September 2017

Student bursary report

10 week report

Investigating the response by the grey field slug (*Deroceras reticulatum*) to environmental factors such as moisture, temperature and pH.

Parent project

Utilising the patchy distribution of slugs to optimise targeting of control; improved sustainability through precision application.

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Under the supervision of Emily Forbes, Harper Adams University

This is the report of a 10 week student bursary project starting in June 2017.

CONTENTS

1. Supervisor's report

This project forms part of a larger AHDB funded project (Project no. 2140009118), which is investigating the patchy distribution of the grey field slug (*Deroceras reticulatum*). There is increasing pressure on grower's to reduce the amount of pesticide used for slug control, one of the main active ingredients, methiocarb, was removed from the market in September 2015 and metaldehyde which now accounts for the majority of the market has been found at levels higher than those permitted under the Drinking Water Regulations (Council Directive 98/83/EC) meaning alternative application methods, which maintain efficacy whilst reducing the amount of pesticide required, are urgently needed. Currently farmers are advised to use refuge traps to monitor slug activity and to inform application of control, however, only a small proportion of the slug population is active on the surface at any one time (South, 1992) and the proportion which is active varies with changing environmental conditions. The patchy distribution of slugs in arable fields is widely acknowledged in the literature (Bohan *et.al*., 2000; Archard *et.al*., 2004; Mueller-Warrant *et.al.,* 2014) and by farmers. The overall project aims to identify a method of identifying the location of patches in arable fields, which if sufficiently stable in time and space could be targeted with pesticide. In the first year patches were located and stability investigated over a growing season. Damage was also measured and correlations between the location of damage and slug patches was examined. It was found that damage was not a reliable indicator of patch location (Forbes *et.al.,* 2017). In the second year soil samples were collected from the same points on the grids where slug numbers were monitored, samples were analysed for soil texture, pH, organic matter and bulk density. Infiltration rates were measured at the same points using an in-field test, soil and air temperatures were recorded on an hourly basis throughout the season and soil moisture was recorded on each slug sampling visit to the field. The work undertaken during the 10 week bursary project aims to support this work by observing slug's behavioural response to different gradients of selected soil characteristics in controlled conditions. Four characteristics were chosen; moisture, temperature, pH and organic matter; these characteristics were chosen as there is evidence in the literature that suggests they may have a role in influencing slug behaviour. The range for each gradient was chosen to cover the ranges which can be observed in arable fields. The experiments were carried out by joining four foil trays each with a different level of the characteristic to be observed; moisture – 50%, 75%, 100% and 125% field capacity, organic matter – 0%, 3%, 6% and 9% and pH – 6, 6.5, 7 and 7.5. Each gradient experiment was carried out in controlled conditions over five days, with slug location recorded three times per day. To maintain the moisture levels throughout the experiment each tray was weighed daily and water added as necessary. Slugs were fed carrot *ad libitum* which was positioned uniformly throughout the trays and replaced daily. Results were analysed using a mixed effects GLM in the statistical package R. The results will be used to improve understanding of behavioural responses observed in the field.

Scarlett was very competent and hardworking throughout the project. She was able to not only carry out the experiments to a high standard with minimal supervision but she also thought carefully about what she was doing which enabled her to see challenges and revise methods where necessary before they became a problem. She has gained a wide range of skills from working in the field to carrying out pH and organic matter analysis in the laboratory, organising experiments and managing equipment requirements.

Archard, G., Bohan, D., Hughes, L. and Wiltshire, C. 2004. Spatial sampling to detect slug abundance in an arable field. Annals of Applied Biology, **145**: 165-173.

Bohan, D., Glen, D., Wiltshire, C. and Hughes, L. 2000. Parametric intensity and spatial arrangement of the terrestrial mollusc herbivores Deroceras reticulatum and Arion intermedius. Journal of Animal Ecology, **69**: 1031-1046.

Forbes, E., Back, M., Brooks, A., Petrovskaya, N., Petrovskii, S., Pope, T. and Walters, K. F.A. 2017. Sustainable management of slugs in commercial fields: assessing the potential for targeting control measures. Aspects of Applied Biology, **134**: 89-96.

Mueller-Warrant, G., Anderson, N., Sullivan, C., Whittaker, G. and Trippe, K. 2014. Can knowledge of spatial variability in slug populations help improve stand establishment? Seed Production Research, Oregon State University, **151**: 4-13.

2. Personal statement

Taking part in this project was an invaluable experience. I have enjoyed these 10 weeks working in partnership with Emily Forbes immensely. During this project I was required to use my initiative to overcome practical problems encountered in the field and during the experiments, sourcing materials and creating the experimental arenas used in the experiments. I have increased my confidence and capability in making difficult decisions and solving problems independently. This project taught me the importance of self-motivation, sticking to a schedule and how to manage my time efficiently in order to overcome the pressure of managing multiple experiments simultaneously. I have become familiar and proficient with scientific procedures and equipment that I had not encountered previously and developed my data analysis skills by working with statistical packages such as R. The weeks following the experiments involved researching previous literature, drawing conclusions from our findings and communicating scientific information effectively both as a report and in presenting our progress to a supervisor.

I have enjoyed learning about an area of which I had no previous knowledge, as well as improving my understanding of subjects I was already familiar with. Having completed this project, I feel better equipped to begin my MSc this September and it has also solidified my desire to pursue a career in scientific research.

3. Abstract

Derocerus reticulatum is one of the most prolific agricultural slug pests in the United Kingdom and Western Europe, resulting in millions of pounds worth of crop losses every year. Their patchy distribution on the soil surface has resulted in difficulties achieving effective targeted control of this species. The behaviour of *D. reticulatum* is largely dependant on physical factors in their environment. This investigation aimed to determine the behavioural response of *D. reticulatum* to different environmental conditions; pH, soil moisture, temperature and organic matter of the soil, with the intention that the results can be used to predict the distribution patterns of these pests. Slugs were randomly allocated to one of the four treatments and their position along a gradient was monitored three times daily for five days. This study found that on average *D. reticulatum* preferred a soil moisture of 125% field capacity, a temperature of 5-14°C and 0% organic matter content, with no preference for pH observed. When coupled with a soil map the results of this investigation can be used to inform a predictive model of the likely distribution of *D. reticulatum* in the field. In this way it may be possible to achieve a more efficient and successful method of controlling this pest species.

4. Introduction

Deroceras reticulatum, or the grey field slug, is a gastropod species in the family Agriolimacidae. It is considered one of the most widely occurring slugs in the north and central European lowlands, Great Britain, and Ireland. Slugs cause approximately £8 million of crop losses per anumn (Port *et al*., 2003) and this species in particular is a significant agricultural pest of cereals, root crops and garden vegetables [1,2]. Previous literature has demonstrated the patchy distribution of slugs [1,3], however, there have been difficulties associated with studying *D. reticulatum* in the field. At any one time only a small proportion of the population will be active on the soil surface [1] and the proportion which are surface-active varies with changing environmental conditions [4]. As a consequence it has proven difficult to achieve targeted application of baits and control measures of these pests. Bohan *et al*. (2000) suggests that, lacking a predictive model, the best that farmers can do is treat the entire field. This technique is becoming less acceptable with recent research and media attention depicting the negative effects of chemical pesticides on biodiversity, agricultural sustainability and human health [5,6]. While slug activity and behaviour is, in part, regulated by endogenous circadian rhythms, literature suggests that it is mostly a direct response to physical environmental conditions, such as soil pH, moisture level, organic matter content, and temperature (Dainton, 1954; Dainton & Wright, 1985; South, 1992).

This investigation aims to determine the behavioural response of *D. reticulatum* to different environmental conditions; pH, soil moisture, temperature and organic matter of the soil. With the intention that the results can be used to predict the distribution patterns of these pests, which will later be tested in the field, and inform control methods.

5. Materials and methods

Soil

Soil was collected from a Harper Adams field site (coordinate SJ701189) using a spade and trowel before being air dried in a drying room (35°C). The soil was then ground in a pestle and mortar and passed through a 2mm sieve.

Slugs

Deroceras reticulatum were collected from traps placed in a Harper Adams field site (SJ701189) and a field site near Wigan (SD532012). Collected slugs were placed individually in clear 250ml plastic tubs (11.5cm diameter, 4.2cm high) where they were allowed to acclimatise for at least a week in the growth chamber. Within each tub was a small (approximately 2 cm x 3 cm) folded section of moist paper towel and a slice of carrot (approximately 3 g). Slugs were randomly assigned to treatment groups (moisture, temperature etc). Slugs chosen to participate in experiments were randomly assigned to different sets of trays (1-10) and randomly allocated to a starting tray position (1-4).

Growth chamber

A Fitotron® growth chamber was set on a schedule of light for 10 hours at 15 °C, followed by 14 hours of darkness at 10 °C.

Trays

Smoothwall foil trays (Top dimensions 220 x 150 x 33mm; bottom dimensions 190 x 120 x 33mm) were used as the experimental arena. Trays were connected in sets of four and labelled 1 – 4. For each experimental condition there were twenty sets of four trays. Soil was placed in these trays up to level of 1cm. A mixture of table salt and Morris K42EP Lithium Multipurpose Grease (4 parts grease to 3 parts salt) was spread around the edges of the trays in order to prevent the slugs from escaping.

Carrot

Each day a slice of carrot was weighed and placed in each tray. The carrots were cut rondelle style approximately 1cm thick.

The experiments

All experiments were conducted in controlled conditions (10 hours light at 15 °C; 14 hours of dark at 10 °C). During each experiment, the position of the slugs along the gradient was recorded three times daily (at 0900, 1330 and 1700) over a five day period. Due to space restrictions in the growth chamber the moisture and pH gradient experiments were observed over two separate five day periods and the temperature gradient experiment over three separate five day periods. After each experiment, all trays and soil were discarded in order to prevent the influence of residual pheromones affecting the following slug's movement [9]. Any slugs that died during the experiment were recorded before being discarded.

Moisture gradient

Field Capacity was assessed using the 1600F1, 5 Bar Pressure Plate Extractor according to the pressure membrane technique (Richards and Weaver, 1944). The pressure was turned on and set at 0.33 bar. After 24 hours the samples were removed and weighed before being placed into a drying oven overnight and reweighed.

Field capacity (%) was calculated using the following equation:-

$$\text{Field Capacity \%} = \frac{(\text{wet soil weight} - \text{dry soil weight})}{\text{dry soil weight}} \times 100$$

The water was added according to the field capacity (%) gradient shown in figure 1.

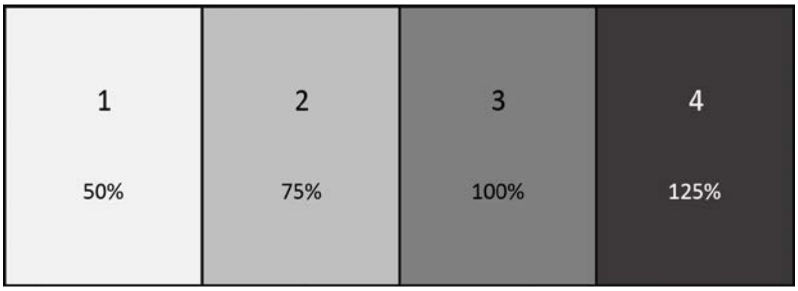

Figure 1. The field capacity (%) gradient shown across the four trays. Whereby Tray 1 is 50%, Tray 2 is 75%, Tray 3 is 100% and tray 4 is 125%.

pH

All trays in this experiment were at 100% field capacity (as calculated using the above method). The pH of the soil samples were tested by adding 25ml of water to 10ml of soil and shaken on the shaking machine for 15mins. The beakers were left to stand for 30 mins, with occasional stirring. The solution was stirred with a glass rod immediately prior to inserting the electrode. After 30 seconds the next stable reading was recorded. Once the pH of the soil was established Calcium Carbonate and Citric Acid were used to raise and lower the pH respectively. Once the weight necessary to change the pH of 100ml of soil was established it was possible to extrapolate this to greater volumes of soil. In this way we could determine the amount needed to raise the pH of each trays to the desired pH. The pH of each tray was retested at the end of the experimental period. The pHs of the trays were made up to be 7.5, 7, 6.5, 6 in trays 1, 2, 3 and 4 respectively.

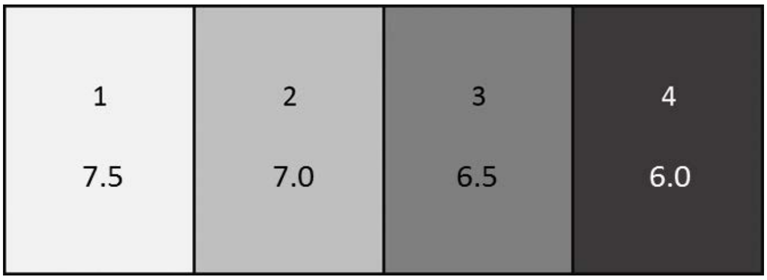

Figure 2. The pH gradient shown across the four trays. Whereby Tray 1 is 7.5, Tray 2 is 7.0, Tray 3 is 6.5 and tray 4 is 6.0.

Temperature

A ceramic heat emitter (MvPower AC 220V 150W Ceramic Emitter Heater Pet Reptile Heat Lamp Bulb Black, Shenzhenshi Musen Shiyefazhanyouxiangongsi, China and Reptile Vivarium Clamp Lamps White 150W, Aquapet, UK) is placed immediately in front of Tray 1. Five Data Loggers (DS1921G-F5 thermochron ibutton, Homechip, UK) were placed within each set of four trays (Figure 3). The average temperature at each position was 24.4°C, 13.6°C, 5.2°C, 4.7°C and 4.4°C

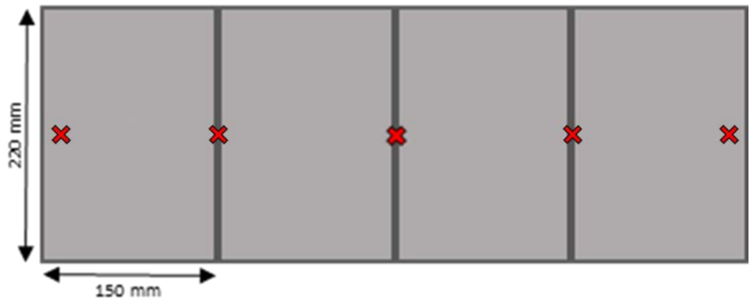

Figure 3. Showing the dimensions and arrangement of one of the sets of four trays along with the positions of the five data loggers, illustrated by the red crosses, used to measure the temperature gradient.

Organic Matter

The sieved air dried soil was put in the furnace for 4 hours at 550℃ to remove all organic matter. The organic matter content of compost was calculated by adding 10g of compost to a crucible and recording the exact weight to nearest 0.001 g. It was placed in a 105°C oven overnight and reweighed before being put into furnace for 4 hours at 550°C, allowed to cool and reweighed. Organic Matter (%) was calculated using the following equation….

$$\text{Organic matter \%} = \frac{(\text{dry weight} - \text{final weight}) \times 100}{\text{dry weight}}$$

Organic matter (%) was increased by adding different amounts of compost (Figure 4).
For 9% organic matter; for every 100 g of soil 10.11 g of compost was added
For 6% organic matter; for every 100g of soil 6.74 g of compost was added
For 3% organic matter; for every 100 g of soil 3.37 g of compost was added

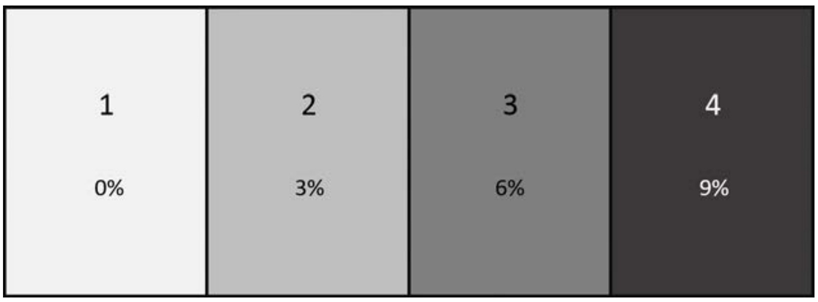

Figure 4. The organic matter content (%) gradient shown across the four trays. Whereby Tray 1 is 0%, Tray 2 is 3%, Tray 3 is 6% and tray 4 is 9%.

Only slugs surviving at the end of the experiment were included in the statistical analyses.

6. Results

Moisture

There was no significant difference between experimental weeks (Figure 5) (Z=-0.5, d.f.=119, p>0.05). 1 2

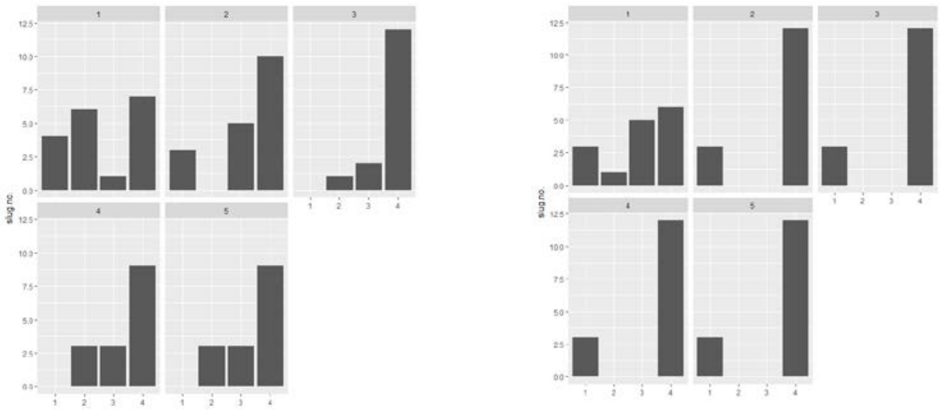

Figure 5. The effect of experimental week on slug position along the moisture gradient. 1 – the total count of slugs in each position during the first 5 day experimental period. 2 – the total count of slugs in each position during the second 5 day experimental period. Tray 1 50% field capacity, Tray 2 75%, Tray 3 100%, Tray 4 125%.

Over the full experimental period significantly more slugs were recorded in Tray 4 (125% field capacity) (Z=6.5, d.f.=114, p<0.001) (Figure 6). A power analysis was carried out which was 100% at 95% confidence interval. The movement of slugs from their starting position to tray 4 was observed quickly, the proportion of slugs in Tray 4 on day 2 was 0.7, 0.8 on day 3, 0.7 on day 4 and remained 0.7 on day 5 (Figure 6).

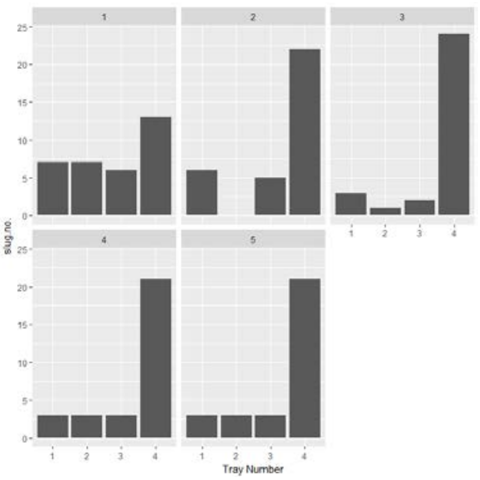

Figure 6. The combined total count of *Deroceras reticulatum* slugs recorded in Trays 1-4, over a period of five days, where graphs 1-5 correspond to days 1, 2, 3, 4 and 5 respectively. Tray 1 represents a moisture of 50% of field capacity, Tray 2 75%, Tray 3 100% and Tray 4 125%.

pH

There was a significant difference between experimental weeks (Z=3.2, d.f.=199, p<0.001), in both weeks tray 3 was significantly different to the other trays (Figure 7), in week 1 there were fewer slugs (Z=-3.3, d.f.=60, p<0.001) and in week 2 there were significantly more slugs (Z=2.6, d.f.=60, p = 0.05).

1 2

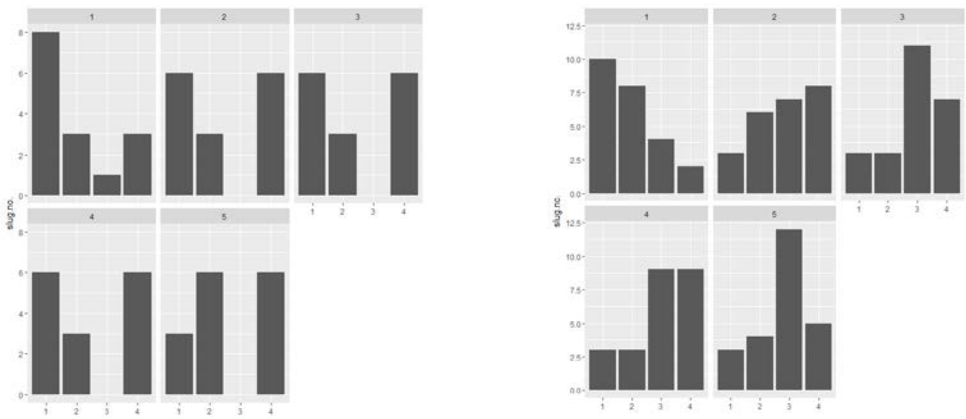

Figure 7. The effect of experimental week on slug position along the pH gradient. 1 – total count of slugs in each position during the first 5 day experimental period. 2 – total count of slugs in each position during the second 5 day experimental period. Tray 1 pH 7.5; Tray 2 pH 7; Tray 3 pH 6.5; Tray 4 pH 6.

When the results of the two experiments are combined there is no significant difference between the number of slugs in each tray (relative to tray 1; tray 2 Z=-0.9, d.f.=199, p>0.05; tray 3- Z=-0.7, d.f.=199, p.0.05; tray 4 Z=0.7, d.f.=199, p>0.05) (Figure 8).

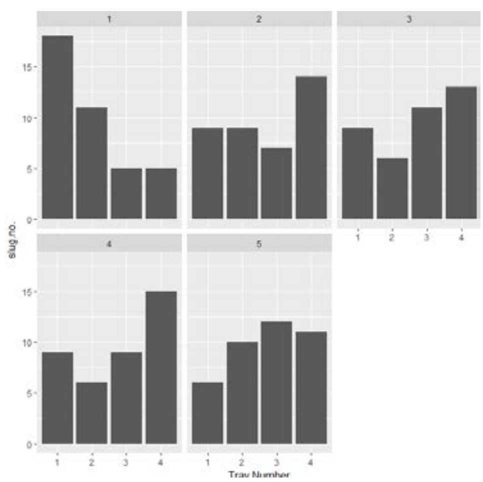

Figure 8. The combined total count of *Deroceras reticulatum* slugs recorded in Trays 1-4, over a period of five days, where graphs 1-5 correspond to days 1, 2, 3, 4 and 5 respectively. Tray 1 pH 7.5; Tray 2 pH 7; Tray 3 pH 6.5; Tray 4 pH 6.

Temperature

There was no significant difference between experimental weeks (z=-3.123, d.f.=594, $p>0.05$) (Figure 8).

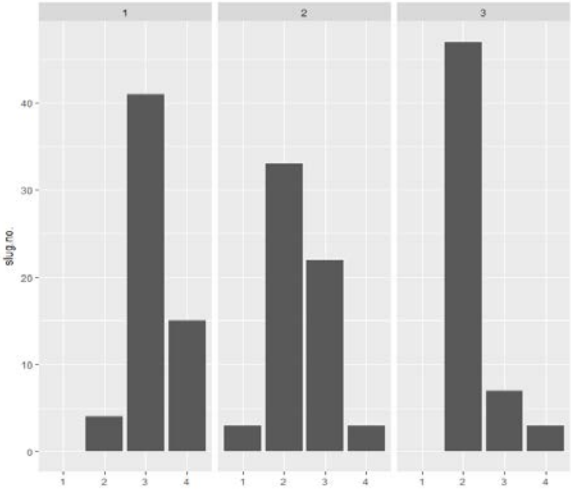

Figure 9. The effect of experimental week on slug position along the temperature gradient. 1 – total count of slugs in each position during the first 5 day experimental period. 2 – total count of slugs in each position during the second 5 day experimental period. 3 – total count of slugs in each position during the third experimental period. Average temperatures; Tray 1 24°C; Tray 2 14°C; Tray 3 5°C; Tray 4 4°C.

Organic Matter

During the single week of experiments *D. reticulatum* slugs were recorded most often in Tray 1 (Figure 10).

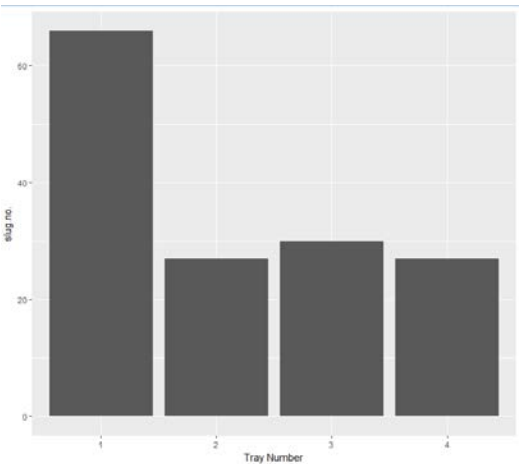

Figure 10. Total count of slugs in each position along a gradient of organic matter content during the 5 day experimental period. Tray 1 0% organic matter; Tray 2 3%; Tray 3 6%; Tray 4 9%.

The number of slugs in tray 1 steadily increased over the five day experimental period as *D. reticulatum* migrated from the other trays. The final day of the experiment shows a clear majority in tray 1 (Figure 11). Each tray showed a significant difference in the number of slugs recorded each day (tray 1 z=-2.5, d.f.=594, p<0.05; tray 2 z=-2.5, d.f.=594, p<0.05; tray 3 z=-2.5, d.f.=594, p<0.05) (Figure 11).

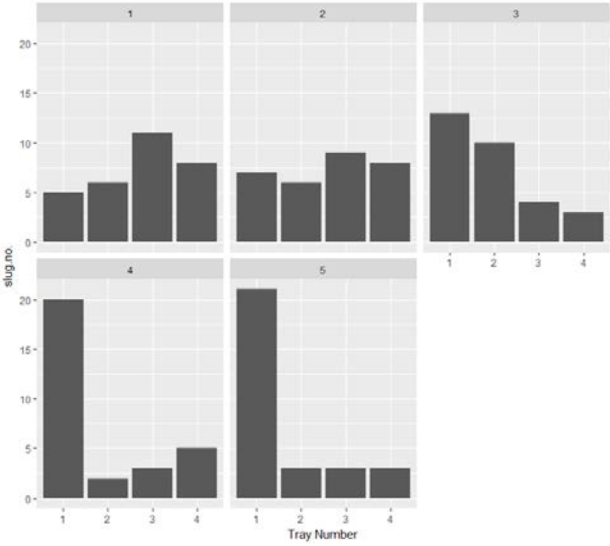

Figure 11. The combined total count of *Deroceras reticulatum* slugs recorded in Trays 1-4, over a period of five days, where graphs 1-5 correspond to days 1, 2, 3, 4 and 5 respectively. Tray 1 0% organic matter; Tray 2 - 3%; Tray 3 - 6%; Tray 4 - 9%.

7. Discussion

Moisture

The permeable outer membrane of a slug makes them vulnerable to conditions that could lead to excessive dehydration or rehydration [2]. Studies have shown that population densities are highly correlated with rainfall and that adult survival is more dependent on rainfall than other environmental factors such as temperature [2,4]. In this investigation slugs were recorded significantly more in Tray 4 – the wettest tray at 125% field capacity. The movement of slugs into Tray 4 occurred very quickly, with 70% being recorded in Tray 4 by day 2. This is a great deal higher than previous literature suggests. Carrick (1942) found that when slugs were exposed to a gradient of flooded soil to almost air-dry soil slugs congregated in 64% saturation. The high results of this study may be in part due to the air filtration system within the growth cabinet. It is possible that this system reduced the humidity within the chamber despite humidity settings, therefore increasing the slug's risk of dehydrating. As a consequence, the slugs may have retreated to the wettest tray. Crawford-Sidebotham (1972) found that activity decreased with increasing vapour pressure deficit (VPD) because VPD is directly proportional to the evaporation of water from the surface of the slug. Papers such as Ondina *et al*. (2004) support that the distribution of *D. reticulatum* is strongly influenced by humidity values. A further consequence of the air filtration system within the growth cabinet may have been a reduction in surface moisture of the soil. Barnes and Weil (1945) found that it was specifically lack of surface moisture that limited slug activity. This was corroborated by Young & Port (1991) who reported that reduced water availability on the surface of the soil limited both the distance travelled by

D.reticulatum and the time the slugs spent foraging. This may be another contributing factor as to why slugs retreated to the wettest tray.

pH

There is some evidence of inconsistency between reported preferences of *D. reticulatum* in regards to pH. Ondina *et al.* (2004) reported that the factors that best explain the distribution patterns of slugs were soil characteristics including moisture, substrate texture and pH. They found that *D. reticulatum* demonstrates a preference for high values of pH, typically occurring in less acid soils with finer texture. Bruijns *et al*. (1959) found that in the Netherlands the sites with a high abundance of *D. reticulatum* were eutrophic soils with a pH greater than 7. These findings have been supported by other papers (Wareborn, 1969; Bishop, 1977; Walden, 1981). Some literature has reported *D. reticulatum* to be more abundant in neutral soils [10,14], while others (Mordan, 1977; Boycott, 1934) found that this species was indifferent to lime, occurring freely in calcareous and non calcareous places. Tattersfield (1990) agreed with this finding, reporting that *D reticulatum* showed no preference between limestone woodland (mean pH range of 7.0 - 8.0) or millstone grit and shale woodland (4.3 - 6.5). This investigation found that in week 1 of experiments there were significantly less slugs in Tray 3 compared to the other trays, while week 2 found significantly more in Tray 3. When the results of the two weeks were collated most slugs were in Tray 3 (pH 6.5) (and, to a lesser extent, Tray 4 pH 6) on day 5 (Figure 8), but the combined results from both weeks of experiments show no significant difference between the amount of slugs recorded in each tray. Consequently, it cannot be determined whether the trend shown in the graphs is due to anything more than chance. Additionally, at the end of each week the pH of the trays were retested. These results differed slightly from the intended pHs. On average Tray 1 was 7.3, Tray 2 was 6.7, Tray 3 was 6.5 and Tray 4 was 6.4. Therefore, the pH value of the trays that slugs spent most time in was 6.5 - 6.4. With this in mind, this data is in line with papers such as Carrick (1942) and Douglas & Tooker (2012) who found that slugs preferred neutral pH soils. These slugs were collected from many different fields. It would be interesting to test the pH levels of the soil in their home field to investigate whether that has an effect on the pH the slugs choose to settle in when presented with a gradient.

Temperature

D. reticulatum, like most invertebrates, are ectothermic and cannot regulate their own body temperature. As a consequence, Dainton (1954) reported that temperature was the most important factor controlling behaviour, development and survival of *D. reticulatum*. Choi *et al*. (2004) reported that juvenile mortality was found as being solely dependent upon temperature. In this current research week 1 of experiments showed most slugs were recorded in Tray 3 (average 5°C). Weeks 2 and 3 reported most slugs were found in Tray 2 (average 4°C). However, statistical tests showed no significant difference between experimental weeks. The preferred temperature of *D. reticulatum* described in this investigation is much lower than is seen in previous literature. Dainton (1954) found this species reaches greatest sizes at 15-18°C. Existing literature supports that the optimum temperature for the growth and development of slugs is within 1-2 degrees of 18°C (Dmitrieva, 1969; South, 1982; Stern, 1975; Douglas & Tooker). Many papers have also looked at the effect of temperature on the rate of movement in *D. reticulatum* and most report that activity is also greatest at approximately 18°C (Wareing & Bailey, 1985; Hogan, 1985; Carrick, 1942). The unexpected results of this study may be due to the drying effect of the heat lamps used to create the temperature gradient. Slugs that stayed in tray 1 (average 24°C) were very quick to dry out and in many cases this resulted in mortality. At high temperatures (25–35° C), slug activity is substantially inhibited by water loss [21]. In the same way the soil in the closer trays lost substantially more moisture overnight than those further away. As previously mentioned, soil moisture is a limiting factor of slug survival and distribution (Barnes and Weil, 1945; Young and Port, 1991; Carrick, 1942). This may explain why slugs were found most commonly in much lower temperatures, because they were forced into trays further away from the heat lamps. Further study could investigate different methods of creating a temperarure gradient that prevents the soil and the slugs from losing moisture.

Organic Matter

Studies show that increased organic matter content is correlated with increased slug numbers (Miles *et al*., 1931; Carrick, 1942; South, 1992). Although decomposing plant material provides a source of food it is more likely that the increased water holding capacity of soils with high organic matter content is the reason for this association (South, 1992; Douglas & Tooker, 2012). In this investigation slugs were recorded most often in Tray 1 (0% organic matter). This result is surprising and is contrary to previous literature cited previously. Douglas & Tooker (2012) reported that slugs are particularly successful in soils with 3% organic matter or greater. The results of the present study are likely related to the texture of the soil once the organic matter was added. The compost added to the soil to increase organic matter was very roughly textured, with large pieces of roughage and tufts of cottony plant material. One can hypothesise that this texture discouraged the slugs from the trays containing organic matter as it restricted movement and compromised the water holding capacity of the soil surface. Research has shown that this species prefers moist, fine grained soils (Ondina *et al*., 2004).

To conclude, this investigation provides further information on the conditions favoured by the slug species *Derocerus reticulatum*. This information can be used to inform current knowledge about their distribution and provide a guide as to the locations best suited for effective targeted control of this pest species.

8. References

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