugs per trial, 5 trials per treatment pellet) and left for 14 hours starting at 18.00 to move freely around the arena and interact with the treatment pellets. At any one time, 3 trials were run simultaneously in separate arenas. A total of 30 slugs were exposed to each treatment pellet type.

Table 3.1 Number of pellet treatments and contents. All laboratory made pellets were made with laboratory grade 99.9% metaldehyde, flour and oil.

Treatment	Pellet Contents
1	1% metaldehyde (laboratory made)
2	3% metaldehyde (laboratory made)
3	5% metaldehyde (laboratory made)
4	No pellets
5	Non-toxic control Pellet
6	Lonza Axcela® 3% Pellet

Each trial lasted a total of 14 hours, with 10 hours being in dark overnight conditions and 4 hours in artificial daylight, 2 hours at the beginning and the end 2 of the trial. During the 10 hours of dark, infrared illumination was used order to be able to video record slug behaviour during the trial.

Slug behaviour was recorded using Brinno BLC200 HDR Laboratory-Cam Time Lapse recording cameras and set at a frame rate of 10 frames per second. Video recordings were analysed in order to confirm interaction with treatment pellets.

3.4.4 Slug Recovery

After the end of the pellet exposure phase of the trial, the health status of each slug was assessed. Slug health status was categorised as follows:

- Not poisoned- no signs of paralysis and poisoning, the skin ripples to touch and slug is moving freely, and no excess of mucus noted.

- Partial paralysis – slug is on soil surface and moving but at a slower pace than expected. Skin still ripples to touch however noticeable mucus secretions around slug.

- Severely paralysed- slug is on soil surface and is barely moving, there may be a large amount of white/ clear mucus surrounding the slug which is likely to be on its side. Skin moves to touch when examined with magnification.

- Dead- no signs of life, skin does not ripple on touch.

Each slug was then moved into an individually labelled Petri dish lined on the bottom with a round of damp filter paper. Petri dishes were stacked and stored in bags in the controlled temperature room in order to maintain day: night cycles and regulate temperature. Each slug was then checked every day for the following three days and a record of their health status was made. Slugs that showed signs of severe paralysis/ death were checked with magnification for ripples on the mantle upon touch.

3..4.5 Statistical analysis

Results were analysed using Chi Squared contingency table analysis in order to compare categorical variables in R (R Core team 2020). For the purpose of this research, only one control treatment (control pellets) was used in the analysis in order to compare only pellet containing treatments.

3.5 Results

After 14 hours exposure to treatment pellets of various metaldehyde contents, the health status of *Deroceras reticulatum* showed great variation dependent on which pellet it had been exposed to. Slugs subject to the non-toxic control pellet and those that had not been exposed to any pellets showed high levels of good health (i.e., not paralysed) with only one slug observed to have died in the “no pellet” treatment (Figure 3.1). Slugs from populations that were exposed to metaldehyde based pellets displayed poorer health conditions, with the majority of slugs showing signs of either moderate or severe paralysis. After 14 hours exposure to pellets, the highest incidence of deaths occurred in slugs that had been subject to 5% metaldehyde pellets (23% death rate) compared to 1%, 3% and Lonza Axcela® death rates (7%, 10% and 10% respectively). Slugs showing no signs of poisoning were more than twice as numerous in the 5% metaldehyde treatment population compared to both 1% metaldehyde and Lonza Axcela® pellets.

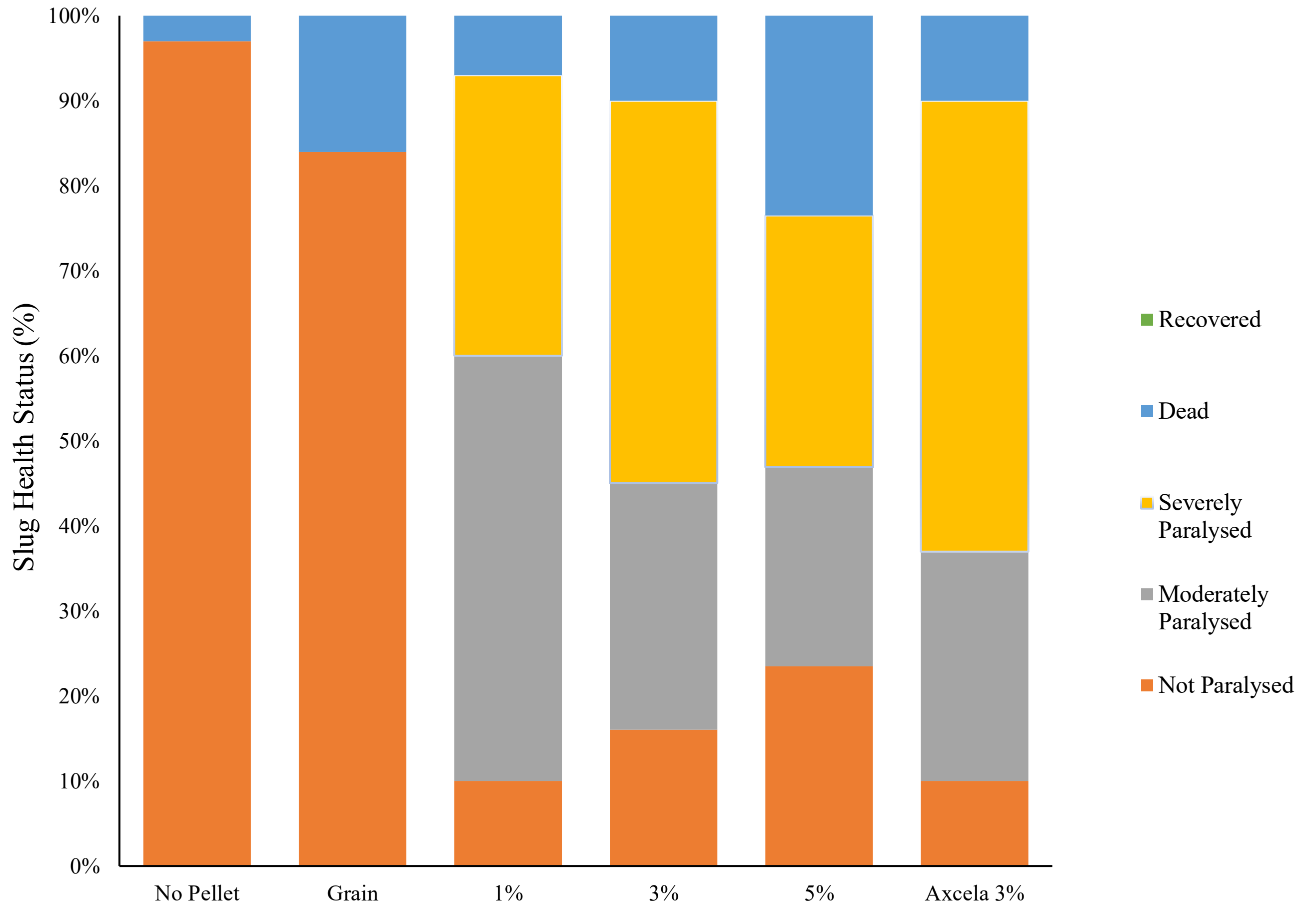

Figure 3.1 Health status of a population of 30 *Deroceras reticulatum* after 14 hours exposure to pellets of varying levels of metaldehyde concentration. Each slug population was subject to only one treatment pellet (n= 30).

After 72 hours placed in individual recovery conditions allowing for slugs to rehydrate, slug populations in all metaldehyde treatments suffered approximately 30% mortality, with the remainder of the populations either paralysed or apparently recovered from the effects of the metaldehyde pellet. A higher concentration of metaldehyde in the pellet did not yield a higher mortality rate, although the numbers of both severely and moderately paralysed slugs was lower in 5% metaldehyde compared to both 1%, 3% and Axcela® treatments (Figure 3.2). The relative mortality between treatments did not change from 14 hours to 72 hours ($X^2_{(4, 150)} = 4.918$, $P = 0.393$). Slugs exposed to metaldehyde pellets recovered in all treatments, and recovery was slightly higher in both 1% and 5% metaldehyde pellets (20% and 23% recovery) when compared to both of the 3% metaldehyde pellet treatments 10% and 13%

recovery rate in 3% laboratory made and Axcela® pellets respectively), though not significantly.

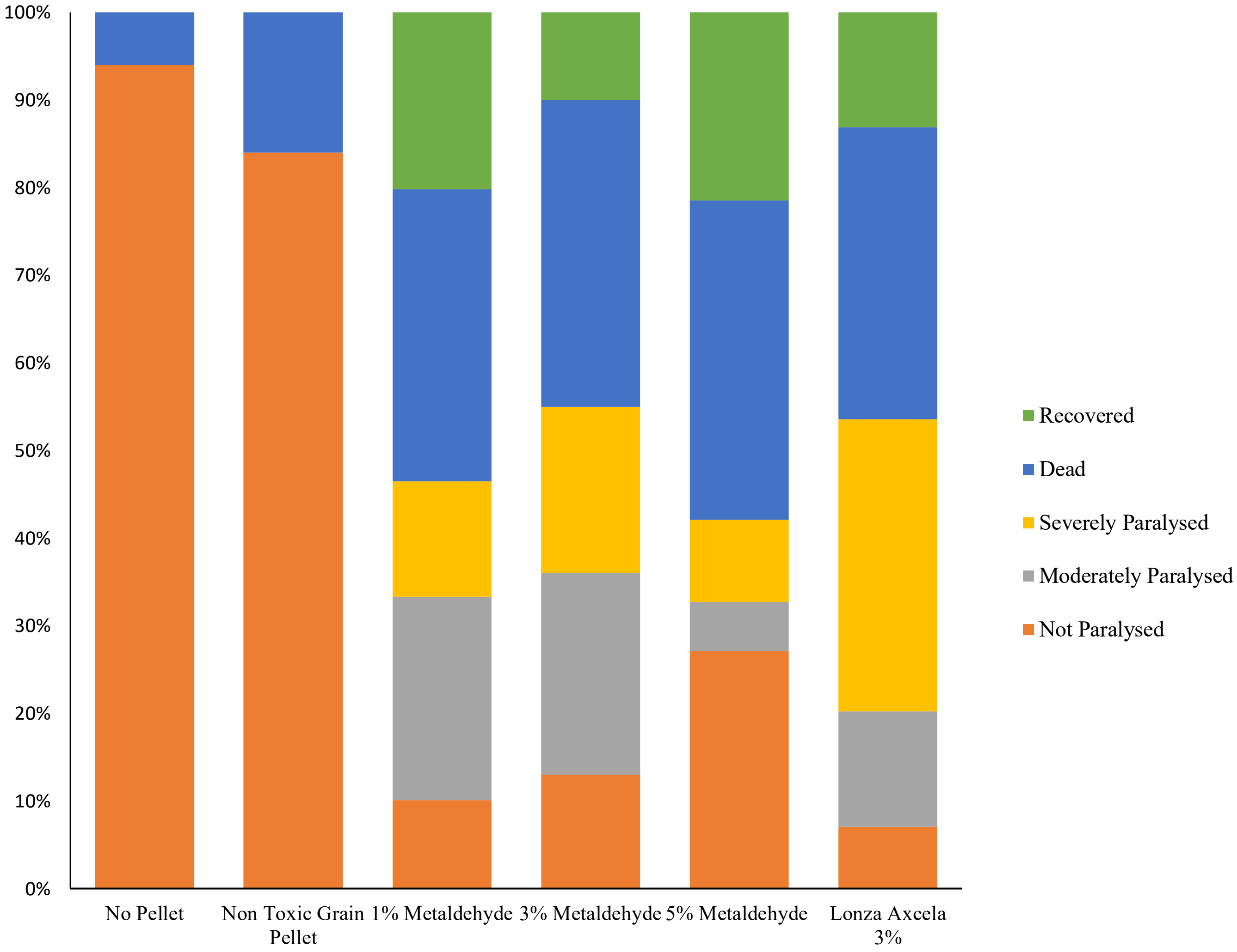

Figure 3.2 Health status of populations of 30 *Deroceras reticulatum* after 72 hours in a recovery environment after exposure to slug pellets of various metaldehyde content. (n = 30).

A proportion of slugs remained paralysed in all metaldehyde treatments after 72 hours of recovery, however there was a higher level of paralysis in lower concentrations of all metaldehyde treatments when compared to 5% metaldehyde. Results of Chi Squared contingency analysis showed that there was no significant difference between treatment and the number of slugs paralysed (moderately and severely combined) before and 72 hours after recovery, $X^2_{(4,150)} = 1.784$, $P = 0.780$. Paralysis proportions across all metaldehyde treatments decreased after recovery compared to proportions initially after exposure. The commercial treatment, Lonza's Axcela® pellet, gave a similar proportion of paralysed slug population to that of 1% metaldehyde treatment however had high proportions of severely paralysed slugs when compared to laboratory made treatments after 72 hours recovery.

3.6 Discussion

This research aimed to provide insight into the effect of metaldehyde on the health of *Deroceras reticulatum* and how the rate of recovery of a poisoned slug placed in ideal conditions may change with metaldehyde concentration.

In this laboratory-based research, *Deroceras reticulatum* were exposed to slug pellets of various metaldehyde concentration over a 14-hour period in order to replicate one full night of foraging in the field. The health status of the slug was then assessed in terms of mortality and paralysis. It was hypothesised that increasing concentration of metaldehyde may have one of three outcomes for the slug behaviourally either, the slug would detect the higher concentration of metaldehyde and reject the pellet entirely (1), or feed on the pellet for a short amount of time causing the slug to be unaffected or moderately paralysed (2), or the slug would feed on the pellet and consume a lethal dose of metaldehyde more frequently than with to lower concentration pellets (3).

Paralysis status was of interest in this study, given the evidence that slugs often succumb to secondary mortality in the field after metaldehyde poisoning (Lagan et al. 2004, Glen et al. 1989).

3.6.1 Not Paralysed

The treatment groups of *Deroceras reticulatum* that were exposed to the non-toxic grain pellets and those that were exposed to no pellets, showed, as expected, good health both after initial exposure and after three days of being in recovery conditions. This is unsurprising due to the lack of molluscicide present in the trials but confirms that for the non-toxic grain pellet (which is identical to the Lonza Axcela® pellet) it is the metaldehyde in the pellet that has the molluscicidal effects (Wright and Williams 1980).

Both 1%, 3% and Lonza Axcela® pellet treatment groups had low levels of slugs not seemingly affected by the pellets (approximately 10% for each group). Over 20% of slugs exposed to 5% metaldehyde were unaffected by the pellets both after initial exposure and three days of recovery. This could suggest that 5% metaldehyde pellet is more easily detected by a *D.*

reticulatum and is more likely to be rejected when encountered in the field, which would support hypotheses 1 and 2 mentioned previously.

3.6.2 Paralysed

Paralysis of *Deroceras reticulatum* was divided into two categories: moderately or servery paralysed. It was predicted that moderately paralysed slugs had consumed less molluscicide (weight for weight) compared to severely paralysed slugs, and therefore would be more likely to recover in the field. On the other hand, severely paralysed slugs are more likely to not recover or be predated upon due to their inability to move. After initial pellet exposure, groups from all treatments had a high proportion of slugs that were moderately or severely paralysed. More than 80% of slugs in the 1% treatment group showed signs of paralysis after initial exposure, which indicates that a large proportion of slugs accepted the low concentration slug pellet and consumed enough pellet in order for the molluscicide to begin to have detrimental effects on the slug's health. In an agricultural setting, it could be that this would be sufficient enough for predation or daylight dehydration to occur within the paralysed population, removing a large proportion of the pest.

Both 3% and Lonza Axcela® pellets yielded slightly higher levels of severely paralysed slugs when compared to 1% and 5% metaldehyde treatment groups after initial pellet exposure. In the field, this could mean that more slugs would be lying on the soil surface and would be likely to succumb to secondary mortality. Fewer slugs were paralysed after initial exposure to 5% metaldehyde, supporting the theory that this high concertation of pellet could be seen to work on an "all or nothing" basis dependent on individual slug behaviour.

After three days of recovery, an extremely small proportion of slugs remained paralysed for 5% metaldehyde, and these individuals had either died or recovered from the poisoning. Levels of paralysis remained relatively high (around 40%) for 1%, 3% and Axcela® pellets, which implies that recovery from metaldehyde poisoning may take longer than anticipated, and that other factors, such as food and nutrient availably may play a role in recovery. Remaining for longer in a paralysed state increases the risk of slugs being predated upon or dehydrating due to weather conditions, and it could be that this is an as effective control by metaldehyde as causing slug mortality. Moderate paralysis was seen in higher proportions after 3 days of recovery for both 1% and 3% metaldehyde groups, which suggests that slugs can survive for longer if only moderately paralysed which again has implications both for secondary mortality and recovery.

3.6.3 Mortality

The highest concentration metaldehyde treatment group (5%) gave rise to the highest proportion of deaths after initial exposure (approximately 20% of the population). This is still a low proportion of a population and is unlikely to translate to noticeable protection of crops. Lower concentrations of metaldehyde resulted in approximately 10% mortality of the treatment groups after initial contact. Low mortality rates imply that the slug has not consumed a sufficient amount of the pellet which would deliver a lethal dose. If a slug is unlikely to consume the lethal dose of a pellet over one night of foraging this may be problematic for growers if there is a rainfall event shortly after application. Metaldehyde is highly mobile and is easily leached out of a pellet after a rainfall event and on contact with soil moisture (Edwards et al. 2009, Zhang et al. 2006, Zhang et al. 2011), and therefore pellets may lose their potency quickly dependent on environmental factors. Ideally, pellet consumption should deliver the lethal dose in the first meal or certainly within that night of foraging in order to avoid issues with metaldehyde loss. The proportion of slugs that died across all metaldehyde treatments after three days of recovery was around 30% and this is similar to previous metaldehyde recovery studies (Wedgwood and Bailey 1988). Mortality is still lower than required in order to produce effective slug control in the field and may provide a reason for growers reporting slug related crop damage despite employing molluscicide pellets to control the population (Iglesias 2003).

3.6.4 Recovery

Recovery for both 1% and 5% treatment groups were approximately 20% of the poisoned population. If almost a fifth of the population can recover from the dehydrating effects of metaldehyde after 3 days of rehydration, this indicates that a substantial proportion of the population may be sustained after exposure to metaldehyde. For both 3% and Axcela® pellets, only 10% of the population recovered, meaning more of the populations exposed to 3% metaldehyde have either suffered mortality or remained paralysed. Ideal recovery conditions in a laboratory-based setting are difficult to simulate due to the many abiotic and biotic factors that could have a positive or negative effect on slug health. Rehydration is likely to be the key factor in determining whether a slug may recover from metaldehyde poisoning (Bourne et al. 1988), and it is for this reason that water availability was the only factor included in the recovery setting. *Deroceras reticulatum* is able to survive for many days without regular feeding (Godan 1983) and therefore it was predicted that a lack of food would not have a significant influence on the ability for the slug to recover.

3.6.5 The Importance of Metaldehyde Concentration

Metaldehyde slug pellets are sold commercially at concentrations ranging from 1- 5% metaldehyde, and the industry standard for agronomic uses tends to be around 3% concentration. While agricultural slug pellet application in England and Wales has remained relatively constant over the past 10 years, there is not a marked decrease in the amount of slug damage to crops at the end of each growing season, as would be expected with successful pesticide use (Garthwaite et al. 2019) Metaldehyde pellets are of major concern to the UK water industry due to the chemical's high mobility in the environment, particularly after heavy rainfall events. Metaldehyde is a pollution risk to surface water and it can be detected in river catchments where concentrations regularly exceed the EU Drinking Water Directive limit of 0.1ug/l of a single pesticide (Castle 2017). On top of the pollution risk, metaldehyde is extremely difficult to remove from drinking water systems once detected and has the potential to cost water bodies millions of pounds in setting up effective treatment systems (Ibrahim et al. 2019). Metaldehyde pellets are a danger to non-target organisms, particularly animals found in similar habitats to slugs or those that feed on invertebrates, such as hedgehogs and birds (Attademo et al. 2016, Gething et al. 2020, Lagnan and Shaw 2006). Organisms that consume poisoned slugs are also at risk of secondary poisoning themselves, and it could be that applying lower concentration pellets in the field will alleviate this risk. On the other hand, the application of lower concentration pellets may result in increased application rates during the growing season, due to fewer fatal poisonings from low concentration pellts.

Considering the risk to the environment, it is imperative that the control of slugs is made as efficient and as effective as possible. Observations of continued slug damage at the end of the growing season indicate that there may be issues at the pest – pellet interaction level that are inhibiting the pellet from working as an effective molluscicide (Howling 1991).

3.7 Conclusion

Although 5% metaldehyde resulted in the highest mortality rate after initial exposure, there was a higher proportion of slugs that had not been affected by the pellet also after exposure. It is likely that a high concentration pellet results in higher levels of molluscicide detection by the slug and is therefore more likely to be rejected by a high proportion of a population in the field. Both the commercial and laboratory made 3% pellet produced similar results in terms of low initial mortality and high levels of paralysis, although after recovery it was noticeable that a higher proportion of slugs subject to the commercial pellet remained severely paralysed and would be arrested on the soil surface when compared to the non-commercial 3% pellet, which implies that pellet formulation is key in producing an effective pellet. Exposure to 1% pellets yielded interested results in that the proportions of slugs paralysed, dead and recovered were not dissimilar to those exposed to 3% pellets.

While 3% pellets are generally observed as the industry standard, research into the effectiveness and stability of a 1% pellet in an agricultural setting that could yield similar mortality and paralysis as 3% pellets may be a way forward in reducing the amount of metaldehyde applied to the environment. Maintaining pellet potency would be essential in designing a 1% pellet, as current formulations allow metaldehyde to leach into surface water and it would be vital that the pellet retained the molluscicide for as long as possible due to its reduced concentration. Paralysis by metaldehyde pellets may be as important as direct mortality due to the existence of natural predators, however the rate of predation is extremely difficult to quantify (Langan et al. 2001). Consumption by predators of slugs that have been paralysed with 1% metaldehyde would also be less likely to cause secondary poisoning and smaller amounts of metaldehyde bioaccumulation (Zhang et al. 2011) in the food chain and therefore the replacement of 3% pellets with 1% pellets may be beneficial not only to the water industry, but to non-target organisms.

Chapter 4: The Potential uses of Inorganic Controlled Release Technology (iCRT) for Metaldehyde Based Molluscicides

4.1 Abstract

Metaldehyde, when consumed by slugs, not only triggers increased mucous production leading to dehydration, but it also causes muscle immobilisation and paralysis. Issues surrounding the ineffectiveness of metaldehyde-based slug pellets are due, in part, because of early detection of metaldehyde by a slug when grazing on a pellet. One solution to early detection could be to “hide” metaldehyde using controlled release technology and delay the release of the substance until further into the slug’s digestive system, which would increase consumption of a pellet and therefore increased chances of a lethal dosage of metaldehyde consumed by a slug. In order to explore the potential of inorganic controlled release technology (iCRT) for molluscicides the palatability of three novel formulations of metaldehyde incorporated into a silicon matrix was assessed by measuring slug consumption. Results of bioassay trials for iCRT formulation 1 suggested that iCRT containing metaldehyde was not palatable and metaldehyde could still be detected by the slugs. There was no significant difference between the median consumption of pellets with iCRT metaldehyde or with metaldehyde alone. Pellets made with iCRT formulation 2, which contained half the amount of metaldehyde of iCRT formulation 1, or made with metaldehyde alone, were again consumed significantly less than those made without metaldehyde. Coating a commercial metaldehyde pellet with iCRT (iCRT formulation 3) did not increase pellet consumption when compared to a non-coated metaldehyde pellet. The results suggest the need for further development of controlled release technology containing metaldehyde.

4.2 Introduction

In order to reduce the risk of poisoning non target organisms, as well as reduce environmental pollution risk, advances in pesticide technology have been focused on creating more species-specific formulations. The creation of a highly specific pesticide requires highly complex technologies in order to create a chemical formulation that presents minimal risk to any organism beside its target, often by targeting specific conditions inside the target animal, for example, gut pH or specific environmental conditions (Park et al). Recently, attention has turned to nanotechnology as a way to create such specific pesticides. Nanotechnology, defined as the study and use of matter from 1-100 nm (Poole and Owens 2003) and is at the forefront of improved pesticide design. Nanopesticides are usually made of a combination of organic and inorganic compounds and are designed to improve to control the release of an active pesticide substance, protect the active ingredient from degradation or increase the solubility of an active ingredient (Ragaei and Sabry 2014).

4.2.1 Controlled Release Technologies in Agriculture

The ability to control the release of an active substance is one of the key attributes in the development of a new and existing pesticide formulations. This branch of nanotechnology is well established in medicine, with controlled release technologies enabling active substances to be released only under specific environmental circumstances or after a defined period of time (Langer and Peppas 2006). Such release technologies allow highly specific spatial and temporal delivery, which has led to significant increases in drug efficiency and treatment success (Bhusal et al 2016, Liechtly and Peppas 2012). Typically, controlled release technologies involve the encapsulation of an active ingredient in a non-organic matrix, for example, silicon which can be engineered to break down under specific environmental circumstances.

The use of controlled release technologies in agricultural pesticides is still very much in its infancy, however in the last decade there has been a noted increase in research surrounding the area of controlled release technologies and pesticides (Singh et al. 2020).

4.2.2 Metaldehyde as a Molluscicide

Metaldehyde based pellets are the most common pesticide used in slug control in western farming. Typically, commercially available from concentrations of 1 – 3% metaldehyde, the grain-based pellets are broadcast over the surface of agricultural land or incorporated into the seed bed at the time of seed drilling. The molluscicidal effects of these pellets are entirely dependent on encounters with the pellets by pest slugs, upon which pellets are ideally consumed by the organism. The molluscicidal effect of metaldehyde is twofold - initially it is an irritant of gastropod mucus glands, causing dehydration and desiccation, and if consumed in large enough quantities, it acts as a nerve poison, immobilising the slug which is then likely to succumb to secondary mortality either from dehydration or predation by natural predators such as birds, ground beetles or hedgehogs.

Risks and problems arising from the use of metaldehyde in conventional farming is a contentious issue. Metaldehyde application presents a large groundwater contamination risk, which has led to pollution of drinking water exceeding the EU Drinking Water Directive maximum limit of 0.01ug/l for a single pesticide in drinking water (European Food Safety Authority 2010). Metaldehyde is also a risk to non-target organisms (Saad et al. 2017), and there is evidence that the molluscicide may persist in soil, although detection was below the minimum detection level (Zhang et al. 2011). In December 2018, the UK Department for Environment, Food and Rural Affairs (DEFRA), announced a UK wide ban of the outdoor use of metaldehyde and for the molluscicide to be phased out over proceeding 18 months because the molluscicide presented an unprecedented risk to wildlife (DEFRA 2018). This decision was condemned by the National Farmers Union, farmers across the UK and molluscicide manufacturers, and a legal challenge by Chiltern Farm Chemicals saw the ban overturned in 2019.

Whilst the fate of metaldehyde use still hangs in balance in the UK, it should be noted that 23 of the EU’s member states still use metaldehyde as a major form of agricultural slug control. Improvements to how the pesticide is manufactured could reduce both the water pollution and environmental risk presented by metaldehyde. In this chapter, the palatability of metaldehyde incorporated into a novel controlled release technology is tested with the grey field slug, (*Deroceras reticulatum*). The pellets tested contained inorganic controlled release technology (iCRT) metaldehyde and a mixture of flour and oil to form a pellet. The grey field slug is the most economically important gastropod pest in the UK, and improvements to molluscicide technology may have the potential to both improve slug control while reducing the negative effects of the pesticide.

4.3 Aims and Objectives

This research aimed to test the palatability and molluscicidal effect of three iCRT metaldehyde formulations. It was predicted that loading metaldehyde into a silicon matrix would mask the molluscicidal effects of the pellet and therefore the slug would be more likely to consume a lethal dose of metaldehyde. The delivery of a higher concentration of metaldehyde to the slug would probably result in an increased likelihood of mortality. For the purpose of this research, palatability of a pellet was inferred by measuring the consumption of a pellet by a slug.

The specific objectives of this research were:

1) To test the palatability of iCRT metaldehyde pellets compared to non iCRT metaldehyde pellets. It was predicted that iCRT metaldehyde would be more palatable to slugs compared to non iCRT due to the controlled release technology delaying detection of the molluscicide by the slug.
2) To test the effect of lower concentrations of iCRT metaldehyde on pellet palatability when compared to higher iCRT metaldehyde concentrations. It was hypothesised that iCRT metaldehyde would be further palatable to slugs at lower concentrations as opposed to higher.
3) To test the palatability of coating a commercial metaldehyde pellet with iCRT compared to the palatability of a non-coated metaldehyde pellet. It was predicted that coating a commercial 3% metaldehyde pellet with an iCRT formulation not containing metaldehyde would act as a palatable enough food source to arrest the slug at the pellet and encourage eventual consumption of the pellet.

4.4 Methodology

4.4.1 Slugs

All slugs (*Deroceras reticulatum*) were collected from amenity grassland in Northumberland, UK. Collection, slug selection and acclimatisation took place following the same methodology described in section 3.4.1 Slugs were collected the evening before the beginning of the trial and stored in a damp refrigerated (3-5^{o}c) environment overnight until use. Slugs were then starved and acclimatised for 24 hours to a controlled temperature room (15 ± 1^{o}C, daylight: night 12:12) where the trial would take place. All slugs were weighed in order to ensure an even distribution of slug sizes between treatments.

4.4.2 iCRT and Pellet Creation

Three iCRT based formulations were created by a manufacturer based on preliminary trials with a novel iCRT metaldehyde formulation. The first metaldehyde formulation, iCRT 1, was formed of iCRT powder (20%) and metaldehyde (4.95%), which equated to a 1% metaldehyde pellet concentration. The second formulation, iCRT was contained half the amount of metaldehyde compared to iCRT 1 (2.5%). The third formulation coated commercial 3% Axcela® metaldehyde pellets in iCRT powder. Acceptance of the iCRT pellets by *D. reticulatum* was compared to iCRT pellets containing no metaldehyde, laboratory made metaldehyde pellets with no iCRT and grain or flour controls control containing no metaldehyde or iCRT. Each formulation starting from iCRT 1 was developed by the manufacturer in response to the results of the previous formulation.

Pellets were made in the laboratory using a combination of plain flour, olive oil and the appropriate treatment powder (Appendix 2). Oil was added to the flour and treatment mix in order to create a firm, but malleable dough, which was then cut into 1g pieces and formed into small pellet like spheres.

4.4.3 Experimental Set Up

Individually labelled 9cm Petri dishes were inverted with a single sheet of filter paper on the base. The filter paper was soaked with distilled water and the excess shaken off. Pellets for iCRT formulations 1 and 2 were placed in plastic lid caps (1cm in diameter) in order to minimising leaching onto the filter paper. One treatment pellet was placed in one lid cap and placed in the centre of each Petri dish and one slug was then introduced per Petri dish. For iCRT formulation 3, one pellet per treatment was placed in the centre of each Petri dish 24 hours prior to the introduction of a slug in order to allow the pellets to soften.

Petri dishes were then stacked in number order so to randomise treatments and placed into clear plastic bags in order to reduce moisture loss. These bags were then stored in the controlled temperature room and left undisturbed until data collection was required.

4.4.4 Data Collection

Petri dishes were checked 24 and 96 hours after the initial introduction of the slugs to the trial. The amount of pellet consumed per Petri dish was recorded as well as the mortality status and condition of the slug. Trials were undertaken in batches and due to accessibility issues 21 slugs were assessed after 24 hours exposure to iCRT 1, but the full 32 slugs were assessed after 96 hours exposure. It was decided that exposure after 96 hours was more likely to represent true palatability of the formulations.

4.4.5 Statistical Analysis

Percentage consumption estimates were converted into a consumption scale in order to standardise results (Table 4.1). Percentage consumption was converted into a scale in order to standardise consumption rates between formulations. Since amount consumed was a subjective measurement, it was agreed that a scale would be more accurate in describing consumption recordings on different days and formulations. For each iCRT formulation median consumptions were analysed using nonparametric one-way analysis of variance (ANOVA) in R (R Core Team 2018).

Table 4.1 Consumption scale for amount of pellet consumed in iCRT bioassay trials. Percentage estimates of pellet consumption were converted into a scale in order to standardise results.

Percentage Pellet Consumption	Consumption Scale
0	0
1-10	1
11-20	2
21-30	3
31-40	4
41-50	5
51-60	6
61-70	7
71-80	8
81-90	9
91-100	10

4.5 Results

4.5.1 iCRT Metaldehyde Formulation 1 Consumption After 24 and 96 Hours

Consumption of all pellets 24 hours in the bioassay was relatively low (Figure 4.1), with slugs only consuming up to 50% of a single pellet and there was no significant difference between the average consumption of pellets between all treatments (F(3,80) = 0.004, p = 0.949). Consumption of metaldehyde pellets appeared to be lower when compared to the non metaldehyde treatments, with the majority of slugs consuming none of the pellets containing metaldehyde. Three pellets from pellets with metaldehyde were consumed up to 20%, indicating that pellets containing 1% metaldehyde could be consumed by slugs. Consumption of non metaldehyde treatment pellets was also low, with a median consumption scale of 1, suggesting that 10% or less of non-toxic pellets had been consumed, however the range of pellet consumption reached up to scale 4 and 6 for the iCRT control and grain control respectively, indicating the potential increased palatability of these pellets when compared to metaldehyde containing pellets.

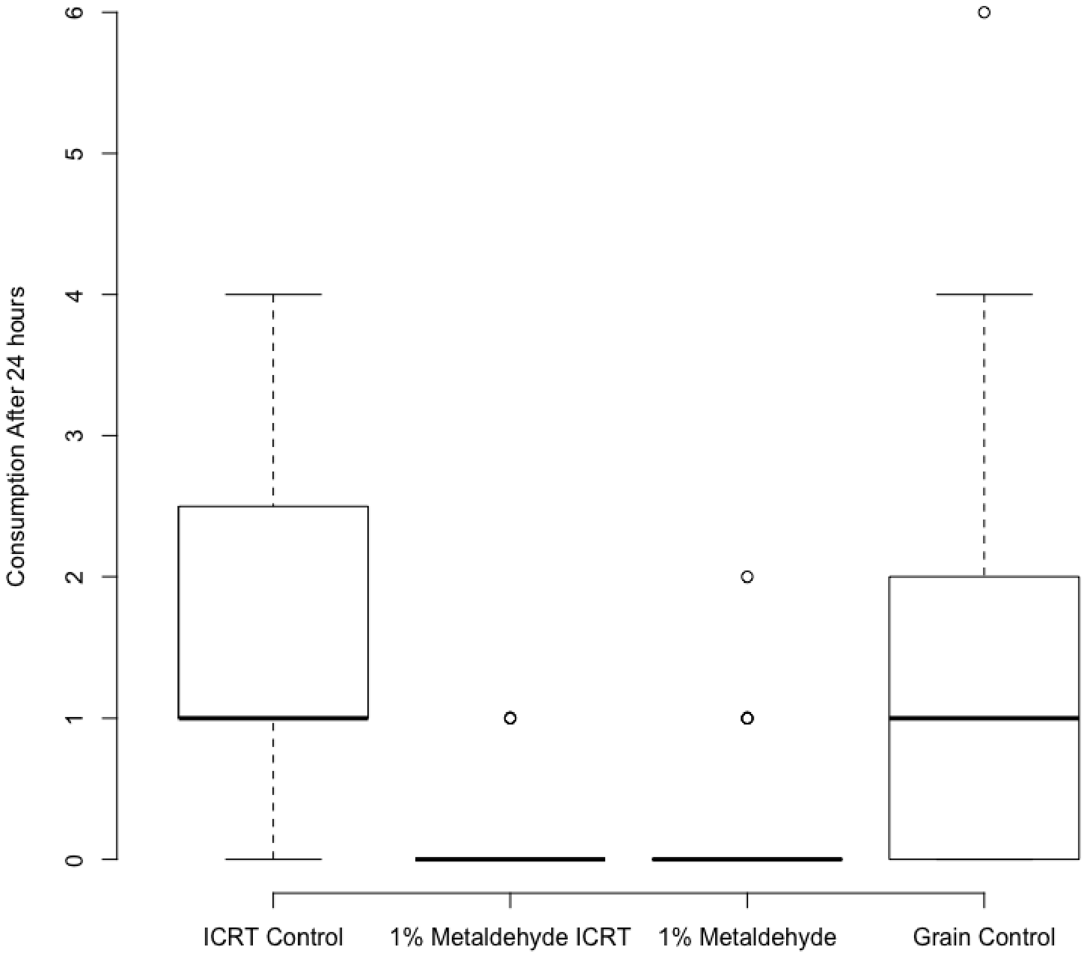

Figure 4.1 Slug consumption of treatment pellets after 24 hours exposure in a Petri dish bioassay. There was no significant difference between the average consumption of each treatment pellet type (F(3,80)=0.004, p=0.949) (n=21).

Pellet consumption after 96 hours increased for both control treatments, and the 1% metaldehyde pellet, and all treatments showed slightly increased pellet consumption (Figure 4.2). Median pellet consumption for non metaldehyde treatments tripled and doubled, with up to 30% of an ICRT control pellet consumed per slug, and up to 20% of a grain control consumed. Pellet consumption for metaldehyde containing treatments remained low (up to

10% of a single pellet consumed) and there was no significant difference in the average consumption of any of the treatment pellets (F(3,124)=2.958, p=0.088).

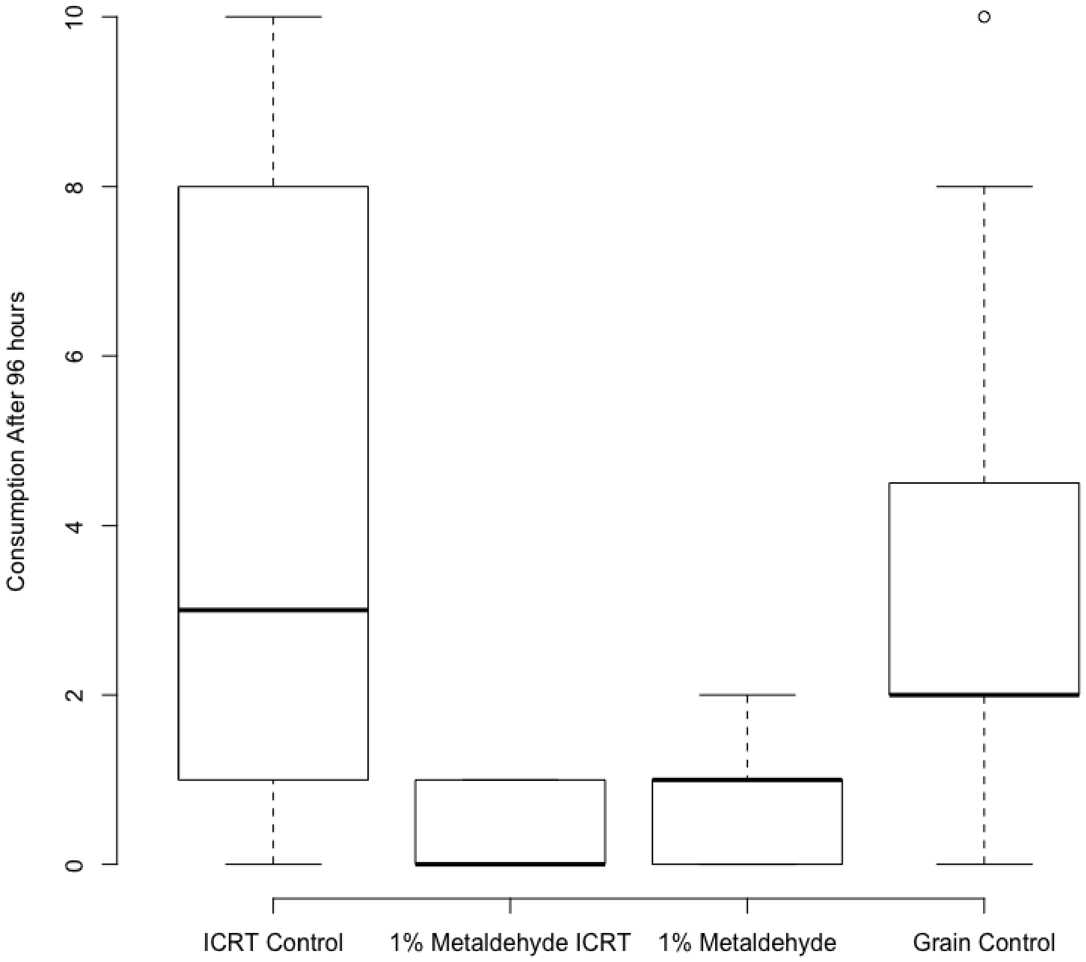

Figure 4.2 Slug consumption of treatment pellets after 96 hours exposure in a Petri dish bioassay. There was no significant difference between the average consumption of each pellet type (F(1,127)=2.958, p=0.088) (n=32).

4.5.2 iCRT Metaldehyde Formulation 2 Consumption After 24 and 96 Hours

After 24 hours, consumption of all pellet types was low with a maximum median pellet consumption of 10% (Figure 4.3). Pellets of each treatment type were consumed by slugs in each bioassay, and on average there was significantly higher consumption of the iCRT control when compared to the three remaining metaldehyde treatments (F (3,124) =6.236, p= 0.014). All metaldehyde containing treatments were not initially consumed by up to 50% of the slugs in each treatment trial, suggesting the detection of the active ingredient in the pellets.

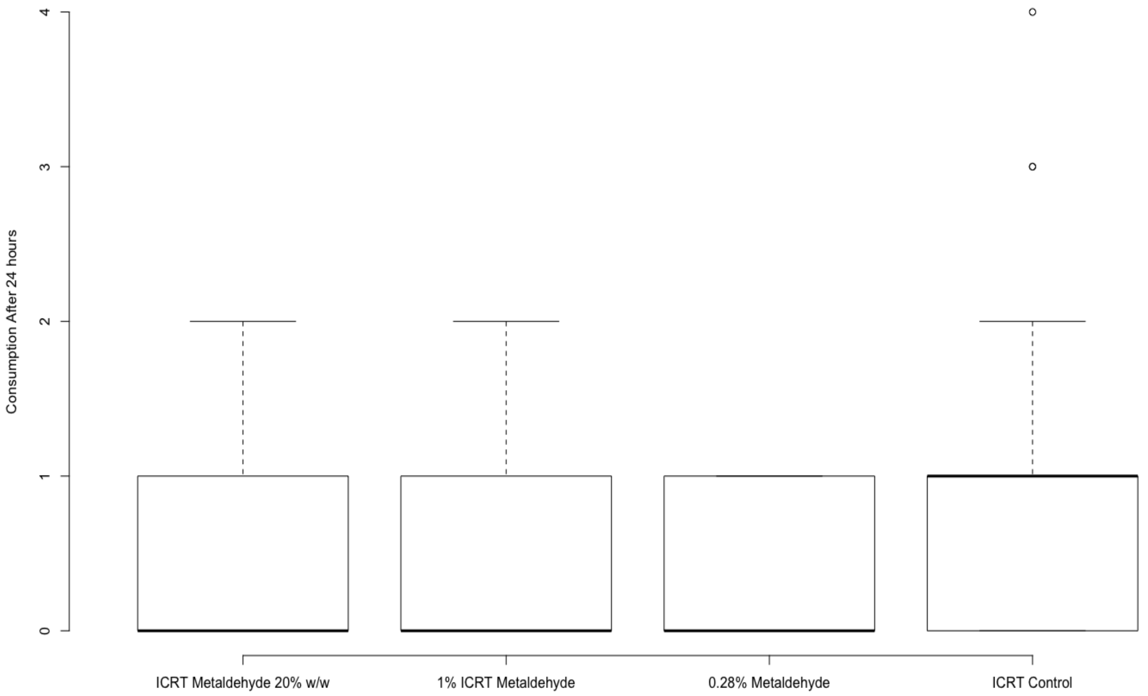

Figure 4.3 *Deroceras reticulatum* consumption of treatment pellets after 24 hours exposure in a Petri dish bioassay (n=32).

Consumption of all pellet types increased 96 hours after the introduction of slugs to the bioassay (Figure 4.4). There were no statistically significant differences between the average consumption of any of the pellet types (F(3,124)= 2.958, p= 0.088), however slugs consumed more of the iCRT control pellet, with double the median consumption of up to 20% of a pellet when compared to treatments containing metaldehyde. Outlying data suggests that iCRT containing pellets were consumed up to between 21-30%, while the non-metaldehyde control was consumed between 91-100%.

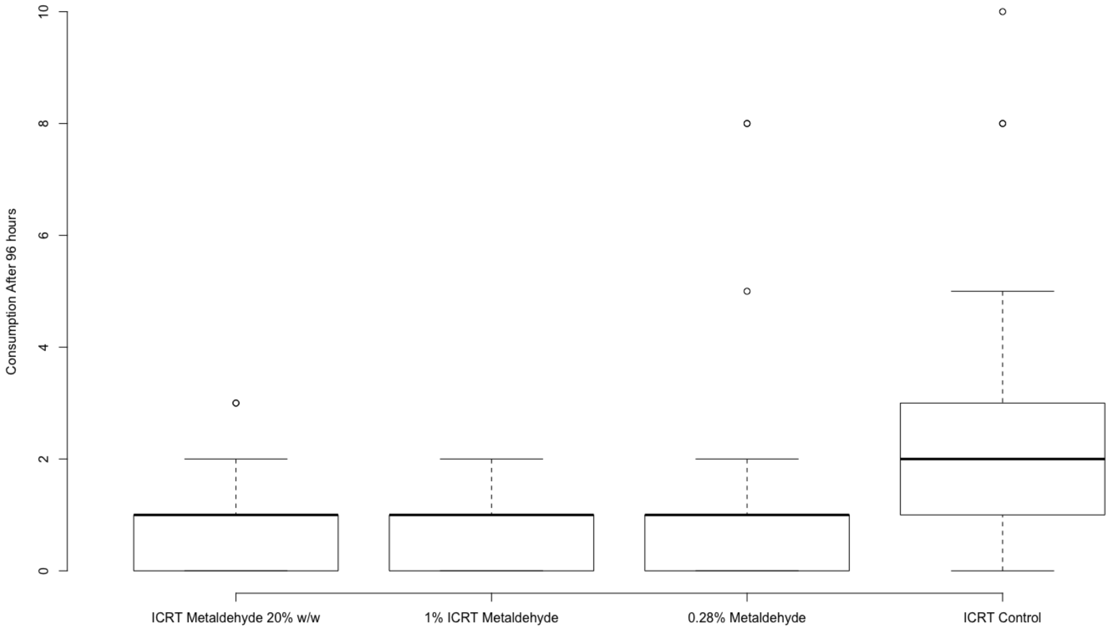

Figure 4.4 *Deroceras reticulatum* consumption of treatment pellets after 96 hours exposure in a Petri dish bioassay (n=32).

4.5.3 iCRT Coated Pellet Formulation Consumption After 24 and 96 Hours

Consumption of all pellets was extremely low 24 hours into the bioassay (Figure 4.5). Metaldehyde containing pellet treatments were not consumed at all by any slug, and up to 50% of slugs subject to the non-toxic control consumed up to 10% of a pellet, although one slug was observed to consume the entire pellet after 24 hours. Average consumption of the control pellet was significantly higher compared to both metaldehyde pellets (F(1,88)=19.75, $p < 0.001$).

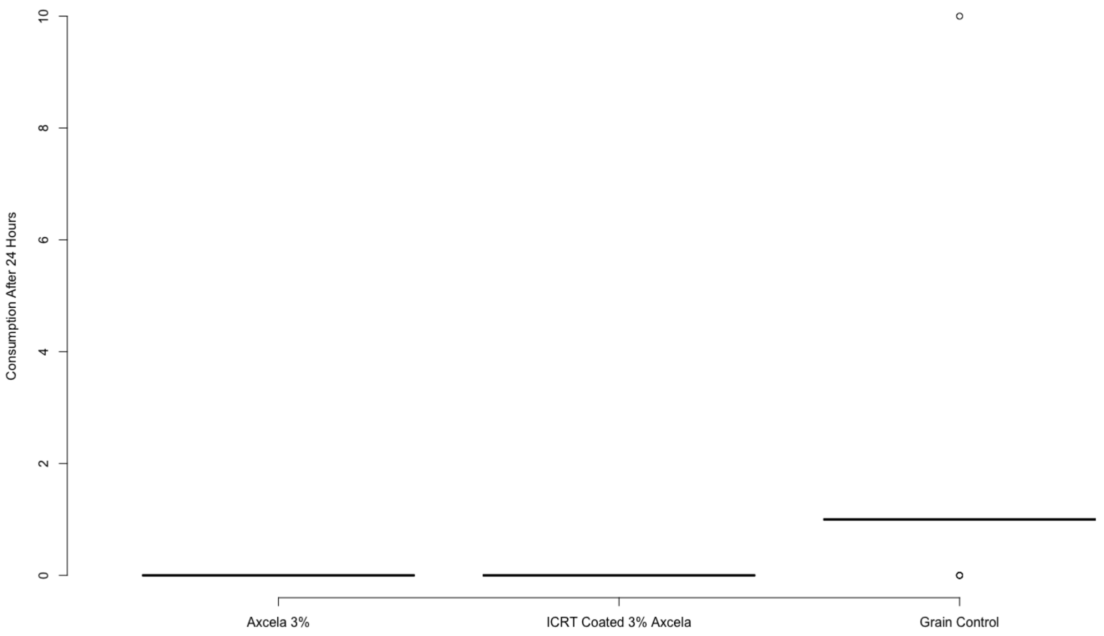

Figure 4.5 Consumption of pellets by *Deroceras reticulatum* in a Petri dish bioassay after 24 hours. (n=32).

Slugs readily consumed all of the non-toxic control pellet after 96 hours, with the exception of one slug which did not consume any of its pellet, which was observed to have died during the trial (Figure 4.6). The non-toxic control pellet was consumed significantly more when compared to both metaldehyde pellets (F(1,88)19.75, $p<0.001$). Up to 50% of slugs in treatment trials containing metaldehyde did not visibly consume any pellet upon observation, however, still more slugs consumed more of the iCRT coated metaldehyde pellet when compared to the commercial pellet alone.

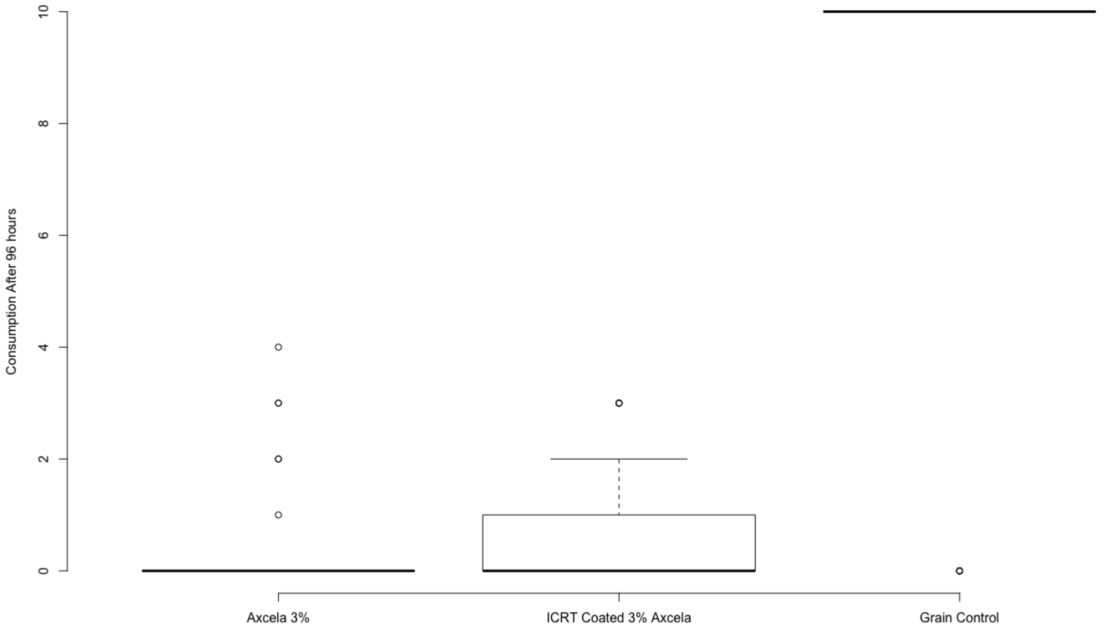

Figure 4.6 Consumption of pellets by *Deroceras reticulatum in* a Petri dish bioassay after 96 hours. (n=32).

4.6 Discussion

This research aimed to test the acceptability of novel metaldehyde slug pellets using inorganic controlled release technology which is currently being used in a number of pesticides and herbicides (Cao et al. 2016, Roy et al. 2014, Sheng et al. 2015). Improvements to metaldehyde based slug pellets are imperative as this widely used molluscicide is a significant water pollution risk and threat to non-target organisms (Hunter 1995). Consumption of each pellet was very low although median consumption of both treatment pellets containing no metaldehyde was higher compared to those containing the molluscicide. After 96 hours, median consumption was still not significantly different between treatment groups, although the median consumption for metaldehyde containing pellets remained at 10% or less. These results suggest detection of the molluscicidal element of the pellet by the slug and therefore suggests that the iCRT formulation containing 4.95% metaldehyde was ineffective in masking the molluscicide from the slug.

Halving the metaldehyde contents of the iCRT formulation was then tested in iCRT 2 treatments. Comparisons between iCRT metaldehyde pellets 0.07%, iCRT metaldehyde pellets 0.28%, metaldehyde no iCRT 0.28% pellets and iCRT pellet with no metaldehyde demonstrated detection of metaldehyde by the slug at extremely low concentrations. Consumption of iCRT pellets containing no metaldehyde was significantly higher after 24 hours into the trial, suggesting that the iCRT formulation could be acceptable to slugs for consumption. After 96 hours, there was no statistically significant difference in the median consumption of each pellet treatment, however treatments containing metaldehyde were consumed half as much as the control iCRT treatment containing no metaldehyde.

While iCRT 1 and 2 formulations with microencapsulated metaldehyde appeared to be unsuccessful at preventing early detection of metaldehyde by the *D. reticulatum*, the results suggested the iCRT formulation without metaldehyde was palatable to the slug. This could infer that slight adjustments to the matrix within the formulation would allow for successful delivery of metaldehyde. Indeed, there are many methods and formulations available to be used in nanotechnology based pesticides (Nuruzzaman et al 2016, Oliveira et al. 2018). A different approach was taken for the final iCRT trail, iCRT 3. Instead of forming pellets with a flour and oil base, pre-manufactured Lonza Axcela® pellets were coated in the iCRT powder formulation, creating an iCRT shell around the 3% metaldehyde pellet. Acceptance of this pellet was compared to uncoated Axcela® pellets and a non-toxic grain pellet equivalent to the Axcela® pellet without metaldehyde. It was hypothesised that coating the metaldehyde pellets in an acceptable food source would arrest the slug upon consumption, meaning the slug would continue to consume the pellet due to the inclusion of an appealing food source. Consumption of the control pellet was significantly higher after both 24 and 96 hours into the trial and suggests that coating the Axcela® pellet in an iCRT formulation had no effect on reducing the detection of metaldehyde by a slug. It could be that during the coating process, a chemical reaction between the iCRT powder and the pellet made the novel pellet unappealing to the slug and it was observed that pellets coated with iCRT powder were a slightly different colour when compared to the non-coated Axcela® pellets. The extremely low consumption of the Axcela® pellet was surprising and contrasts with results observed in the previous chapters of this research. On stark contrast between the previous chapters and this research trial was that the Axcela® pellet was on soil for previous chapters, as opposed to on filter paper. It could be that the damped filter paper increases the leaching process of metaldehyde out of the pellet and therefore the slug detects the presence of metaldehyde earlier than on soil, meaning the pellet is more likely to be rejected. The rationale behind performing this research on filter paper in Petri dishes was to avoid any external factors that may impact palatability, however in doing so, the leaching of metaldehyde may have been increased as a result. This research could be repeated on soil conditions similar to methods described in chapters 3 and 4 and compared to the results from this chapter.

This research has further demonstrated that *D. reticulatum* may be able to detect metaldehyde at extremely low level, even less that 1% concertation (Wedgewood and Bailey 1988). This may give some insight into the issues surrounding the ineffectiveness of slug control by use of metaldehyde pellets in agriculture. It could be that detection in soil from pellet leaching could deter a slug from feeding, or that metaldehyde could be detected by olfactory cues. It is difficult to determine the point at which a slug may detect metaldehyde, however it could be that in this research metaldehyde may have leached onto filter paper (although this is unlikely due to pellets being placed in plastic caps in order to avoid this), or that the slug can detect metaldehyde before physical contact. Research suggests that slugs persistently sample food sources in their environment (Whelan 1982) and it is likely that slugs may have encountered the novel pellets, taken a “test bite” and decided it was unpalatable when pellet consumption was shown to be extremely low.

4.7 Conclusions

iCRT metaldehyde proved to be no more effective in being an acceptable food substance than metaldehyde pellets alone. Chemical reactions when including metaldehyde into the iCRT matrix are likely to have occurred which may have altered the chemical structure of both substances, potentially rendering the iCRT matrix ineffective in masking the molluscicide. When coating Axcela® pellets with iCRT, pellets showed a number of inconsistences in coating thickness which may have led to some pellets being less consumable compared to other pellets. Seed dressing are a potential area of future research for iCRT metaldehyde (Simms et al. 2002) due to the apparent low palatability of encapsulated metaldehyde.

Though the inclusion of metaldehyde in the iCRT formulations in this research suggests that the molluscicide was not successfully encapsulated in order to increase delivery to the target organism, it highlights the need for further exploration of other formulations and technologies in this area. The development of nano-pesticides is still very much in its infancy, and even after the development of a formulation that is successful in its effect on the target organism, broader issues and questions arise around the application of these technologies in the field such as the persistence of its residues in water, soil and crops (Arias- Esteves et al. 2007). Evidence from this research suggests that slugs will readily consume pellets made with iCRT formulation that does not contain metaldehyde, which could suggest the potential for iCRT metaldehyde pellets being a viable option if alterations are made to the inclusion of metaldehyde in the formulation.

Chapter 5: General Discussion

5.1 Thesis Overview

This research provides knowledge of the impact molluscicides have on the movement, feeding and health of *Deroceras reticulatum*, with particular focus on the concentration of the molluscicidal ingredient. Pellet based molluscicides are likely to remain the most common form of slug control in agriculture due to the nature of pest slug invasions and limited molluscicides available for large scale control. It is for this reason that it is essential that the pest-pellet interaction between *D. reticulatum* and a molluscicide pellet is well described and understood, in order to inform for enhancements to pellet design leading to improved slug control. Understanding the relationship between slug and pellet, also provides knowledge of how better to use and apply pellets in order to maximise control. This research suggests *D. reticulatum* is able to detect metaldehyde prior to pellet contact, and it could be that this is due to leached metaldehyde present in the substrate that the pellet is on. Findings in chapters two and three suggest that low concentrations (1% as opposed to 5% metaldehyde) are more palatable to the slug.

Studying the behaviour of *Deroceras reticulatum* in a laboratory-based setting allows for more control over external factors that may influence behaviour when compared to a field based setting. Behavioural studies that concentrated on the movement and foraging behaviour of the slug (chapter 3) took place in arenas filled with damped agricultural clay- loam soil, which allowed the slug free movement around the arena unrestricted only to the limits of the arena boundaries. It was important that slugs were allowed to interact with pellets in the most natural way possible, and so for this reason in chapters 3 and 4 slugs were exposed to pellets over a 14-hour period over-night, in order to replicate how foraging may take place out in the field. No plants or food sources were included in these behavioural assays, as observations based solely the effect of the molluscicide was on slug behaviour was the targeted outcome of this research. It is likely that external food sources will impact on slug foraging behaviour (Cordoba et al. 2018) however this research aims to provide behavioural information surrounding the pest- pellet interaction.

5.2 Summary of Chapters

The acceptability of pellets containing metaldehyde, ferric phosphate or non-molluscicide to *D. reticulatum* in chapter 3 appeared high with the majority of slugs encountering and feeding on a pellet at least once throughout the 14 hour trial however the palatability of pellets was differentiated with the average time *D. reticulatum* spent feeding on each pellet type. *D. reticulatum* spent significantly longer feeding on both 1% metaldehyde and ferric phosphate pellets when compared to 3, 5% and Axcela® pellets. It could be that 1% metaldehyde pellets are more palatable compared to higher concentration pellets because the slug does not detect the molluscicide during consumption, whereas higher concentrations will deliver a high dose of metaldehyde per bite which may cause irritation of the mucus glands after fewer bites compared to 1% pellets. It could also be that after consumption of a higher concentration metaldehyde pellet, slugs are incapacitated on the soil surface and unable to locate and feed on subsequent pellets. Observations of pellet reacceptance showed that slugs exposed to 3, 5% and Axcela® pellets were around 25% less likely to reaccept a pellet compared to slugs exposed to 1% and ferric phosphate. Feeding time was significantly higher for Ferric phosphate pellets compared to 3%, 5% metaldehyde and Axcela® pellets, and this is likely that due to the contrasting modes of action between ferric phosphate and metaldehyde. Mortality by ferric phosphate tends to occur 5-7 days after pellet consumption (Horgan et al 2006) and therefore it is unlikely that slugs are able to detect the molluscicidal effect of the pellet during consumption.

Recovery from metaldehyde pellets is predicted to be one of the main reasons inhibiting effective slug control (Crawford- Sidebotham 1970, Glen and Orsman 1986). In chapter 4, the recovery rates of *D. reticulatum* after exposure to varying concentrations of metaldehyde pellets indicated that recovery was higher for slugs exposed to 1% and 5% metaldehyde compared to the 3% metaldehyde pellets. It could be that at both concentrations the lethal dose of metaldehyde is not being delivered to the slug by pellet consumption. At 1%, too little metaldehyde may be delivered to the slug so that it can dermally/ orally rehydrate after moderate paralysis from the pellet. At 5% metaldehyde, it could be that the slug detects the molluscicide more quickly due to the higher dose delivery per bite and stops feeding before a lethal dose can be delivered. After the recovery time, a higher percentage of slugs for 1%, 3%

and Axcela® pellets were either moderately or severely paralysed compared to 5% metaldehyde pellets, where a greater proportion of slugs were either dead or had recovered. Paralysis on the soil surface is likely to play a large role in slug control by metaldehyde pellets (Howitt and Cole 1962), facilitating predation from natural predators or increasing the risk of dehydration from sunlight.

Trials of an inorganic controlled release technology (chapter 5) showed that development of microencapsulated metaldehyde is still very much in its infancy, however there is much opportunity for further research and development in this area. More targeted and precise delivery of slug pellets will be essential in improving the efficiency of slug control while reducing risk to the wider environment (Tsuji 1999). Coating a commercial slug pellet was predicted to hide the pellet temporarily from the slug so that it could be arrested into feeding on the iCRT coating and eventually the pellet, however it is likely that chemical reactions between the pellet and coating made the pellet unpalatable to the slug. Pellet acceptance and palatability was decided to be the most crucial aspect of testing a novel molluscicide, and for this reason iCRT pellets were presented to individual slugs in Petri dishes. By placing *D. reticulatum* in close proximity to the pellet the chance of pellet encounter was greater when compared to in an arena, and also allowed clearer measurements of pellet consumption to be made. Though pellet consumption levels were subjective, the consumption scale developed allowed for accurate measurements of consumption to be taken.

5.3 Metaldehyde Ban

On the 19th of September 2018, it was announced by the Department for Environment, food and Rural Affairs (DEFRA) that the outdoor use of metaldehyde would be banned as of Spring 2020, with usage only permitted to permanent greenhouses. The ban was announced upon the advice of UK Expert Committee on Pesticides (ECP) and the Health and Safety Executive (HSE), reasoning that metaldehyde presents “an unacceptable risk to birds and mammals” (DEFRA 2018). Growers and manufacturers were given an 18-month time frame in order to sell metaldehyde until June 2019 and use up supplies until the following June 2020. The announcement by DEFRA stated that the ban may reduce the possibility of polluting drinking water but emphasised that this was not a factor in the decision making from the advice of the ECP and HSE.

Shortly after the ban was announced, Chiltern Farm Chemicals, who are one of the U. K’s largest suppliers of slug pellets, started judicial proceedings in order to challenge the government’s decision. The ban was declared unlawful and overturned due to flawed decision making (BC Legal 2019). The overturning of the ban was of much relief amongst farmers and growers, from whom had described the ban as a “massive blow” for the industry (Case 2019) due to the predicted higher costs of application of ferric phosphate. The ban has subsequently been reinstated as of 18th September 2020, and that the outdoor use of metaldehyde will be banned as of the end of March 2022 (DEFRA 2020).

The ban is likely to lead to the increase in the application of ferric phosphate on arable land as the main commercial molluscicide available for large scale agricultural use. Ferric phosphate is, on average, more expensive to apply at £17-24/ ha compared to metaldehyde at £10-15/ha, which is likely to have a large impact on farming translating to increased produce costs. There is little research into the effects of increased application of ferric phosphate on land, and while it is sold and marketed as an organic pesticide, it is likely that the included chelating agents (usually EDTA) are responsible for the binding on iron phosphate inside the slug gut which interferes with calcium metabolism causing slug mortality (Rae et al. 2009, Speiser and Kistler 2002). Increased application of ferric phosphate may lead to an increase in non-target poisonings, and there is evidence that the molluscicide causes increased mortality in earthworm populations (Langan and Shaw 2006).

The ban of metaldehyde in the UK will lead to challenges for growers seeking alternative slug control that is economically viable and may also lead to an increase in evidence of non-target poisonings, which the government stated the reason being for the ban of metaldehyde. The removal of metaldehyde from agricultural land should lead to a decrease in metaldehyde pollution in water systems, reducing pressure from water companies.

5.4 Final Conclusions

Metaldehyde is still used by growers in 23 EU member states and research into improving the stability of pellets is essential in order to reduce water pollution risks after application. New technologies such as inorganic controlled release substances allow for the potential for molluscicides such as metaldehyde to be included into a pellet in a way that makes the pellet more stable for holding metaldehyde, but also more palatable to slugs and therefore more effective as a control means. This research has demonstrated that *D. reticulatum* is extremely sensitive to metaldehyde, however, will accept and feed on pellets of low concentrations but not necessarily receive a lethal dose after feeding. Delivery of a lethal dose of metaldehyde is challenging, and manufactures should consider that lower concentration pellets that will arrest the slug in feeding potentially long enough for a substantial dose of metaldehyde to severely paralyse the slug on the soil surface, while slugs are more likely to reject metaldehyde pellets of higher concentrations. Encouragement of natural predators by farmers (increasing field margins, hedges and set aside) will aid in the control of slugs alongside molluscicide use, as should be included in any integrated pest management scheme (Macfadyen et al. 2014).

Advances in integrated pest management (IPM) could see the reliance on synthetic pesticides for agricultural crop pest control reduced in the years to come. Improvements to biological control, pest forecasting, mechanical and farming practices (Buhler et al. 2000, Huffaker 1985, Yenumula and Prabhakar 2012) as well as improvements to in field data collection, and the incorporation of economic analysis to management plans could inform growers on better farming practices to achieve maximum crop yield with less dependence on synthetic pesticides (Lima et al. 2011).

The likely increase in ferric phosphate use in UK agriculture should be monitored for its effects on water and soil pollution and quality, as well as impacts on non-target organisms. More research confirming the efficacy of ferric phosphate by a combination of laboratory and field studies could predict a more accurate slug mortality rate, which could be used to educate growers about the potential benefits of ferric phosphate as a molluscicide. The removal of metaldehyde from the UK market presents an opportunity for further innovation in the slug pellet industry and more sustainable agriculture, with a focus on molluscicides that are not only effective at controlling slug populations, but present minimal risk to the environment.

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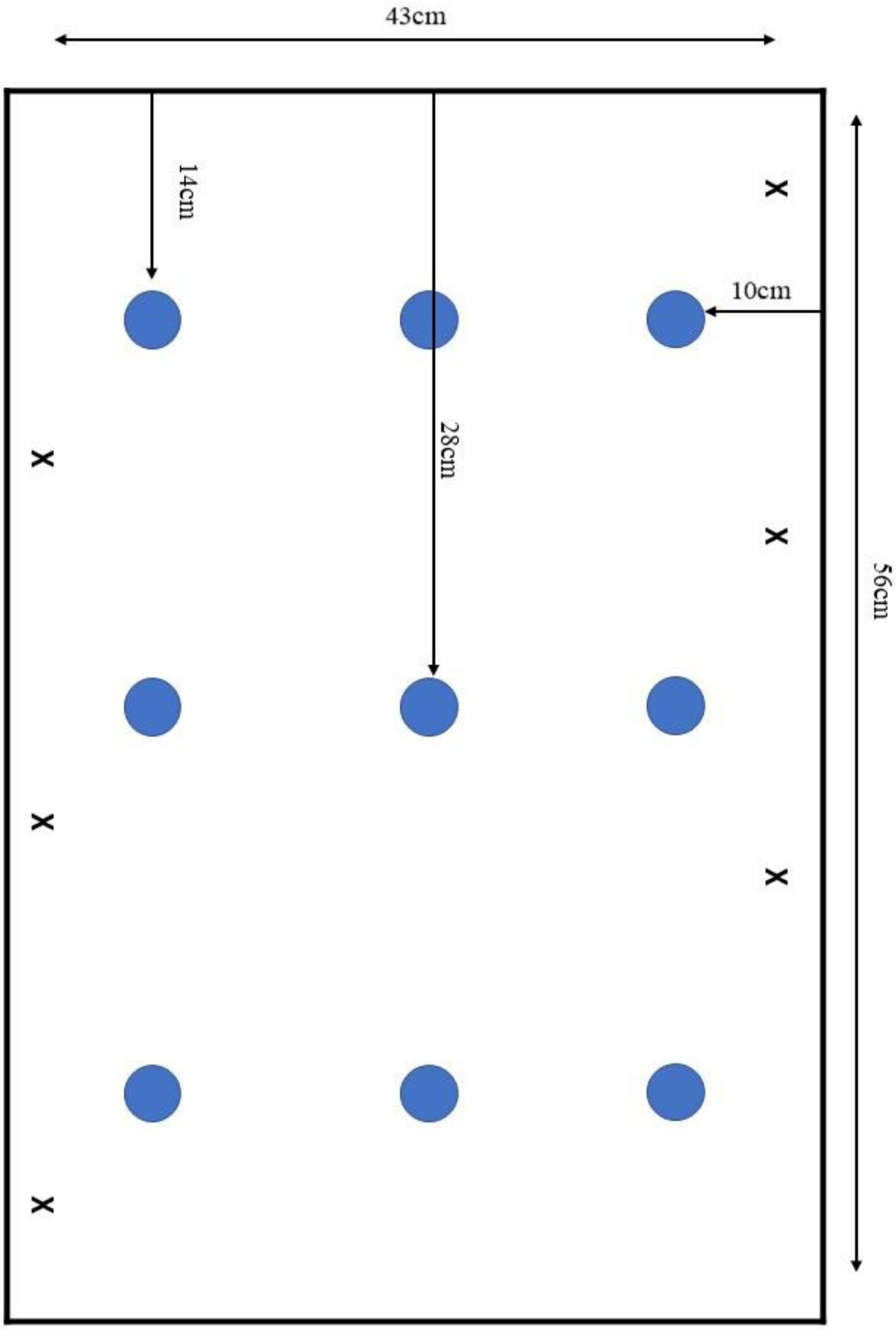
Appendix 1: Pellet Baiting Points



Appendix 1 Pellet baiting points and slug location in arena. Slugs are represented by "X" and slug pellets by blue circles. Slugs were placed at equidistant locations around the outside of pellets at the beginning of each trial.

Appendix 2: Slug Pellet Formulations

	Flour	Oil	99.9% Metaldehyde	Commercial formulation	iCRT Metaldehyde	iCRT control
1% metaldehyde	✓	✓	✓			
3% metaldehyde	✓	✓	✓			
5% metaldehyde	✓	✓	✓			
3% Axcela® metaldehyde				✓		
Non toxic pellet				✓		
iCRT metaldehyde	✓	✓			✓	
iCRT Axcela® coated Pellet				✓		✓
iCRT control pellet						✓
Sluxx ferric phosphate 3%				✓		

Appendix 2 Contents of pellets used in chapters 3, 4 and 5. Olive oil was added to flour containing pellets in 1 ml drops in order to form a malleable dough. Inorganic control release technology (iCRT) was provided by Lucideon LTD at metaldehyde concentrations of 4.8 and 2.4%.