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

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1. Abstract

The grey field slug (*Deroceras reticulatum*) is an agricultural pest, causing economic damage to a range of crops in the UK. Legislation has led to a reduction of registered active ingredients and increased pressure to reduce pesticide usage. Discontinuous distributions of slugs in arable fields offers the potential to target control applications, reducing pesticide use, while maintaining efficiency. This report investigates the stability of patches and methods for locating them.

Significant aggregations of slugs were found at all field sites, with stable areas of higher slug densities occurring in the same area of the field at all five fields sites during the 2015–16 season. In the subsequent two seasons, slug numbers were lower. However, similar patterns of stability were observed in the fields with the largest populations. Stability of patches between seasons requires further work.

Alternative methods of locating areas of higher slug densities were investigated. Using crop damage from grazing was not found to be suitable. The highest correlation between slug numbers and damage was $r = 0.52$. No positive correlation was found in the field with the largest population. Using soil characteristics was also investigated, with organic matter, pH, bulk density and soil texture found to be significantly different at some field sites within and outside of slug patches, providing potential candidates for further investigation.

A method of identifying individual slugs was developed to improve understanding of patch formation. Radio frequency identification tags were used to track slugs in the field over two five-week periods. Slugs were found to remain close to their release point. The maximum distance moved from the point of release was 78.7 cm in April 2017 and 101.9 cm in November 2017.

The combination of results from this work suggests there is strong potential for targeting molluscicides to areas of higher slug densities.

2. Introduction

2.1. *Deroceras reticulatum*

The grey field slug, *D. reticulatum* (previously named *Agriolimax reticulatus* (Müller)) is the most economically important slug species, accounting for the majority of damage to crops in the UK (Ramsden *et al.*, 2017). *Deroceras reticulatum* has two main peaks of reproductive activity, one in spring and one in autumn, however, it is an opportunistic species which reproduces whenever favourable mild, wet conditions occur, resulting in overlapping generations/life stages throughout the year (Port and Port, 1986).

2.2. Discontinuous distribution of slugs

The aggregation of *D. reticulatum* in arable fields is widely reported (South, 1992; Bohan *et al.*, 2000a; Archard *et al.*, 2004) with areas of high slug densities dispersed among areas of lower density. There is limited and conflicting research into the longevity of high density patches. Bohan *et al.*, (2000a) did not detect patch stability from a series of six assessments carried out between March 1997 and March 1998 in a winter wheat crop, whereas Mueller-Warrant *et al.* (2014) found stable patches in five grass fields when analysing between 8 and 15 assessments taken between October 2014 and February 2015. The differences observed in these two studies could be due to sampling method, (defined area traps compared to refuge traps).

Non-uniform distribution of slug populations may offer the potential for reducing molluscicide use in agricultural fields. If such patches are found to be sufficiently spatially and temporally stable, and a commercially viable method of identifying their location and dimensions can be established without the need for refuge trap counts then control measures may be targeted at high slug density patches alone, leaving areas with lower slug numbers untreated.

2.3. Understanding the mechanism underlying slug patch formation

Few studies have investigated the behavioural responses that influence the formation of areas of higher slug densities (patches) or their spatial or temporal stability. Difficulties associated with studying and effectively tracking *D. reticulatum* in the field have hampered investigations. A large but variable proportion of the slug population in arable fields is located beneath the soil surface, with a smaller proportion active on the soil surface (South, 1992), resulting in the number of surface-active slugs varying widely under different environmental conditions. In cold or dry weather a smaller proportion of the population will be observed on the soil surface as slugs move down the soil profile where conditions remain more constant (Choi *et al.*, 2006). Various techniques have been developed to assess populations, including surface searching, refuge traps, hand sorting of soil, soil flooding, defined area traps (DATs) and capture-recapture approaches (South, 1992), and have been used to confirm the non-uniform distribution of *D. reticulatum*. The lack of data on slug

population size and individual movement beneath the soil surface, contributes to there being no conclusive findings on the mechanisms underpinning the formation, stability or location of higher density patches (Forbes *et al.*, 2017).

Radio Frequency Identification (RFID) tags have been used to track movement in a range of vertebrate and invertebrate species, including fish (Roussel *et al.*, 2000), honey bees entering and exiting hives (Henry *et al.*, 2012) and vine weevils (Pope *et al.*, 2015), and the technology allows individuals to be uniquely identified. Grimm (1996) injected RFID tags into the foot of *Arion lusitanicus*, a much larger (<13 cm long) slug species than *D. reticulatum* (<5 cm), and demonstrated that tag insertion had no effect on survival and egg laying, although no work was done to establish the impact on either feeding or locomotor behaviour. The technique has since been employed by Ryser *et al.* (2011) to assess field survival rates of *A. lusitanicus* and *A. rufus* and by Knop *et al.* (2013) to investigate locomotor activity of *A. lusitanicus* and *A. rufus* in arable fields. In both cases, however, the method was used as in a mark-recapture technique rather than for tracking the movement of individuals. The use of RFID tags to study the behaviour of the much smaller slug species such as *D. reticulatum* has not been investigated to date. In order to be an effective method for tracking individual slugs, the method of marking must not affect key aspects of biology and behaviour, must allow for differentiation between individuals, and be sufficiently long-lasting.

2.4. Slug damage to wheat crops

Slugs cause damage to wheat crops primarily by seed hollowing, with the level of damage being dependent on seed depth at drilling, and the condition of the seed bed. Cloddy seed beds will allow slugs easy access to the seeds compared to a fine seed bed, and shallow drilled seed is more susceptible to slug damage than deeper drilled seed (Glen *et al.*, 1990). After germination seedlings are also vulnerable to slug damage by leaf shredding until GS21 (main shoot and one tiller, AHDB, 2016). Crops are able to compensate for low levels of damage but even at low levels of damage slugs can be economically damaging (Table 2.1). It is questionable whether at the early growth stages the relatively low, but still economically important, levels of damage would be sufficient to identify patches of crop which would need to be treated.

Table 2.1. Percentage plant and yield losses following different levels of simulated slug damage in winter wheat. Losses are expressed as a % reduction in treatment plots with simulated slug damage compared to untreated control plots (Jessop, 1969).

Simulated damage (%)	Plant loss (%)	Yield loss (%)
25	20	4
50	65	19
92	82	34

2.5. Pesticides

Current EU and UK policy aims include optimising pesticide use in crop production to protect the environment and human health whilst maintaining agricultural efficiency. The Pesticides Authorisation Directive (PAD) (Council Directive 91/414/EEC) was introduced in 1993 and since its introduction there was a 50 % decrease in registered pesticides available for use between 1993 and 2012 (Carlile, 2006; Hillocks, 2012). Several other EU directives and regulations also govern the registration and use of pesticides, including Water Framework Directive (Council Directive 2000/60/EC), Sustainable Use Directive (Council Directive 2009/128/EC), Groundwater Directive (Council Directive 2006/118/EC), Regulation (EC) No 1107/2009 (approval of products), Regulation (EC) No. 396/2005 (maximum permitted levels in food), Regulation (EC) No. 1272/2008 (classification, labelling and packaging of pesticides) and Regulation (EC) 1185/2009 (statistics on PPPs). Whilst the focus of legislation is on health and the environment, it is important that the decrease in available pesticides does not have damaging consequences for farming productivity (The Andersons Centre, 2014).

Historically slugs have been managed through common farming practices including ploughing and crop rotations. Crop husbandry has changed over time, with many modern day farming practices being less beneficial in the reduction of slugs. For example, the prohibition of stubble burning (The Crop Residues (Burning) Regulations 1993), a move towards minimum tillage and direct drilling (Kennedy *et al.*, 2013), shorter rotations and larger areas of OSR grown mean there is more demand for effective slug control methods. Stubble burning aided the control of slugs by directly killing individuals and reducing the amount of food available on the soil surface (Glen and Symondson, 2003). The reduction in cultivations reduces the number of slugs killed by the mechanical action of the machinery (Port and Port, 1986; Kennedy *et al.*, 2013) and fewer slug eggs are exposed to the surface where they become desiccated (Glen and Symondson, 2003). Crops following OSR in rotation are more susceptible to damage from slugs, partly due to the dense canopy of the OSR crop, which provides ideal environmental conditions for increases in population size resulting in larger numbers being present during the often susceptible establishment period (Port and Port, 1986, Glen *et al.*, 1993). The timing of planting and rotations also affect damage levels; winter grown crops are more susceptible to slug damage as growth is slower, lengthening the time for which vulnerable stages are exposed to the pest (South, 1992). Of the range of commercial products available for slug control in arable crops, molluscicide pellets are the industry's preferred approach. In 2016, 37 % of winter and spring sown wheat, 88 % of OSR and 100 % of potatoes were treated with a molluscicide in the UK (Garthwaite *et al.*, 2018).

For many years there have been at least two active ingredients (methiocarb and metaldehyde (discussed below)) concurrently available for the control of slugs. The primary method of application is in a bran based pellet containing the active ingredient, with the bran acting as an attractant to the slugs. Pellets can be broadcast across the field or mixed and drilled with the seed (South, 1992).

Methiocarb was one of most widely used active ingredients, especially in high value crops until it was removed from the market in September 2015 (HSE, 2014) due its detrimental effect on farmland birds (Clarke, 2014). Metaldehyde has been the most widely used active ingredient for slug control, representing 75 % of the UK slug pellet market in 2016 (most recent figures; Garthwaite *et al.*, 2018), the implications of its likely removal from the market will be significant for slug management. The removal of metaldehyde from the market was announced in December 2018 (Defra, 2018), in July 2019 an appeal meant the ruling was overturned on a technicality. Although the ban was lifted, it will likely be reinstated in the near future (Appleby, 2019; Pickstone, 2019), therefore for the purposes of this report it will be assumed that metaldehyde will not be available long term for slug control. Ferric phosphate is a relatively new product, approved for use in the UK since 2005 (AHDB, 2010). Ferric phosphate acts as a stomach poison, iron deposits in the digestive gland cause slugs to stop feeding and ultimately death (Triebkorn *et al.*, 1999).

2.6. Current guidelines for pesticide application for slug control

Current guidelines for the application of slug control products recommend using refuge traps (upturned plant pot saucers) baited with chicken layers mash when the soil surface is moist and temperatures are between 5 and 25°C. In crops of up to 20 ha nine traps set in a 'W' shaped transect across the field are recommended (13 in larger fields). Traps should be left overnight and the number of slugs counted the following morning (AHDB, 2016). Established thresholds for OSR and wheat recommended that control measures are applied when a mean of 4 slugs per trap are recorded in a standing cereal crop (AHDB, 2016). The number of slugs active on the surface and so found in surface refuge traps varies widely according to weather conditions (Choi *et.al.*, 2006; Hommay *et.al.*, 1998; Willis *et.al.*, 2008). Using a 'W' shaped transect may reduce inaccuracies associated with the discontinuous distribution of *D. reticulatum* resulting in more accurate assessment of slug populations (Petrovskaya *et al.*, 2012) but it does not distinguish sufficiently accurately between discrete areas of high and low slug densities to allow targeting of controls.

2.7. Slug traps

For the purposes of this study, the trapping method adopted must enable the accurate identification of the location and dimensions of discrete patches with higher slug densities whilst allowing for temporal variation in slug activity and distribution above and below the soil surface, sample and map a sufficiently large area of the field to facilitate comparisons between the various characteristics assessed in relation to these patches, and allow any slug population movement across the field to be determined. A key component of this study involved the investigation of the temporal stability of slug populations and their distribution within arable fields. To reduce the risk of the trapping technique affecting the size of populations following a series of assessments taken in a restricted area of a field crop, a non-destructive sampling method was sought. The criteria for

assessing the advantages and disadvantages of methods used for slug trapping are typically based on their suitability for farmers i.e. ease of use, cost effectiveness etc. For the purposes of research, methods are required that take account of key aspects of the biology and behaviour of slugs. Refuge trapping, used in protocols which incorporate assessment of surface activity and population size over time without removing slugs from the study area, offers a technique that addresses some of the major constraints of the work reported in this report. The selected standard trap was therefore, unbaited refuge traps consisting of upturned terracotta plant pot saucer 18 cm diameter (LBS Horticulture Supplies, Lancashire, UK).

Determining an appropriate trapping frequency across fields is important if accurate assessment of the characteristics of the discontinuous distribution of slugs to be achieved (Clark and Evans, 1954). If a grid which is too fine or too coarse is used then populations can appear uniformly or randomly distributed and the ability to detect patches even if they exist is lost (Bohan *et al.*, 2000a). A method is needed which allows sufficiently large areas of fields to be monitored to facilitate identification of crop areas which lie between and within patches of higher slug density. Additional consideration of the time required to sample each grid, on each assessment day, was essential to allow the slug distribution in a number of fields to be carried out at regular intervals. The models developed during the analysis of Petrovskaya *et al.* (2018) predicted that that a coarser sampling grid (internode intervals of greater than 10 metre intervals) can be used to obtain accurate trap count estimates in fields with larger slug populations. Petrovskaya *et al.* (2018) also found that a further reduction in the number of traps to a 3 x 3 grid (30 metre internode interval) and a 2 by 2 grid (40 metre internode interval) increased the error to 27 and 40% respectively. Although the errors incurred by using a coarser grid would be similar to those normally recorded in population monitoring (Meir and Fagan, 2000), a finer grid, which allowed the edges of patches to be more clearly defined was required for the purposes of this research. Therefore, to ensure that the most accurate information regarding slug patches and population estimates, can be collected using the available resources under this project, a 10 by 10 grid of traps was a more suitable option.

2.8. Alternative methods of locating slug patches

Methods of relating the location of slug patches to other factors in the field have been investigated by Bohan *et al.* (2000b) who related the distribution of slugs to carabid beetle activity as a basis for conservation biocontrol strategies. In this study destructive soil samples were taken to measure slug abundance, which meant that the grid for mapping slug populations was offset by 2.5 m from the grid for measuring beetle populations and the position of the grid had to move by 1.5 m on each sampling date to avoid the area where the soil had previously been removed. This technique allowed a comparison of slug and beetle populations on two sampling visits but the destructive nature of the sampling would not support long term studies. Mueller-Warrant *et al.* (2014) investigated slug numbers in relation to damage in Oregon, USA and found a weak correlation between slug counts and damage in clover fields, using a minimum of 30 slug blankets per field

spaced at one blanket per acre. Counts of slugs were carried out weekly over a 19-week period and the percentage loss of crop stand was assessed. Using non-destructive surface refuge traps the authors were able to repeatedly sample the slug distribution over time, however, this paper does not provide details of the frequency of crop assessments or the proximity to the slug counts. Literature sources (Bohan *et al.*, 2000a; Glen *et al.*, 2003) suggest that refuge traps would need to be positioned on a finer grid than used by Mueller-Warrant *et al.* (2014) to obtain accurate assessments of the slug spatial distribution and damage assessments would need to be carried out in close proximity to the refuge traps.

2.9. Slug distributions and soil characteristics

Carrick (1942) first suggested that edaphic factors, such as pH, soil moisture and organic matter, may influence the location of areas of higher slug numbers in arable fields. Few field studies investigating the relationship between slugs and soil characteristics have been conducted subsequently, with the majority of research being carried out under laboratory conditions and focusing on individual soil characteristics (moisture and temperature, Getz, 1959; pH, Wareborn, 1970; temperature, Wareing and Bailey, 1985; organic matter, Speiser, 1999; temperature, Cook, 2004). The emerging trend from the literature suggests that pH, soil moisture and factors affecting seed bed condition are the key to understanding the distribution of slugs in arable fields.

South (1965) considered the discontinuous distribution of *D. reticulatum* in relation to several environmental factors in a grassland field, including distance from the headland, organic matter content of the soil, moisture and the stone coverage (as a percentage of the soil surface), but none of these factors were found to be significantly correlated with the distribution of slugs. More recently, the distribution of 17 species of terrestrial gastropods was related to a combination of soil characteristics in 10 km by 10 km grid squares in Iberia, using three samples from 124 grid squares taken in each year of the three-year study (Ondina *et al.*, 2004). Three groupings of slugs were identified, the first showing a preference for acidic soil with a high proportion of coarse sand (>56.4 %), the second (including *D. reticulatum*) were associated with wetter, less acidic soil with high proportions of silt and clay and a third group which showed no preference. More specifically, *D. reticulatum* were found to occur in higher numbers in soils with high pH (5.6-8.5) and calcium levels (5.3-26.0 %), an intermediate level of moisture (36.8-41.6 %) and gravel fraction (8.3-14.0 %) and a low coarse sand fraction (14.7-24.2 %), low level aeration (22.3-27.1 %) and aluminium content (0.1-0.6 %) (Ondina *et al.*, 2004). Further work is required to investigate the influence of a range of selected factors, both individually and in combination on the distribution of *D. reticulatum* in arable fields in the UK.

3. Materials and methods

3.1. Slug patches and their stability

3.1.1. Field sites

During the first year of the study (2015-2016) experimental work was conducted at five field sites within close proximity of each other (maximum 17.4 km apart), and each with a similar crop rotation (fields 1-5; Table 3.1). In the second year of the study (2016-2017) fields were selected from a wider geographical area (incorporating eastern counties; Lincolnshire, Nottinghamshire and Leicestershire, as well as Shropshire and Lancashire) and different crop rotations (fields 2-13; Table 3.1). In the final year (2017-2018) a sub-set of these fields were studied, maintaining the geographical spread, with the addition of two new sites not previously sampled (fields 2, 6, 7, 14 and 15; Table 3.1) and facilitating comparisons in successive cropping years.

Table 3.1. Summary of field locations and crop rotations for field sites used during 2015–16, 2016–17 and 2017–18. In each case, the crop grown prior to the commencement of experimental work is also reported. Crops two years prior to the commencement of assessments and post-assessment completion are not shown (shaded grey).

Field no.	County	Field	Cropping season			
			14–15	15–16	16–17	17–18
1	Shropshire	Adeney (Corner)	oilseed rape	winter wheat		
2	Shropshire	Adeney (Middle)	oilseed rape	winter wheat	winter barley	oilseed rape
3	Shropshire	Lynn (Badjics)	oilseed rape	winter wheat	fallow	
4	Shropshire	Lynn (Stoney Lawn)	oilseed rape	winter wheat	fallow	
5	Shropshire	Uppington (1)	oilseed rape	winter wheat	fallow	
6	Leicestershire	Oadby		oilseed rape	winter wheat	cover crop
7	Lancashire	Wigan		oilseed rape	winter wheat	fallow
8	Lincolnshire	South Kyme (1)		oilseed rape	winter wheat	
9	Lincolnshire	South Kyme (2)		spring wheat	spring wheat	
10	Lincolnshire	Dog Dyke		winter wheat	winter wheat	
11	Leicestershire	Hoby		oilseed rape	winter wheat	
12	Nottinghamshire	Flawborough		oilseed rape	winter wheat	
13	Shropshire	Bridgnorth		winter wheat	oilseed rape	
14	Shropshire	Uppington (2)			oilseed rape	winter wheat
15	Lincolnshire	Belchford			spring beans	winter wheat

3.1.2. Trap counts

A 10-by-10 grid (10 m between nodes) was used at all field sites to assess the number of active slugs on the soil surface. Grids were positioned within fields to include an area where the grower had historically found high numbers of slugs and to extend beyond to an area with historically fewer slugs (based on the grower's knowledge of the field). The minimum distance of the grid from the field boundary was 20 m, situated away from any in field trees and the topology of the area was as uniform as possible. Refuge traps (upturned terracotta plant pot saucers (LBS Horticulture Supplies, Lancashire, UK), 18 cm diameter) placed at each node of the grid were not baited (in order to not attract slugs to a food source). In 2015 – 16 slug assessments were carried out at 14-day intervals, with additional assessments at approximately 5-day intervals in two of the fields;

Adeney (Middle) and Lynn (Stoney Lawn) between January and February 2016. In 2016–17 and 2017–18 slug assessments were carried out at approximately monthly intervals.

3.1.3. Slug classification

At each assessment, the slugs found in each refuge trap were identified (as *D. reticulatum*, *Arion* spp or *T. budapestensis*), counted and recorded under three size categories (large (>100 mg), small (<100 mg and >5 mg) or very small (<5 mg)). Slugs were counted *in situ* and were immediately released under the trap in which they were found.

3.1.4. Statistical analysis of slug patches and stability within and between growing seasons

All statistical analysis was carried out in R Version 3.3.1. (R Core Team, 2013).

Slug counts

ANOVA was used to determine differences between slug counts in different fields and years. Post-hoc Tukey's test was used to determine where differences occurred.

Maps of slug counts created using the `interp` and `filled.contour` functions in R. The number of slugs in between traps was calculated by polynomial interpolation.

Hotspot analysis

The presence of hotspots was determined using the `ScanLRTS` function in R.

Taylor's Power Law

Taylor's Power Law (Taylor, 1961) was used to calculate an index of aggregation during each growing season. The mean and variance for each assessment date were calculated and then the log of each was taken. The correlation between the log (mean) and log (variance) was calculated using Pearson's correlation coefficient (r) and was calculated for the assessments within each field season (2015-16, 2016-17 and 2017-18) as well as for the three field seasons combined. In each case the coefficient of the line of best fit was calculated, which equates to the index of aggregation.

Patch stability

The stability of patches was investigated using Pearson's Product Moment Correlation coefficient (r). Areas of higher slug densities were identified by locating the highest trap counts on each sampling occasion and where these occurred in the same area on more than 50 % of assessments the area was identified by a red box.

3.2. Slug patches and damage

The standard experimental (10 by 10 traps with 10 m intervals between nearest traps) was established in five commercial fields in Shropshire, UK each sown with winter wheat following a previous crop of OSR (Adeney (Middle), 52°46'2.535"N -2° 26' 38.85"E, cv. Reflection; Adeney (Corner), 52°45'56.9268"N -2°26'40.4736"E, cv. JB Diego; Lynn (Badjics), 52°43'44.8746"N -2° 20' 11.8392"E, cv. Reflection; Lynn (Stoney Lawn), 52°44'11.9112"N -2°21'2.2818"E, cv. Reflection, Uppington (1), 52°40'37.0848"N -2°34'49.296"E, cv. Horatio). All fields were cultivated using a subsoiler and disc harrow followed by rolling. At each site crop husbandry followed normal farm practice with between 1 and 3 applications of molluscicide.

3.2.1. Experimental design and slug assessments

Grids were established at a minimum of 20 m from the nearest field edge and with the nearest edge of the grid parallel to the field edge. The number of slugs under each refuge trap was counted at approximately 14-day intervals between week commencing 30 November 2015 (week 1) and 15 February 2016 (week 12), and thereafter monthly until week commencing 23 May 2016 (week 26). These counts were used to investigate the relationship between slug numbers and crop damage.

3.2.2. Damage assessments

The percentage leaf area damaged by slugs (slug damage identified following the definitions of AHDB (2014)) was recorded from 20 randomly selected leaves located within a circle (50 cm radius) centred on each refuge trap. The mean leaf damage was calculated for each trap at each sampling visit. Sampling for damage levels was extended beyond the period in which slug controls might usually be applied to test the relationship between damage and a wider range of slug populations, as reflected by the catches of the surface refuge traps.

3.2.3. Analysis of slug distribution and crop damage

Maps of slug numbers and damage distributions were produced using the `interp` function of R version 3.3.1. (R Core Team, 2013), a polynomial interpolation between the grid nodes. Hotspot analysis was used to identify areas of the field with significantly higher numbers of slugs than would be expected in a random distribution. The correlation between trap counts on different dates, and between trap counts and leaf damage assessments at each trapping point were quantified using Pearson's Product Moment correlation coefficient r . Statistical analysis comparing slug population size in different fields was conducted using analysis of variance, post hoc Tukey's HSD tests were carried out to identify where significant differences were occurring.

3.3. Relating location of slug patches to soil characteristics

3.3.1. Laboratory experiments

Eighty adult slugs (*D. reticulatum*) were collected from two field sites (Harper Adams University (52°46'01.26"N 002°34'50.14"W) and Wigan (53° 30' 22.66" N 002° 42' 25.54" W)) and returned to the laboratory for a 48 hour acclimatisation period in a Fitotron (Sanyo SGC097.CFX.F - Fitotron Temperature/Humidity Test Chamber, Weiss Technik UK Ltd, UK; 14 hours light at 15°C: 10 hours dark at 10°C, 60% humidity). As slugs are active at night, experimental assessments would need to be made during the scotophase. To facilitate this all individuals were acclimated to a light dark cycle in which scotophase commenced at 08:00.

Individual slugs were maintained in a rearing enclosure comprising a 250 ml circular plastic container (11.5 cm diameter; 4.2 cm high) with eight 1 mm diameter holes drilled through the lid. The base of each enclosure was covered with a 4 cm² disc of damp paper towel (2 ply blue centre feed roll, Cater4you, UK), moistened with 1 ml of distilled water, which was replaced daily. Slugs were fed ad-libitum on 1 cm thick slices of carrot which was replaced daily. Stepped soil gradients (investigating soil moisture, pH, organic matter or temperature; Table 3.2) were established. Each gradient consisted of 4 compartments (15 cm x 22 cm x 3.3 cm) made from foil trays (D-272-33, Cater For You, UK), connected by making 2.5 cm cuts in the corners of one of the long edges and folding them to create a join between adjacent compartments (Figure 3.1). A 2 cm band of Vaseline and salt mixture (ratio 4:3) was applied to the edge (but not the connecting platform) of each compartment in order to contain the slugs within the experimental arena.



Figure 3.1. Stepped soil gradients used to investigate slug preference for soil moisture, pH, organic matter (pictured) or temperature were established. Four compartments (15 cm x 22 cm x 3.3 cm) with a 2 cm band of Vaseline and salt mixture (ratio 4:3) was applied to the outer edges in order to contain the slugs within the experimental arena. Each compartment contained 330 cm³ of air dried soil and 1 cm slices of carrot were placed in the centre of each compartment to ensure slugs had access to food at all points along the gradient.

Each individual tray was weighed before 330 cm³ of air dried (for a minimum of 72 hours at 35°C) and sieved (through 2 mm mesh) soil (collected from 52°46'01.26'N 002°34'50.14'W) was added to each tray, providing a 1 cm deep layer, with its surface level with the joint between compartments. The compartments were then reweighed to calculate the exact weight of the soil contained. Distilled water was added to all compartments to elevate the moisture content to field capacity (16 %), apart from those used in the moisture gradient experiment (in which water was added at varying rates to create the gradient described in Table 3.2). Field capacity of the soil was calculated using the pressure membrane technique (Richards and Weaver, 1944). A total weight of each assembled compartment (including foil tray, soil, Vaseline/salt and water) was recorded for each compartment, to allow for any water lost through evaporation to be replaced at daily intervals.

Food was offered *ad-libitum* and consisted of a singular circular disc (approximate diameter 2 cm; depth 0.5 cm) of carrot, placed in the centre of each compartment. These discs were of sufficient size to ensure a constant food supply was always available in each compartment throughout the experiment. Experiments for each soil characteristic were run simultaneously and slugs were randomly assigned to an experimental gradient (soil moisture, temperature, pH or organic matter). In each replicate, a single pre-weighed slug was released into a compartment determined using a random number generator. Experiments were carried out in a Fitotron under the same conditions as those used during the acclimatisation period and commenced simultaneously 1 hour after the start of the scotophase. The position of each slug within each experimental arena was recorded 3 times per day at 0900, 1300 and 1700 for a five-day period.

Table 3.2. Levels of five soil characteristics tested in a stepped gradient to investigate substrate preferences of adult *Deroceras reticulatum*. Moisture = % field capacity; organic matter = % by weight.

Characteristic gradient	Compartment 1	Compartment 2	Compartment 3	Compartment 4
Moisture (low)	50 %	75 %	100 %	125 %
Moisture (high)	125 %	200 %	290 %	370 %
pH	5.86	6.26	6.51	6.97
Organic matter	0 %	3 %	6 %	9 %
Temperature	4°C	5°C	14°C	25°C

Organic matter

Air dried and sieved soil (1.5 kg) was heated in a furnace (AAF11/18, Carbolite Gero, UK) for 4 hours at 550°C to remove organic matter (Ministry of Agriculture, 1986), before being placed in the compartments of the stepped gradient. A gradient was created by adding known percentages of compost (97 % organic matter content, Godwin's multi-purpose compost, E. J. Godwin (Peat Industries) Ltd). To calculate the organic matter content of the compost it was air dried and 10 g

was added to a pre-weighed crucible. The weight of compost was recorded to nearest 0.1 µg (Precisa 262SMA-FR, Precisa Ltd, UK) before being placed in a 105°C oven (LCO/42H/DIG, Genlab, UK) for 24 hours and reweighed. The sample was then put into an ashing furnace (AAF11/18, Carbolite Gero, UK) for 4 hours at 450°C, then allowed to cool before being reweighed. The following equation was used to calculate the organic matter content.

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

(Ministry of Agriculture, 1986)

No compost was added to the soil in compartment 1 and increasing rates of compost were added to compartments 2, 3 and 4 to create a gradient (Table 3.2).

pH

The pH of the soil collected from the field was determined. Ten grams of air dried and sieved soil was placed into a 100 ml beaker and 50 ml water was added before the cap was secured on the beaker and placed on an orbital shaker (HS 501 digital, IKA, Germany) at 240 RPM for 15 minutes. The pH meter used (3510 pH meter, Jenway, UK) was recalibrated after every 50 samples, using pH 4.0 (Buffer Colour Coded Solution pH 4.00 (Phthalate) Red, Fisher Scientific, UK) and pH 7.0 buffer solutions (Buffer Colour Coded Solution pH7.00 (Phosphate) Yellow, Fisher Scientific, UK). The electrode was placed in test solutions until the pH reading became stable, and was then rinsed with distilled water between samples (Ministry of Agriculture, 1986). Using pre-weighed volumes of soil and water, either citric acid or calcium carbonate was added in known quantities and the pH of the soil retested until the target pH was achieved. This was confirmed by adding the known quantity of either citric acid or calcium carbonate to 10 soil samples, for each pH level and retesting the soil pH. Using this data the amount of citric acid or calcium carbonate required to alter the pH of the soil in each compartment of the pH arena to create the pH gradient detailed in Table 3.2 was calculated.

Moisture

The field capacity of the soil was calculated using the pressure membrane technique. Air dried and sieved soil was soaked overnight in distilled water, 10 rubber rings (5.4 cm diameter, 1 cm high) were placed on a porous plate and then filled with saturated soil. The samples were placed in the pressure plate apparatus (5 bar pressure plate extractor, Soil Moisture Equipment Corporation, USA). A pressure differential of 1.3 atmospheres was applied for 6 hours. The soil within each ring was then weighed and placed into a crucible, the samples were then oven dried at 105°C until a constant weight was achieved. The samples were then reweighed and the soil moisture content of

the samples after the pressure differential had been applied were calculated (Richards and Weaver, 1944). Soil moisture at field capacity was calculated as 16 %. The amount of distilled water to be added to each compartment to create the gradient (initially 50 – 125 %) was calculated as a percentage relative to field capacity (Table 3.2). A second moisture gradient from 125 to 370 % was established following the results of the initial moisture gradient experiment in order to include soil with standing surface water (370 %).

Temperature

In order to create a temperature gradient, the experimental arenas were constructed in a cold room (SCS Group, UK) set at 4°C and 14h light: 10h dark cycle. Heat lamps (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) were placed at one end of the gradient and data loggers (DS1921G-F5 thermochron ibutton, Homechip, UK) set at 5 cm intervals on the soil surface along the gradient to monitor the temperature throughout the experiment.

3.3.2. Field study

Soil samples (approximately 250 g) were collected from three fields (Oadby, Leicestershire; South Kyme (1), Lincolnshire and Wigan, Lancashire) at the end of the 2016-17 growing season and three fields (Adeney (Middle), Shropshire; Uppington (2), Shropshire; Wigan, Lancashire) at the end of the 2017-18 growing season from each point on a standard grid (described in Chapter 2). The fields were selected because detailed, cropping season long assessments of slug numbers would be available from refuge trapping conducted using the same grids to investigate the presence and spatial stability of patches of higher slug numbers (reported in Chapter 3). Samples were taken from each grid point and returned to the laboratory for analysis of organic matter, pH, soil texture and particle density along with a separate sample for bulk density. In addition, at each grid point infiltration rates were measured at the time of soil sample collection, and soil moisture was recorded on each visit to the field for slug assessments.

On return to the laboratory soil samples were air dried for a minimum of 36 hours at 35°C, ground using a pestle and mortar and passed through a 2 mm sieve prior to analysis.

Organic matter and pH

Organic matter content and pH of each soil sample was determined using the methods described in sections 5.2.1.1 and 5.2.1.2 respectively (Ministry of Agriculture, 1986).

Soil texture

Air dried and sieved soil (10 g) was placed into a 600 ml laboratory beaker before 20 ml hydrogen peroxide was added and the soil left to soak overnight for a minimum of 15 hours. An additional 10 ml hydrogen peroxide was then added and the beaker placed on a hot plate (SD 500 digital hotplate, Stuart Equipment, UK) set at 90°C for one hour, being stirred at 10-minute intervals and the volume maintained at 25 ml by adding distilled water as required, the solution was then boiled for 2 minutes to complete the breakdown of organic matter before being allowed to cool to the laboratory ambient temperature. The solution was poured into a beaker, ensuring all soil from the beaker and the rod were included, before 10 ml of a dispersing agent (35 g sodium hexametaphosphate and 7 g sodium carbonate in 1 L distilled water) was added and the solution placed on the orbital shaker for 10 minutes. In a pre-weighed crucible, 10 ml of dispersing agent was oven dried overnight to determine the residual weight. After shaking, all the contents from the beaker were poured into a 500 ml measuring cylinder through a 63 µm sieve. The contents of the sieve were transferred into a pre-weighed crucible (sample a) and oven-dried (60°C). The contents of the measuring cylinder were made up to 500 ml and mixed thoroughly. A 25 ml sample was taken from 90 mm depth and transferred to a pre-weighed crucible (sample b) and oven-dried (60°C). After the solution had been allowed to settle for 7.5 hours, a second 25 ml sample from 90 mm depth was taken (sample c) and transferred to a pre-weighed crucible and oven-dried (60°C). The samples were reweighed at 24-hour intervals until they reached a constant, once a constant weight was reached the weights for sand (sample a), silt (weight sample b minus sample c) and clay (sample c) were recorded (Ministry of Agriculture, 1986; Kettler *et al.*, 2001).

Sand % = $\frac{\text{weight sample a}}{\text{total weight sample a} + \text{sample b} \times 20} \times 100 \%$

Silt % = $\frac{\text{weight sample b} - \text{sample c} - \text{residue weight} \times 20}{\text{total weight sample a} + \text{sample b} \times 20} \times 100 \%$

Clay % = $\frac{\text{weight sample c} - \text{residue weight} \times 20}{\text{total weight sample a} + \text{sample b} \times 20} \times 100 \%$

Particle density

Oven dried and sieved soil (40 g) was placed in a pre-weighed 100 ml flask and the weight recorded before 50 ml of water were added. The mixture was allowed to stand for 5 minutes before the total volume was recorded. The total volume of soil solids and particle density calculated using the equation below.

$$\text{Particle density} = \frac{\text{oven dry weight of soil (g)}}{\text{Volume of soil (ml)}}$$

(Tan, 2005)

Bulk density

Within 10 cm of each field grid point a metal soil corer (7.5 cm diameter x 7 cm height) was fully inserted into the ground. The soil sample contained within the core removed, and returned to the laboratory in a plastic bag, where it was transferred to a paper bag and dried for 72 h (or until a constant weight was recorded in successive assessments) at 105°C in an oven. The volume of the soil was calculated using the volume of the cylinder ($\pi r^2 h$) and bulk density was calculated using the equation below.

$$\text{Bulk density} = \frac{\text{dry weight of soil (g)}}{\text{volume of soil (ml)}}$$

(Wood, 2006)

Soil porosity

Following analysis of the soil particle density and bulk density soil porosity was calculated, using the following equation

$$\text{Soil porosity} = \frac{\text{particle density} - \text{bulk density}}{\text{Particle density}} \times 100 \%$$

(Tan, 2005)

Infiltration rate – simplified falling head method

When the soil was close to field capacity, a metal corer (15.3 cm diameter x 14.5 cm high) was inserted 5 cm into the ground and soil moisture inside the cylinder was measured using a soil moisture probe (Field Scout TDR, Spectrum Technologies Inc., USA). Water (500 ml) was added to the cylinder and the time for this water to drain from the container was recorded using a stopwatch. The soil moisture inside the cylinder was re-measured and the rate of infiltration was measured using the following equation.

$$K_{fs} = \frac{(\Delta\theta)}{(1-\Delta\theta) t_a} \left[\frac{D}{(\Delta\theta)} - \frac{\left[\frac{D+1}{\alpha^*} \right]}{(1-\Delta\theta)} \ln \left[1 + \frac{(1-\Delta\theta)D}{(\Delta\theta) \left[\frac{D+1}{\alpha^*} \right]} \right] \right]$$

Where $\Delta\theta$ = difference between field-saturated water content and the initial water content, α^* = constant, D = Volume of water / cross-sectional area of the infiltrating surface, t = time

(Bagarello *et al.*, 2004)

Soil moisture

Soil moisture was recorded at 5 cm depth at each grid point on each sampling visit to the field using a soil moisture probe (Field Scout TDR, Spectrum Technologies Inc., USA).

3.3.3. Statistical analysis

All statistical analyses were conducted using R 3.3.3. (R core Team, 2015). All residuals were tested for normality and equal variance.

Laboratory experiments

The analysis of the four gradient experiments was carried out using a generalized linear mixed effect model (GLMER). Non-significant terms were removed from the model to reach a minimum adequate model.

Field study

Maps of slug counts created using the `interp` and `filled.contour` functions in R. The number of slugs in between traps was calculated by polynomial interpolation. The presence of hotspots was determined using the `ScanLRTS` function in R. The areas of higher slug densities were identified using the analysis carried out in Chapter 3. A Student's t test was carried out to analyse the results of each soil characteristic within areas of higher slug density compared to areas of lower slug density. Where the assumptions of normality and equal variance were not met a non-parametric Wilcoxon Mann-Whitney test was carried out.

3.4. Using Radio Frequency Identification tags to track the movement of *Deroceras reticulatum* above and below the soil surface

Deroceras reticulatum were collected using surface refuge traps baited with approximately 75g chicken feed pellets (Young, 1990) from two field sites in Uppington (52°40'36.68"N 002°34'50.14"W) and Adeney (52°46'01.26"N 002°34'50.14"W), Shropshire, UK during the two-week period before the start of each experiment (between January 2016 and November 2017). Slugs weighing over 300 mg were returned to the laboratory and maintained individually in 250 ml circular plastic rearing containers (11.5 cm diameter; 4.2 cm high) with 1 mm diameter puncture holes in the lid. The base of each container was lined with paper towel (approximately 2 cm x 3 cm) moistened with 5 ml distilled water, which was replaced daily. Lettuce leaves (cv. Romaine) were offered *ad libitum* to each slug as food, and replaced with fresh leaves daily. Slugs were maintained in a controlled environment room under standard rearing conditions of 60 % humidity, 10:14 hour light: dark cycle, and at 15°C during the light phase and 10°C during dark, to reflect UK conditions in autumn and spring, and allowed a 48-hour acclimatisation period before being used in experiments.

3.4.1. Laboratory experiments

Insertion of RFID Tag

To insert an RFID tag, each slug was removed from its rearing container and placed individually into a smaller circular lidded plastic container (28 ml, height 33 mm, top diameter 44 mm, base diameter 31 mm) with a 5 mm hole drilled through the top. CO₂ was gently released through the hole into the container using a Corkmaster CO₂ dispenser and 8 g CO₂ bulb (Sparklets, UK), for approximately 20 seconds or until the slug was fully extended. The anaesthetised slug was then removed from the pot and held between the thumb and index finger either side of the mantle with the head facing away from the technician. The needle of an MK165 implanter (Biomark, USA) was then positioned at an approximately 30° angle to the body wall (left side), level with the top of the keel, and $\frac{3}{4}$ of the way along the length of the slug from the anterior end. With the tip of the needle pointing toward anterior end, it was inserted through the body wall and when no longer visible, the tag (a chip and antenna coil encased in glass, 8 mm long and 1 mm wide) (HPT8 tag, Biomark, USA) was released before withdrawing the needle from the slug.

Treatments

Five treatments, with 20 slugs per treatment, were used to assess the effect of different aspects of the tagging process:

Tagged (T) + CO₂ + Glue (G) – slugs were anaesthetised using CO₂, an RFID tag inserted and glue (Loctite Precision Max, Loctite, USA), applied over the insertion site to seal the wound.

Tagged (T) + CO₂ - slugs were anaesthetised using CO₂ and an RFID tag was inserted.

CO₂+ - slugs were anaesthetised and the implanter needle was inserted through the body wall but no tag was injected.

CO₂ - slugs were anaesthetised with CO₂ only.

U - untreated control (slugs were maintained in the rearing cages without any part of the tag implanting process being applied).

Slug survival

Following RFID tag insertion, slugs were returned to their individual rearing containers and maintained under the conditions described above for 28 days. During this period, slug mortality, defined as a lack of response to a mechanical stimulus, coupled with a characteristic change in body form following death (body extended and shrivelled), was recorded at 24 h intervals throughout the experiment. Mortality assessments were confirmed when similar observations were recorded for three consecutive days). The experiment was replicated three times.

Feeding

RFID tagged slugs were maintained under the conditions described above for 28 days.

To assess relative rate of food consumption between treatments, each slug was offered pre-weighed lettuce (approx. 1.5 g). After 24 h the remaining lettuce was re-weighed and consumption estimated by subtraction and replaced with fresh lettuce. The procedure was repeated throughout the 28-day experimental period.

Production of egg batches

The impact of implanting RFID tags on rate of reproduction was assessed by recording the number of egg batches laid at 24-hour intervals throughout the 28 days period following treatment.

Locomotor behaviour

Slugs were maintained in the laboratory for a 48-hour acclimatisation period following tag implantation under the conditions described above, before they were randomly allocated to one of two treatment groups. Slugs in the first group were implanted with an RFID tag and those allotted to the second treatment remained untagged (controls). All tags were inserted using the procedure described above (T + CO₂; no glue was applied to the insertion site), and both tagged and untagged control slugs were then maintained under the standard rearing conditions for 14 days before being used for behavioural recordings. Lettuce was fed *ad libitum* and replaced daily throughout this period.

On days 14, 21 and 28 after insertion of the RFID tags, the slugs were released individually at the centre of a 50 cm diameter arena comprised of a circular plywood board painted with white gloss paint (Colours Pure brilliant white Gloss Wood & metal paint B&Q, UK). The recordings took place between 2 and 8 hours after the lights came on in the controlled environment rooms, with the order

of slugs being randomised on each recording occasion. A video-camera (SONY HDR-CX240E Handycam, SONY, Japan) was positioned at 100 cm above the centre of the arena and focussed to record slug activity over the whole arena. Slug behaviour was continuously recorded for 60 minutes or until it had left the arena, whichever occurred first. Video recordings were uploaded into Ethovision XT (Noldus, The Netherlands) and analysed for total distance moved and mean velocity. Distance moved was assessed using the centre point of the slug, which risked additional distance being added when the slug contracted and the size of its profile changed. To control for this the Ethovision settings were adjusted to ensure that a new point along the track was only recorded once the slug had moved more than 0.25 cm. The length of time it took for the slug to leave the arena was also recorded.

3.4.2. In-field tracking

Locomotor behaviour of D. reticulatum in winter wheat

The behaviour of the slugs was investigated in commercial winter wheat and oilseed rape crops in Shropshire, UK (52°46'01.26"N 002°34'50.14"W), in spring (April; 9 slugs) and autumn (November; 20 slugs) 2017. A 4 x 5 grid of refuge traps (as described in Chapter 2) was established in the study area, with 2 m between adjacent traps. Slugs were collected from these traps and the grid node at which each individual was caught recorded. After sufficient specimens had been collected, the traps were removed and each was replaced with a fibreglass flexi-cane to mark the grid nodes.

Slugs were returned to the laboratory where an RFID tag was inserted (each with a unique identifying code) into individual slugs using the technique described above (T + CO₂; i.e. without the application of glue), before being maintained under the standard rearing conditions for a 14-day recovery period. Individual slugs were then released (at sunset) back into the study grid at the node from which they were originally collected.

Movement was tracked after release by recording the location of the slugs at predetermined intervals using a HPR Plus reader (Biomark, USA) and a combination of two antennae (BP Plus Portable antenna; Racket antenna; Biomark, USA). Initially the racket antenna (which has a smaller read range (up to 10 cm) facilitating more accurate determination of location) was used to systematically search the area within a 1 m radius of the last known location of the slug. If the slug was not found the larger BP Plus Portable antenna (read range up to 20 cm) was used, allowing the area contained within ever larger concentric circles to be searched efficiently until the slug was located. In cases where the BP Plus Portable antenna was used to find the RFID tag in a wider area, a more precise location was then determined using the racket antenna. When an RFID tag was detected the identity of the slug was confirmed using the unique identifying code, its precise position was confirmed visually (if on the surface), and its position marked using a labelled peg recording the identifying code, assessment number, and the time of the observation. In addition, records of the slug presence above or below the soil surface, and its current activity, (leaf eating,

linear locomotion, etc.) were made. Slugs were tracked at approximately 20-minute intervals for two hours post release in April 2017 and for 8 hours post-release in November 2017. In November 2017 slugs were also tracked on the following two nights for 8 hours. Following these initial periods of intense monitoring, slugs were tracked daily, and then at weekly intervals for a maximum of 38 days or until a period of 2 weeks had elapsed without any movement being observed.

Immediate accurate measurement of the distances travelled by *D. reticulatum* were more challenging during evening assessments. Accordingly, the distance between sequential marker pegs were measured the following morning. To avoid accumulation of errors that may accrue if measurements were made between sequential marker pegs, the location of each marker peg in relation to the original release point (marked by the flexi-cane on the grid node) were determined before the distance between sequential marker pegs was calculated. The location of each peg was also recorded using a hand-held GPS accurate to 18 mm (Leica RX1220T, Germany). On each night of tracking and on subsequent visits to the field, soil moisture was recorded (three points across the grid) using a soil moisture probe (Field Scout TDR 100, Spectrum technologies, Inc, USA) and soil and air temperature were recorded at 30-minute intervals using data loggers (iButton DS1921G-F5 thermochrons, Maxim integrated Products, USA).

3.4.3. Statistical analyses

Effect of implanting RFID tags on survival, feeding and production of egg batches

Following tests for normality and heterogeneity of the data (using the diagnostic plots in R to check residuals vs fitted values, Q-Q plots, scale-location plots and residual vs leverage plots), the effect of treatment on mortality rate, lettuce consumption and production of egg batches was investigated using repeated measures ANOVA.

Effect of implanting RFID tags on locomotor behaviour

Following tests for normality and heterogeneity of the data (using the diagnostic plots in R to check residuals vs fitted values, Q-Q plots, scale-location plots and residual vs leverage plots), the effect of treatment on mean velocity and total distance moved was investigated using ANOVA.

Locomotor behaviour of *D. reticulatum* in winter wheat

Maps of individual slug movement in the field were created using the 'plot' function in R. The mean total distance moved over the experimental period and the mean distance from the start point at the end of the trial period were calculated. Distances moved were calculated using linear interpolation of the x and y coordinates of two consecutive tracking points, the distance between each point and the total displacement (distance between the final location and the original release point) were calculated using Pythagoras' theorem. The distances between each point were added

together to give a total distance moved. Daily temperature and rainfall were correlated with the number of active slugs using Pearson's Correlation Coefficient.

4. Results

4.1. Slug patches and their stability

4.1.1. Slug counts

The number of slugs detected varied between assessment dates within fields, between fields and years. There was a significant difference between the mean slug counts in each season ($F=41.74$, $d.f.=2, 157$, $p<0.001$). The mean number of slugs at each field site was $451.3 (\pm 63.8)$ in 2015-16, $55.5 (\pm 7.0)$ in 2016-17 and $145.7 (\pm 40.2)$ in 2017-18. There was also a significant difference between fields in all years, identified by a post-hoc Tukey's test, in 2015-16 Lynn (Stoney lawn) had a significantly higher mean number of slugs than the other fields ($F=12.10$, $d.f.=4,41$, $p<0.001$), in 2016-17 the mean number of slugs in Dogdyke had significantly fewer slugs than Wigan and Uppington (1) and the mean number of slugs was significantly higher in Uppington (1) than Lynn (Badjics), Flawborough, Lynn (Stoney Lawn), Bridgnorth, South Kyme (2) and Adeney (Middle) ($F=3.89$, $d.f.=311,77$, $p<0.001$). In 2017-18, Uppington (2) had a significantly higher mean number of slugs compared to the other fields sampled ($F=8.14$, $d.f.=4,27$, $p<0.001$).

In general numbers of slugs were lower in assessments carried out in August, September and October and then, a small peak occurred between November and January followed by a larger peak in the spring (between March and May), where assessments continued after May numbers decreased. In 2015-16 assessments started in December, a peak in the spring was observed with a significantly higher number of slugs occurring in March ($F=16.79$, $d.f.=5, 30$, $p<0.001$). Although there appears to be some indication of the general trend for an autumn and spring in the 2016-17 and 2017-18 sampling periods there were no significant differences in the number of slugs observed in different months. The number of slugs recorded in the 2016-17 and 2017-18 sampling periods were significantly lower ($F=24.27$, $d.f.=2, 134$, $p<0.001$).

4.1.2. Hotspot analysis

Hotspot analysis was carried out for all sampling visits to all field sites over three seasons. Irrespective of the variation between the size of slug populations discrete areas of higher slug densities were observed in all fields (except on one occasion in each of Adeney (Middle) (August 2016), Belchford (June 2018), Dogdyke (April 2017), Hoby (September 2016), South Kyme (1) (August 2016) and Wigan (March 2018)). An example of the results of the hotspot analysis is shown in Figure 4.1. The hotspots (areas of significantly higher numbers of slugs than expected in a random distribution) in 2015-16 season in Adeney (Middle) appear in the same area of the field in 10 of the 11 assessments and in Lynn (Badjics) on 7 out of 7 assessments (Figure 4.1). In 2016-

17 the hotspots at Uppington (1) occur in three areas of the field, with those in the largest of the three areas occurring in all 7 assessments. Of the two smaller areas, one area contains a hotspot on 5 of the 7 assessments and the other 4 of the 7 assessments. In Wigan, hotspots occurred in the same area of the field on all 10 assessment dates. In 2017-18 the hotspots in Uppington (2), hotspots were detected on all assessment dates, occurring in the same area of the field on all 7 occasions. Although hotspot analysis indicated that in most fields in most years areas of higher slug densities consistently appeared in the same locations in the arable fields some exceptions occurred. For example, in In Wigan hotspots were not consistently found in one area of the grid and on one assessment date no hotspots were present.

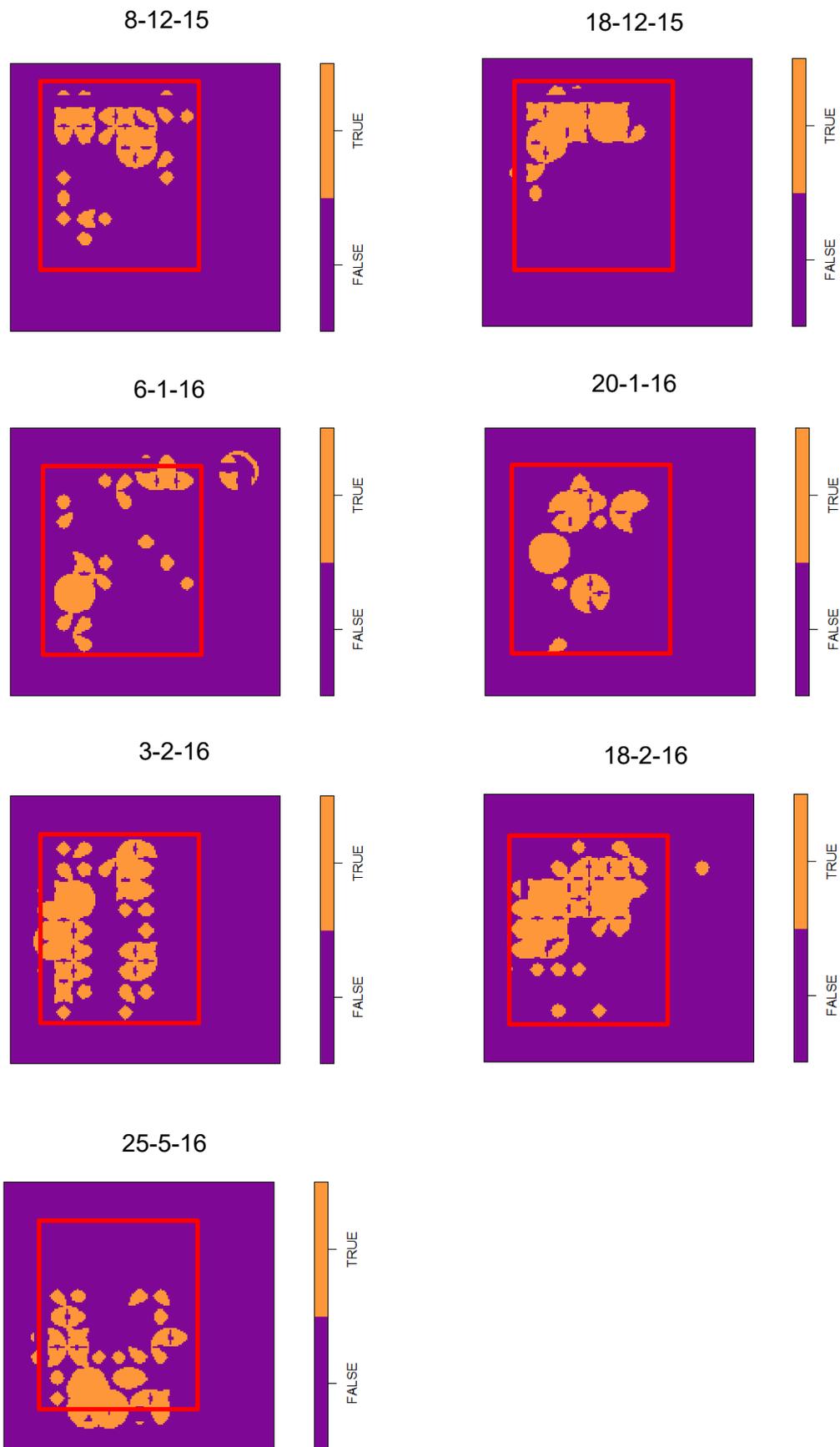


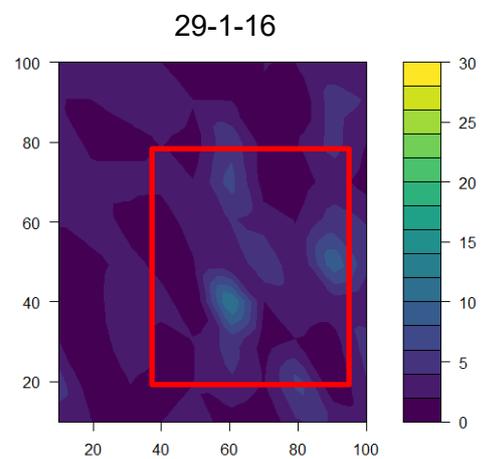
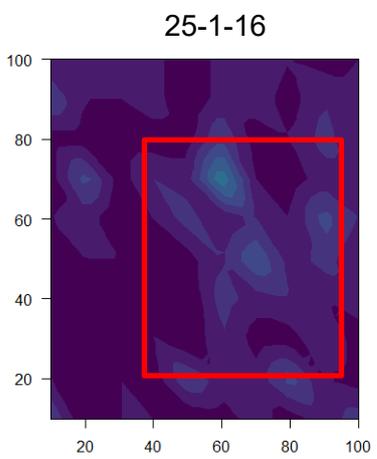
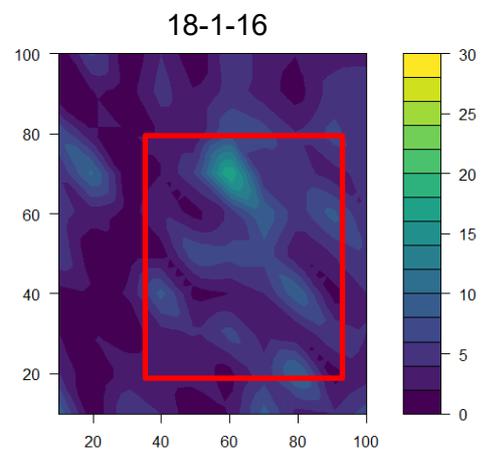
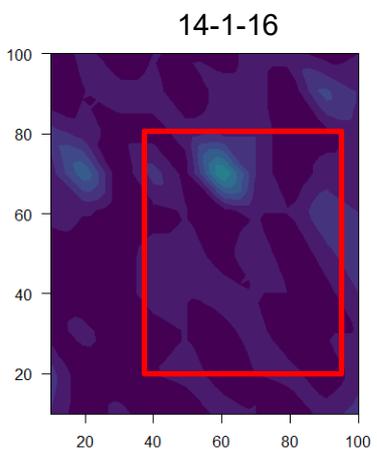
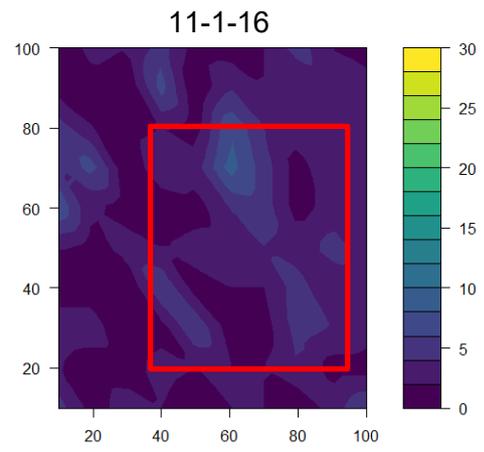
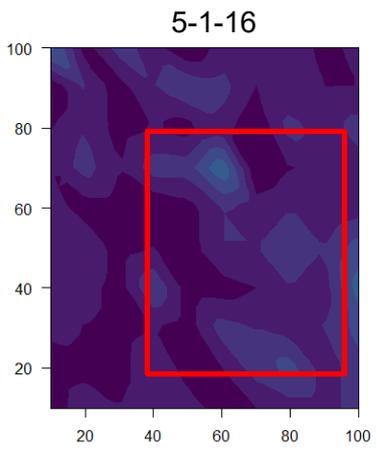
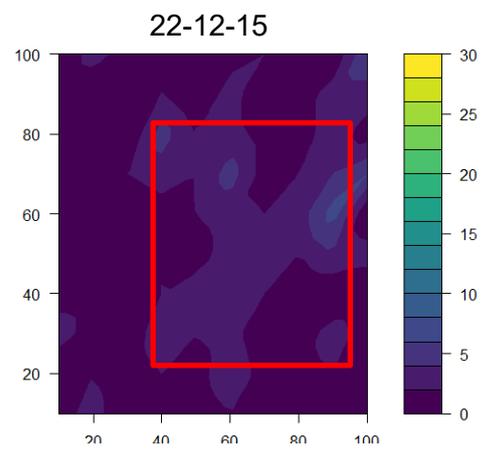
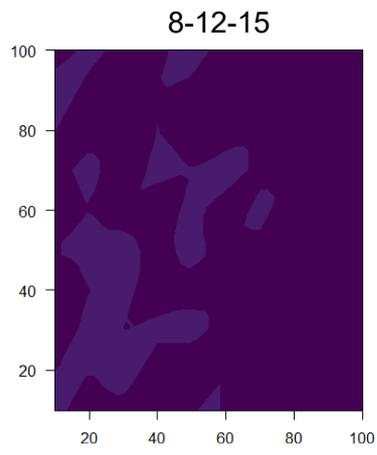
Figure 4.1. Hotspot analysis for Lynn (Badjics) for assessment dates throughout the 2015-16 growing season. Orange represents significant aggregations of slugs, areas where more slugs were found than would be expected if the slugs were randomly distributed across the field at $p < 0.05$ significance level. Purple shows the areas of the field where the number of slugs observed was not significantly different to that expected if they were randomly distributed. The red boxes highlight the areas where hotspots most frequently occur.

4.1.3. Within season patch stability

2015-2016

There was variation in the number of slugs that were active on the soil surface (reflected in refuge trap catches) between assessment visits and field sites. Patches of higher slug numbers were located in all five fields during the 2015-16 season, the size and shape of these patches varied between fields but remained spatially stable within each field, with patches ranging from 300 to 7000 m². Throughout the 2015-16 growing season the locations of individual slug patches were highly correlated between assessments. At Adeney (Corner) correlations as high as $r = 0.38$ ($t=4.09$, $d.f.=98$, $p<0.001$) were observed between assessments, at Adeney (Middle) the highest correlation was $r = 0.65$ ($t=8.46$, $d.f.=98$, $p<0.001$), Lynn (Badjics) $r = 0.53$ ($t=6.20$, $d.f.=98$, $p<0.001$), Lynn (Stoney Lawn) $r = 0.85$ ($t=15.81$, $d.f.=98$, $p<0.001$) and Uppington (1) $r = 0.3$ ($t=3.12$, $d.f.=98$, $p=0.002$). High correlations were not only observed between assessment sites in temporal proximity to each other but also across the season, for example, at Adeney (Middle) a correlation of $r = 0.41$ ($t=4.46$, $d.f.=98$, $p<0.001$) was observed between the assessment on 14/1/16 and 26/4/16, at Lynn (Badjics) a correlation of $r = 0.4$ ($t=4.29$, $d.f.=98$, $p<0.001$) was found between the assessment on 8/12/15 and 3/2/16 and at Lynn (Stoney Lawn) there was a correlation of $r = 0.43$ ($t=4.68$, $d.f.=98$, $p<0.001$) between assessments on 18/12/15 and 15/3/16. The correlations between trap counts demonstrates that the highest trap counts are reappearing in the same location, further work investigated whether traps in close proximity to each other displayed similar stability.

Within the stable area of higher slug numbers, the individual trap recording the highest count could vary between assessment dates. A similar pattern of stability was observed at the other field sites monitored in the 2015-16 season, with the area of the field with the highest number of slugs occurring in the same location in nine out of eleven assessments of Adeney (Middle) (shown as an example in Figure 4.2), six out of seven in Lynn (Badjics), 11 out of 13 in Lynn (Stoney Lawn), and of the two areas containing hotspots in Uppington (1), one occurred on six out of eight assessment dates and the other four out of eight.



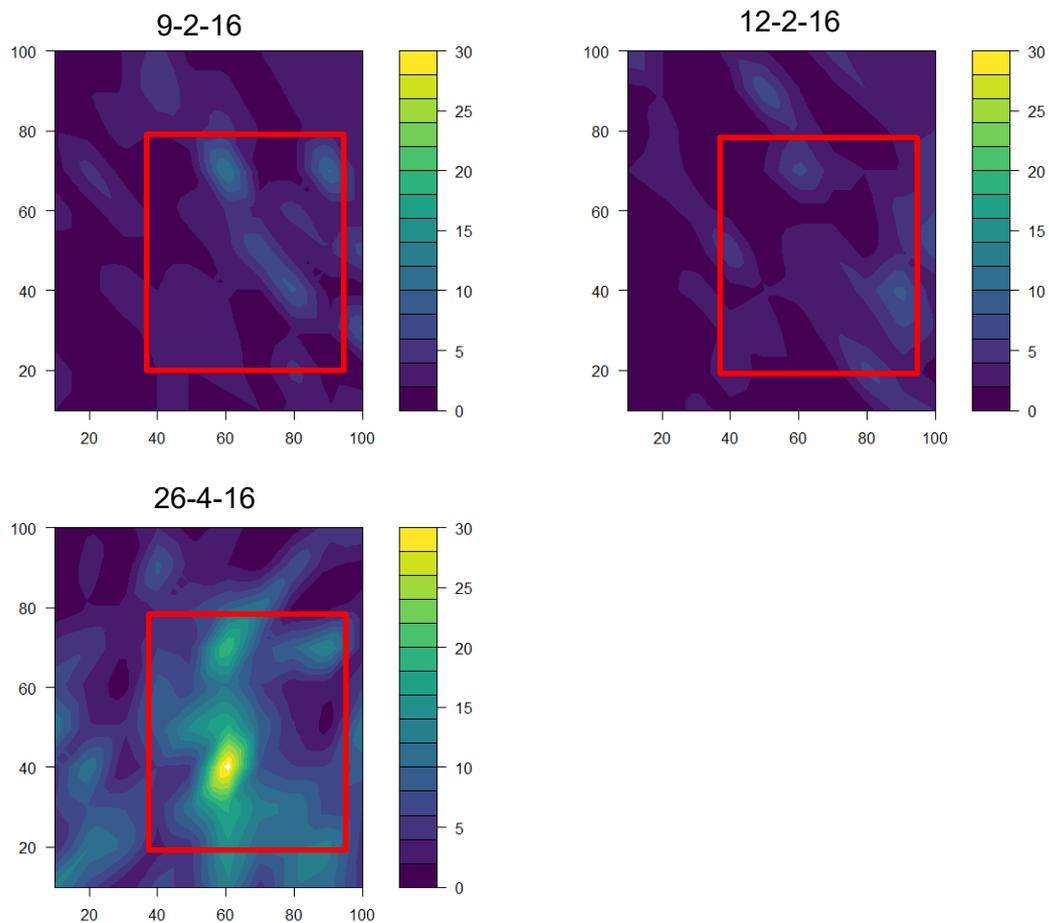
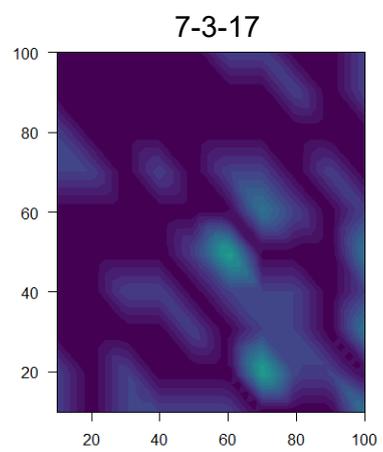
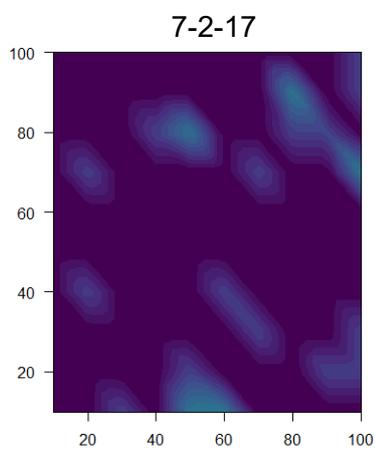
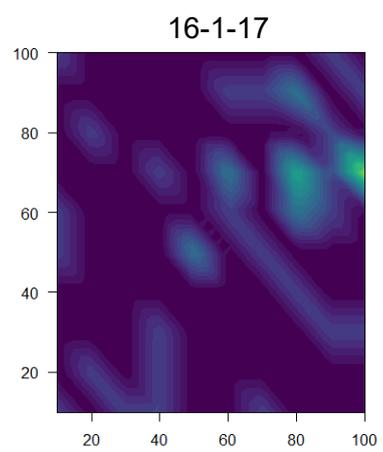
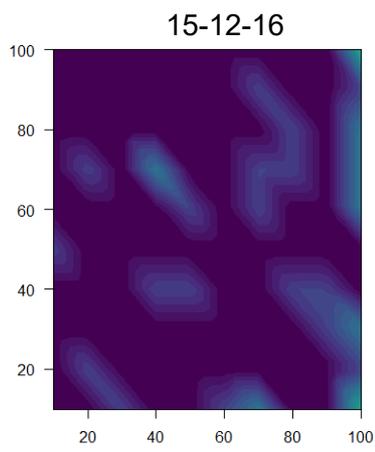
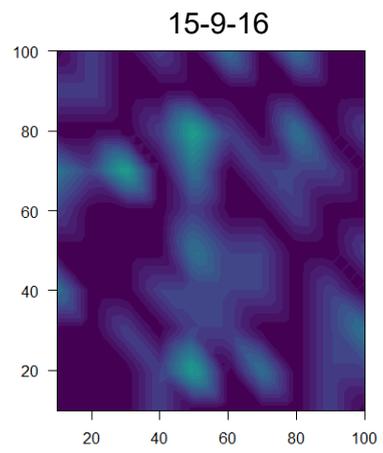
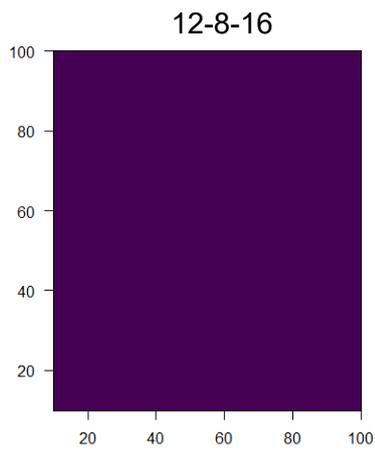


Figure 4.2. Heat maps showing slug distribution at Adeney (Middle) from assessments carried out between December 2015 and April 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation. The areas highlighted in red shows the location of the traps with the highest slug counts.

2016-2017

The total number of slugs observed in 2016-17 was significantly lower ($F = 41.74$, $d.f. = 2, 157$, $p < 0.001$) than the 2015-16 season (mean of 55.5 ± 7.0 slugs recorded per assessment date compared to 451.3 ± 63.8 in 2015-16). The average number of slugs per trap was below the AHDB threshold level (average 4 slugs per trap in a standing crop, (AHDB, 2016)) on all assessment dates at all field sites. In fields where slug numbers were lowest there was little variation between the maximum and minimum slug catches in individual refuge traps. The generally lower catches in individual refuge traps also resulted in no distinct patches being detectable in some fields, as illustrated by the heat map for Adeney (Middle). Despite the low slug populations, in the two fields with the highest maximum total counts (Uppington (1) and South Kyme (1)) and in one field with a low population (Wigan), some correlations between trap counts on different assessment dates were found. The highest correlations were in South Kyme (1), $r = 0.33$ ($t = 3.41$, $d.f. = 98$, $p < 0.001$) between assessments on 17/2/17 and 9/3/17, Uppington (1), $r = 0.38$ ($t = 4.11$, $d.f. = 98$, $p < 0.001$) between assessments on 13/9/16 and 28/2/17 and Wigan, $r = 0.53$ ($t = 6.23$, $d.f. = 98$, $p < 0.001$)

between assessments on 12/4/17 and 10/5/17. In these fields, similar patterns of stability to those detected in the 2015-16 field season were observed, in Uppington (1) on all seven assessments. Slightly lower populations resulted in clusters of patches of higher slug areas being more difficult to identify, but the areas they formed were still visible in South Kyme (1) on five out of nine assessments and in Wigan on seven out of ten assessments.



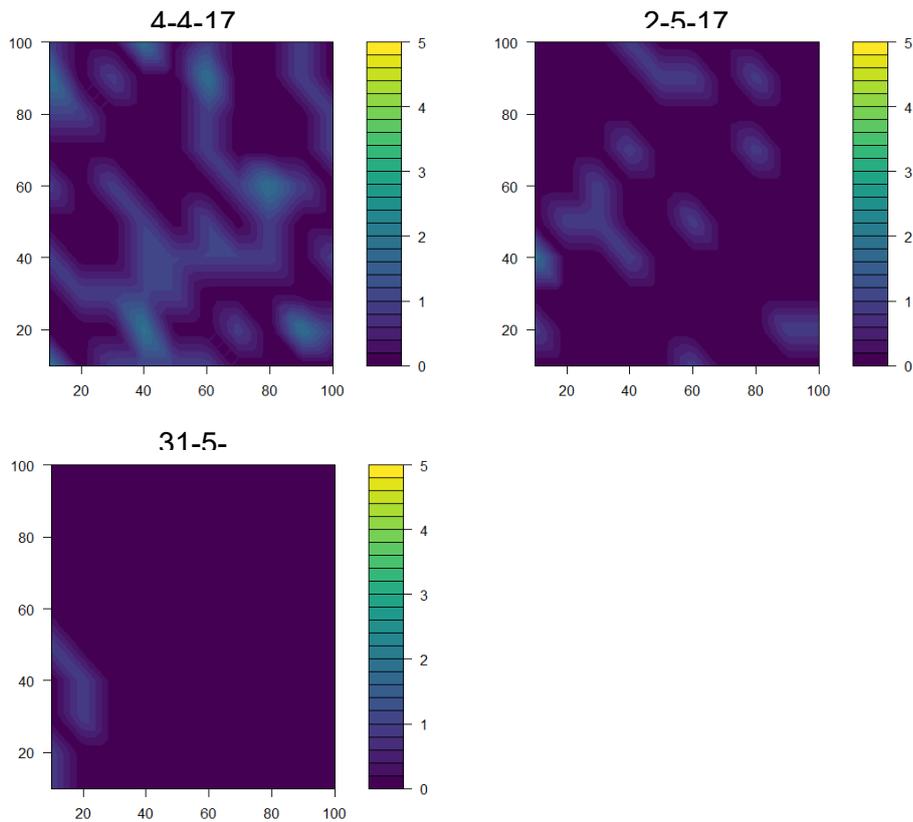


Figure 4.3. Heat maps showing slug distribution at Adeney (Middle) from assessments carried out between August 2016 and May 2017. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, with the numbers in between traps calculated by polynomial interpolation.

2017-2018

The mean number of slugs observed at each assessment in 2017-18 was significantly higher than in 2016-17 and lower than in 2015-16 (mean of 145.7 ± 40.2 slugs at each assessment compared to 55.5 ± 7.0 in 2016-17 and 451.3 ± 63.8 in 2015-16) ($F= 41.74$, $d.f.= 2, 157$, $p<0.001$). The variation between fields was high, the mean total count, for each assessment visit, at Uppington (2) was 457 slugs compared to 26 at Oadby. At four of the five field sites where the slug counts were low (mean number of slugs per trap <2.7 , in each case, lower than the AHDB 4/trap action threshold) no stable patches were detectable (Adeney (Middle), Belchford, Oadby and Wigan). In Uppington (2), where the highest mean number of slugs was detected higher density patches were present and occurred in the same areas of the field on all seven assessment dates. The correlations between trap counts on different assessments dates were similar to the 2016-17 field season, the highest correlation was in Uppington (2) where $r = 0.47$ ($t=5.33$, $d.f.=98$, $p<0.001$) between assessments on 18/4/18 and 26/4/18.

4.1.4. Between season patch season

Of the six fields where assessments were carried out in multiple years there were two fields, Uppington (1) and Adeney (Middle) where stable patches were detected in multiple seasons. In Uppington (1) the patches located in 2016-17 were largely located in a different part of the grid to the patches located in 2015-16. The correlation between counts in 2015-16 and 2016-17 at Uppington (1) was low. There were only three significant correlations between seasons, two were positively correlated between 7-12-15 and 18-10-16 ($r = 0.23$, $t=2.34$, $d.f.=98$, $p=0.021$) and between 1-2-16 and 20-12-16 ($r = 0.22$, $t=2.018$, $d.f.=98$, $p=0.031$), and one was negatively correlated between 19-1-16 and 13-9-16 ($r = -0.28$, $t=-2.93$, $d.f.=98$, $p=0.004$). Although patches were not detectable in Adeney (Middle) in 2016-17 or 2017-18 due to the low number of slugs recorded, when the area of the field with the highest slug counts in 2015-16 was compared with the 2016-17 and 2017-18 data on two out of nine and three out of eight assessments respectively the traps with the highest slug counts were located within the same area. The highest correlation was recorded between assessments on 18-1-16 and 7-3-17 ($r = 0.33$, $t=3.50$, $d.f.=98$, $p<0.001$). In 2016-17 at Lynn (Stoney Lawn) patches were not detectable, however, on the assessment date with the highest individual trap count (10 on 16-12-16) the area of the field with the highest number of slugs appeared in the same location as the highest trap counts in 2015-16. There were no significant correlations between the trap counts on the 16-12-16 and any of the assessments in the 2015-16 field season. The data collected in this study does not provide sufficient evidence that the level of stability in the location of higher density slug patches over the life cycle of an individual crop (identified in earlier sections of this report), is reflected in similar stability between crops or cropping seasons.

4.2. Slug patches and damage

4.2.1. *Deroceras reticulatum* populations

Slug numbers varied with time in the winter wheat fields studied. Significantly lower numbers of slugs were recorded between 30 November and 22 December 2015 ($F=6.47$, $d.f.=1,40$, $p=0.015$), with a mean trap count of 204.6 ± 44.4 slugs per trap on each assessment date compared to 451.6 ± 72.2 for the period 4 January to 29 April 2016. Post hoc (Tukey's HSD) analysis showed slug numbers at Lynn (Stoney Lawn) were higher than in the other fields assessed during the period 4 January to 29 April 2016. The mean total number of slugs recorded in the sampling grid at each assessment between 4 January and 29 April 2016, was 257.5 ± 94.8 at Uppington and corresponding figures of 319.7 ± 47.8 at Adeney (Middle), 284.2 ± 42.1 at Lynn (Badjics), and 960.0 ± 145.4 at Lynn (Stoney Lawn). Slug populations in Adeney (Corner) were not significantly different from Uppington, Adeney (Middle) and Lynn (Badjics) but did not follow the pattern of the other fields and remained low throughout this period with a mean of 72.8 ± 18.3 slugs per assessment

during this later period. Due to the low number of slugs recorded in Adeney (Corner), no further analysis of the data collected was undertaken. Irrespective of the variable population sizes of slugs at in different fields, hotspot analysis detected discrete areas of higher slug densities in all fields investigated (Figure 4.4).

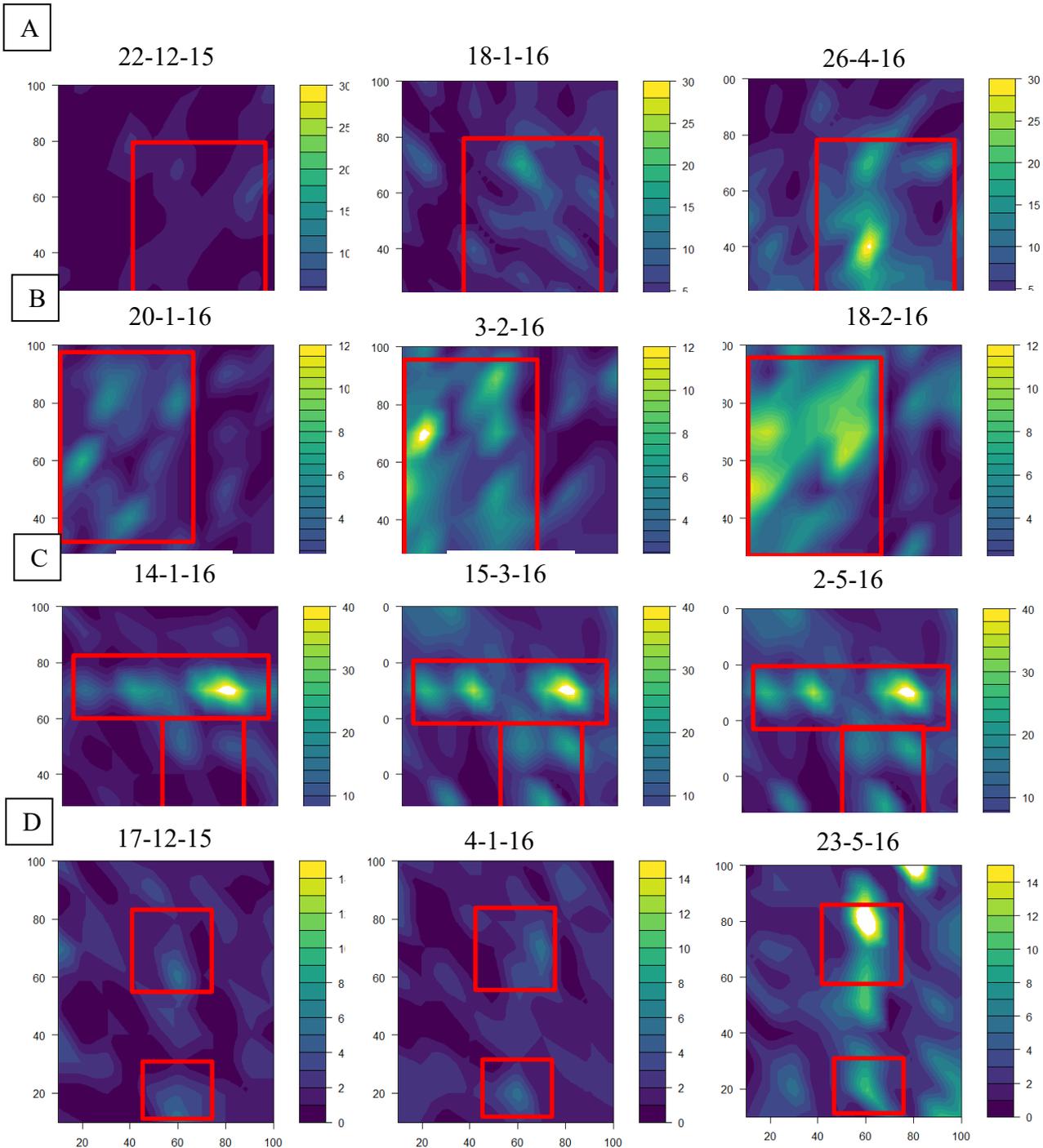


Figure 4.4. Heat maps showing slug distribution at (A) Adeney (Middle), (B) Lynn (Badjics), (C) Lynn (Stoney Lawn) and (D) Uppington (1) from assessments carried out between December 2015 and May 2016. The numbers along the x and y axis show dimensions of the sampling grid in metres. 100 refuge traps were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs, numbers in areas between traps were calculated by polynomial interpolation. The areas highlighted by red boxes show the location of the traps with the highest slug counts.

4.2.2. Correlation between location of damage and slug counts

Slug feeding damage varied significantly between fields ($F=8.90$, $d.f.=3,25$, $p<0.001$). Post hoc (Tukey's HSD) analysis showed the field with the highest percentage leaf area damaged was recorded in Lynn (Stoney Lawn), reflecting the high slug numbers recorded in refuge traps. No significant differences in percentage leaf damage occurred between any of the other fields assessed. Damage scores decreased in all fields over the growing season (Figure 4.5) reflecting the increase in plant size, whereas the number of slugs per trap increased throughout the season.

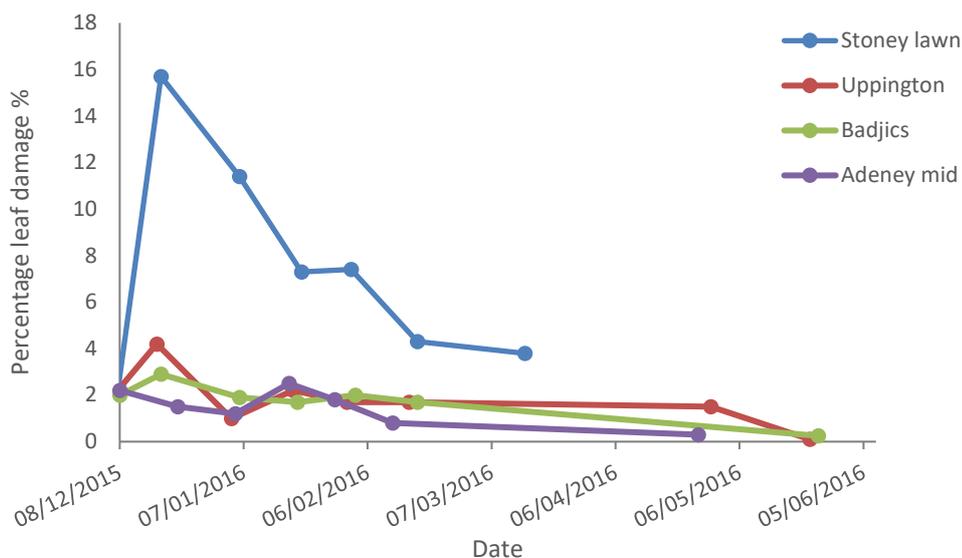
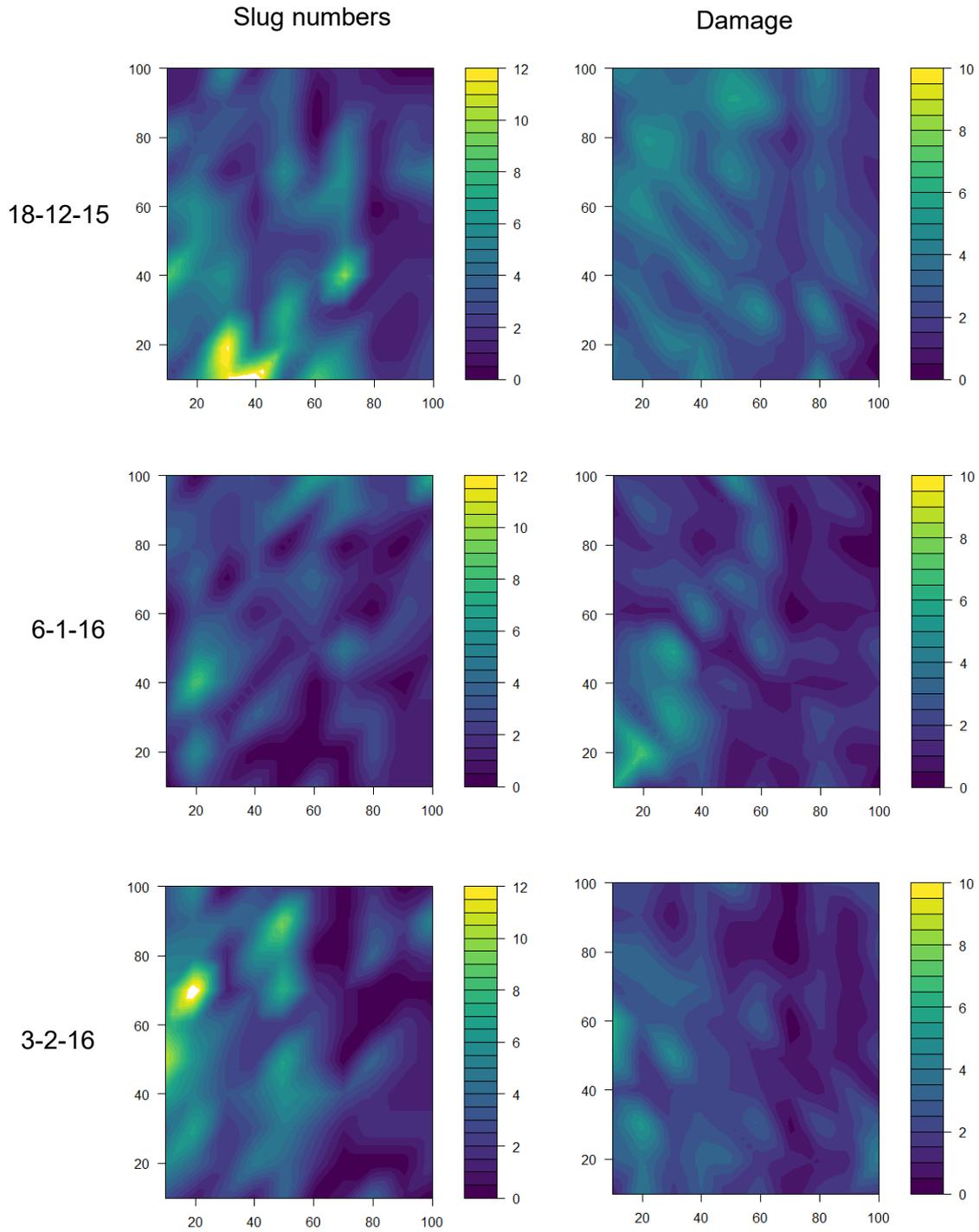


Figure 4.5. Mean percentage leaf area damaged on each assessment date between December 2015 and May 2016 at each field site. Damage was assessed as a percentage leaf damage of 20 leaves within a 50 cm radius of each refuge trap across a 10 by 10 grid.

Significant correlations between the percentage feeding damage recorded on plants and slug catches in refuge traps at each grid point were found in each field. At Lynn (Stoney Lawn), the field with the highest number of slugs, there was one significant correlation on 2/2/16, which was negative. The correlations between damage and slug numbers were positive in the other three fields; in 6 out of 7 assessments at Adeney (Middle), six out of seven assessments at Lynn (Badjics) (Figure 4.6) and three out of eight assessments at Uppington (1). Pearson's Correlation Coefficients at Adeney (Middle) varied between $r = 0.24$ (26/4/16) and $r = 0.43$ (29/1/16), at Lynn (Badjics) between $r = 0.23$ (6/1/16) and $r = 0.52$ (18/12/15) and at Uppington (1) between $r = 0.18$ (7/12/15) and $r = 0.52$ (17/12/15). Although statistically significant the correlations between the damage assessments and slug counts were weak ($r < 0.39$) or moderate ($r = 0.40-0.59$) and no positive correlation was found at Lynn (Stoney Lawn) where the field had the highest number of slugs. The weak relationship between apparent slug damage and numbers of slugs caught in refuge traps suggests that visible slug damage is a poor indicator of the location of patches of higher slug densities, even in winter wheat fields with higher slug populations.



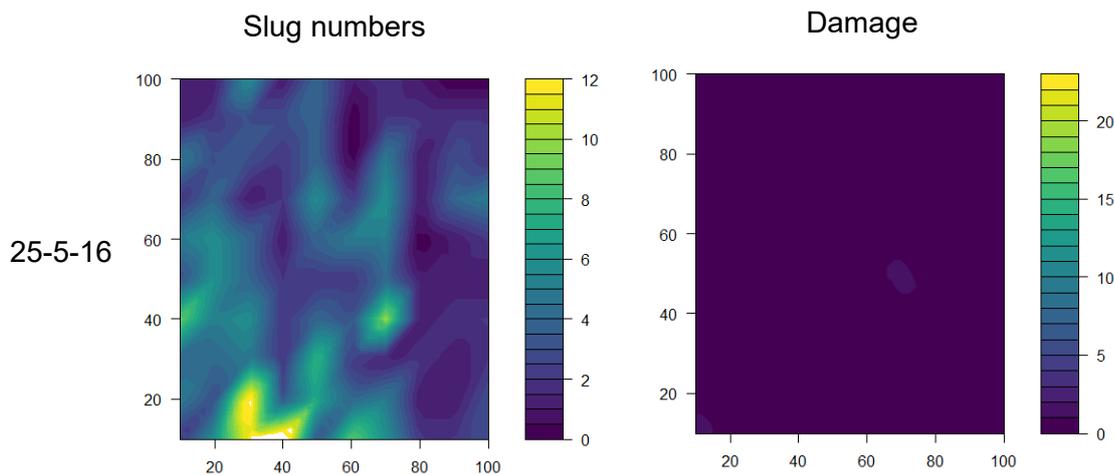


Figure 4.6. Heat maps of slug distributions and percentage leaf damage at Lynn (Badjics) on assessments between December 2015 and May 2016. The numbers along the x and y axis show distance in metres. Sampling points were positioned at 10 metre intervals in a 10 by 10 grid. Colour scale represents the number of slugs and percentage damage, with the numbers in between calculated by polynomial interpolation. Damage assessments were carried out on 20 leaves in a 50 cm radius of each refuge trap.

4.3. Relating location of slug patches to soil characteristics

4.3.1. Laboratory experiments

In experiments investigating slug responses to characteristics of the physical environment, the number of slugs recorded in different compartments of the stepped gradients at the end of the 5-day exposure varied. Statistically significant differences in slug numbers between compartments were found in experiments investigating organic matter content, low moisture, high moisture and temperature gradients, but no significant difference was observed between the numbers of slugs in each compartment of the pH gradient (Table 4.1).

Table 4.1. Proportion of individual slugs recorded at the end of the 5-day experimental period in each of the four compartments of the stepped gradients used to investigate the responses of slugs to organic matter content of soil, or soil pH, moisture or temperature. Figures followed by different letters were significantly different ($p < 0.05$). Each soil characteristic were investigated separately and conditions in each compartment (C1-C4) in the five experiments were; Exp A: Organic matter content – C1= 0%, C2=3%, C3=6%, C4=9%; Exp B: pH – C1=5.86, C2=6.26, C3=6.51, C4=6.97; Exp 3: Moisture (low) – C1=50% of field capacity, C2=75%, C3=100%, C4=125%; Exp 4: Moisture (high) - C1=125%, C2=200%, C3= 290%, C4=370%; Exp 5: Temperature – C1=4°C, C2=5°C, C3=14°C, C4=25°C.

	Compartment 1	Compartment 2	Compartment 3	Compartment 4
Organic matter	0.70 ^a	0.10 ^b	0.10 ^b	0.10 ^b
pH	0.15 ^a	0.23 ^a	0.31 ^a	0.31 ^a
Moisture - low	0.10 ^a	0.10 ^a	0.10 ^a	0.70 ^b
Moisture – high	0.37 ^a	0.37 ^a	0.18 ^a	0.08 ^b
Temperature	0.00 ^a	0.46 ^b	0.46 ^b	0.08 ^a

Organic matter

The number of slugs recorded in the section of the gradient with 0 % organic matter at the end of the experiment was significantly higher than in all other compartments ($z=3.28$, $d.f.=54,3$, $p=0.001$; Table 4.1). Differences in slug distribution between compartments started to emerge during day 2 of the experiment and increased in magnitude thereafter.

pH

No significant differences were detected between the number of slugs observed in the different compartments of the pH gradient at the end of the experiment (when compared to the compartment with soil pH 5.86; the compartment with soil 6.26 - $z=-0.73$, $d.f.=3,84$, $p=0.47$; soil pH - 6.51 $z=1.77$, $d.f.=3,84$, $p=0.078$; soil pH - 6.97 $z=0.89$, $d.f.=3,84$, $p=0.37$; Table 4.1).

Moisture

In the experiment in which the lower range of soil moisture was investigated (50-125 % field capacity), a significantly higher number of slugs were recorded in the gradient compartment offering the highest moisture content (125 %; $z=4.93$, $d.f.=3,72$, $p<0.001$; Table 4.1). Differences between the compartments with different soil moisture were apparent from early in the experiment and was maintained thereafter. When the experiment was repeated using a gradient with soil moisture ranging from 125 % to 370 % of field capacity, significantly fewer slugs were recorded in the compartment with 370 % field capacity ($z=2.99$, $d.f.=3,58$, $p=0.003$; Table 4.1). Differences between compartments containing the two higher soil moistures, and those with lower moisture levels appeared to start emerging during day 2 of the experiment and were largely maintained thereafter.

Temperature

A significantly higher number of slugs were recorded in the 5 and 14°C compartments of the stepped gradient used to investigate responses to temperature than in the more extreme temperatures offered (when compared to the compartment with the lowest temperature, 4°C; 5°C $z=2.87$, $d.f.=3,12$, $p=0.004$; 14°C $z=2.49$, $d.f.=3,12$, $p=0.013$; 24°C $z=0.64$, $d.f.=3,12$, $p=0.64$; Table 4.1). In addition, 3 slug mortalities were recorded in the compartment with the lowest temperature (4°C; 2 on day 1 and 1 on day 2) and 5 mortalities in the highest temperature section of the gradient (24°C; 2 on day 1, 2 on day 2 and 1 on day 3). No mortalities were recorded in the 5 and 14°C compartments. Differences between the numbers of slugs in different compartments emerged from day 2 of the experiment and were maintained thereafter until the final assessment.

4.3.2. Field study

Inter-field variation between the soil characteristics assessed was detected in the six arable fields investigated (organic matter, $F=834.3$, $d.f.=5,594$, $p<0.001$; pH, $F=120.5$, $d.f.=5,594$, $p<0.001$; bulk density, $F=298.2$, $d.f.=5,594$, $p<0.001$; particle density, $F=3.6$, $d.f.=5,48$, $p<0.008$; porosity, $F=329.0$, $d.f.=5,594$, $p<0.001$; infiltration rate, $F=29.3$, $d.f.=4,453$, $p<0.001$; moisture, $F=508.5$, $d.f.=5,594$, $p<0.001$). In four of the six fields significant differences were detected in the level of at least one of the soil characteristics between traps located in the patches of high or low slug densities, as defined in section 4.1 (Table 4.2). For example, in the 2016-17 season in Oadby bulk density ($t=-2.13$, $d.f.=98$, $p=0.036$) and porosity ($t=2.08$, $d.f.=98$, $p=0.040$) and in Wigan organic matter content ($t=2.40$, $d.f.=98$, $p=0.018$) and pH ($t=2.03$, $d.f.=98$, $p=0.045$) were found to differ significantly between patches of higher slug numbers and the spaces between these patches which contained lower slug densities. In the 2017-18 season pH in Adeney (Middle) ($t=2.51$, $d.f.=98$, $p=0.014$) and the maximum soil moisture content at the Wigan site ($t=2.04$, $d.f.=98$, $p=0.044$) were also found to vary significantly between areas in which slug patches had formed and those areas not incorporated into patches. In addition, assessments of soil texture were taken at a single field site (Adeney (Middle)) and significant differences were recorded between percentage sand ($t=-3.89$, $d.f.=21$, $p<0.001$), percentage silt ($t=3.41$, $d.f.=21$, $p=0.002$) and percentage clay ($t=3.12$, $d.f.=21$, $p=0.005$) recorded in soil samples from within patches of higher slug densities and the areas between these patches.

Table 4.2. The range of soil characteristics in areas of fields assessed for patches of higher slug densities. In each field stable slug patches were detected using catches of refuge traps set at each node of a standard 10 by 10 grid, with a 10 metre interval between nodes. Soil assessments were taken at the same nodes. Figures represent mean slug count across the whole trapping grid, or for the maximum and minimum value for each soil characteristic. Values highlighted in grey indicate significant ($p < 0.05$) differences between areas within and outside slug patches. * = no measurements taken.

Fields 2016-17	Mean slug count	Organic matter %	pH	Texture-sand %	Texture - silt %	Texture - clay %	Bulk density gcm^{-3}	Particle density gcm^{-3}	Porosity %	Infiltration rate mms^{-1}	Mean moisture %	Max. moisture %	Min. moisture %
Oadby	0.57	6.4-9.6	5.2-6.7				0.97-1.52	2.4	36.7-65.0	0-32	26-44	36-57	11-33
South Kyme (1)	0.97	7.1-12.4	5.8-7.9				0.95-1.33	2.2	39.5-56.8	0.1-58	39-57	46-69	20-52
Wigan	1.06	3.9-9.3	5.5-6.8				1.02-1.46	2.4	38.8-58.3	0-48	23.5-36.5	31-51	4-29
Fields 2017-18													
Adeney (Middle)	0.53	3.6-6.8	5.2-6.9	28.7-49.8	16.6-29.4	31.4-43.3	1.0-1.55	2.3	32.4-56.5	0-75	28-45	33-52	17-38
Uppington (2)	4.67	2.4-4	5.5-7.5				1.36-1.79	2.4	35.4-43.1	0-90	20-37	24-47	11-29
Wigan	1.00	2.2-7.2	6.1-7.3				1.12-1.52	2.4	36.6-53.2	*	25-42	29-50	20-37

Organic matter

The organic matter content of individual soil samples in the six fields sampled varied from 2.2 % (Wigan 2017-18) to 12.4 % (South Kyme (1) 2016-17; Table 4.2). Although laboratory experiments showed no differences in slug activity between soils with enhanced organic matter content, a significant correlation between slug numbers and soil characteristics was detected at one of the six study sites. The spatial variation in the organic matter content of the soil at the Wigan site in 2016-17 is illustrated in Figure (A). The discrete patches of higher slug densities were defined using the method described in Chapter 3, an example of the distribution at the Wigan site is shown in Figure (B). The level of organic matter in soils adjacent to refuge traps set within patches of high slug densities (7.06) was compared with and found to be significantly greater than those in areas of lower slug densities (6.55 %; $t=2.4$, $d.f.=98$, $p=0.018$). The Wigan site displayed the largest range of organic matter (3.9-9.3 %; Table 4.2) potentially indicating that sufficiently large spatial variation is required before the effect of the factor can be detected using the methods employed. South Kyme (1) displayed a range that was only slightly lower than at Wigan (7.1-12.4 %; Table 4.2), but with no significant correlation between slug numbers and soil organic matter. The lowest level of organic matter content in this case was higher (7.1 % in South Kyme (1) compared to 3.9 % in Wigan).

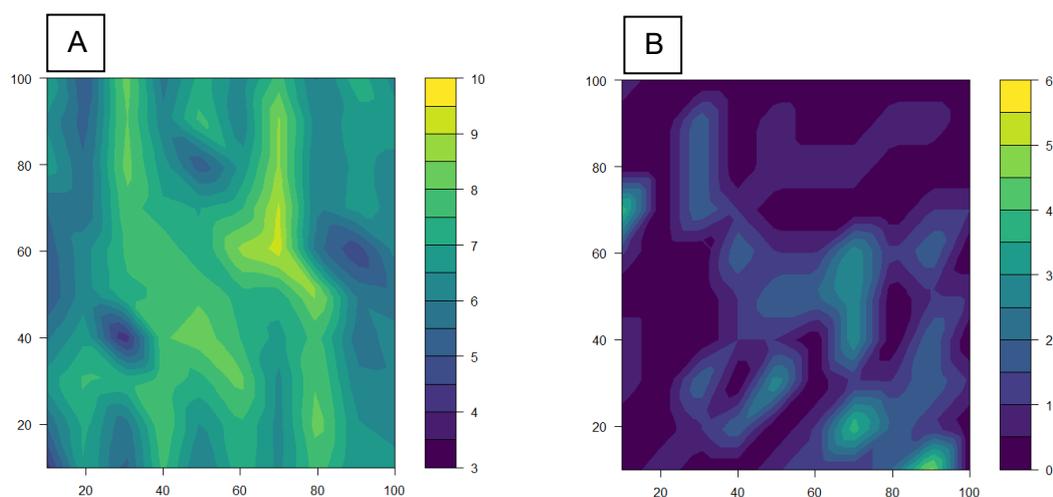


Figure 4.7. The percentage of organic matter (A) and an illustration of the distribution of slugs in Wigan (2-3-17) (B) at each point on the standard sampling grid. The x and y axes show dimensions of the sampling grid in metres. Slug distributions were determined from 10 assessments carried out between October 2016 and June 2017 using 100 refuge traps positioned at 10 metre intervals in the 10 by 10 grid. Soil samples for organic matter content were taken from the same positions. Colour scale represents the organic matter (A) and number of slugs (B), the numbers between traps was calculated by polynomial interpolation.

pH

The pH of individual soil samples in the six fields sampled varied from 5.2 (Oadby 2016-17 and Adeney (Middle) 2017-18) to 7.9 (South Kyme (1) 2016-17; Table 4.2). There was a significant difference in the pH level of the soil samples taken from areas of higher slug density when

compared to those with lower slug density at two of the sites investigated (Table 4.2). The mean pH of soil samples taken within slug patches (6.28) was found to be significantly higher than in those samples taken from areas with lower slug densities (6.16; $t=2.03$, d.f.=98, $p=0.045$). In Adeney (Middle) in the 2017-18 comparison of the spatial variation in soil pH across the sampling grid showed that the average pH in patches containing higher slug densities (6.32) was also significantly greater than in areas with lower slug densities (6.16; $t=2.51$, d.f.=98, $p=0.014$).

Moisture

Soil moisture was assessed at each node of the standard sampling grid, on each assessment visit to all six field sites. The individual soil moisture measurements recorded in the fields ranged from the lowest of 4 % (Wigan, 2016-17) to the highest of 69 % moisture content (South Kyme (1) 2016-17; Table 4.2). Soil moisture measurements varied between assessments, for example, at the Wigan site the mean soil moisture content was 27.7 % on 12-4-17, 9.5 % on 10-5-17 and 30.9 % on 8-6-17. No significant differences in either the mean soil moisture (mean for each point calculated from individual measurements from each assessment) or minimum soil moisture (minimum recorded for each point on the grid on any assessment date) assessments between the areas of higher or lower slug densities were identified (Table 4.2). A significant difference was detected between the maximum moisture content of the soil in areas of higher slug density (43.1 %) when compared to areas of lower slug density (40.6%) in a single site, Wigan (2017-18) ($t=2.76$, d.f.=98, $p=0.010$).

Soil texture

Due to limited resources soil texture analysis was carried out on a subset of samples in only one field (Adeney (Middle) 2016-17). There was a significant difference in the percentage of each particle size fraction between soil taken from areas containing higher slug densities and those with lower densities, clay ($t=3.12$, d.f.=21, $p=0.005$), silt ($t=3.41$, d.f.=21, $p=0.003$) and sand ($t=-3.89$, d.f.=21, $p<0.001$). The average proportion of clay in soils taken from within the area of higher slug density was significantly higher (41.3 %) than in those from areas with lower densities (37.5 %). Similarly, a higher proportion of silt was found in samples from within the area of higher slug density (25.5 %) compared to other areas (21.7 %). Conversely, the proportion of sand in the area of higher slug density was lower (33.2 %) compared to the area of lower slug density (40.8 %).

Bulk density

Bulk density recorded in individual samples from all six experimental fields ranged from 0.95 gcm^{-3} (South Kyme (1) 2016-17) to 1.79 gcm^{-3} (Uppington (2) 2017-18; Table 4.2). A significant difference in the bulk density of the soil was detected between areas of higher slug density compared to areas of lower slug density in only one field. At Oadby in the 2016-17 season the average bulk density of the soil in patches of higher slug density was 1.18 gcm^{-3} compared to 1.22

gcm^{-3} in the patches with a lower density ($t=-2.13$, $\text{d.f.}=98$, $p=0.036$). The bulk density measurements at Oadby covered the largest range of those recorded in all six study sites.

Particle density

A subset of samples were analysed for particle density, little variation between individual samples was detected, for example, the mean of 26 samples taken from Wigan 2017-18 was $2.41 \pm 0.03 \text{ gcm}^{-3}$. As the initial analysis indicated that the differences between samples ($\pm 0.03 \text{ gcm}^{-3}$) were less than the accuracy of the technique ($\pm 0.2 \text{ gcm}^{-3}$) and time resources were limited it was decided not to continue with particle density measurements at individual grid points. Instead sample points were collated from nine areas of the grid and the mean particle density for the field used in the calculation for soil porosity measurements.

Soil porosity

Soil porosity is a measure of the proportion of a defined volume of soil that is taken up by pores and can be calculated from measurements of bulk density and particle density, and as such is not independent of these two factors. The soil porosity recorded in individual soil samples taken from the six fields investigated ranged from 32.4 % (Adeney (Middle) 2017-18) to 65.0 % (Oadby 2016-17). A significant difference in the porosity of the soil was detected in areas of higher slug density compared to areas of lower slug density at one site, Oadby (2016-17). At Oadby the average soil porosity in patches of higher slug density was 50.8 % compared to 49.1 % in the areas with lower slug density ($t=2.08$, $\text{d.f.}=98$, $p=0.040$).

Infiltration rates

Infiltration rates were assessed in five of the fields investigated and outcomes ranged from the lowest individual assessment of 0 mms^{-1} (occurring in all fields except South Kyme (1) 2016-17) to the highest rate of 90 mms^{-1} (Uppington (2) 2017-18). No significant difference in the infiltration rate was detected in areas of higher slug density compared to areas of lower slug density in any of the fields tested.

4.4. Using Radio Frequency Identification tags to track the movement of *Deroceras reticulatum* above and below the soil surface

4.4.1. Laboratory experiments

Survival

Over the full experimental period a significantly lower survival rate of *D. reticulatum* was recorded in treatments in which RFID tags were implanted into slugs (T + CO₂ + G and T + CO₂) ($F=45.8$, $\text{d.f.}=4,10$, $p<0.001$; Figure 4.8). During the seven days after tag insertion, a mean of 5.8 ± 1.7 of the

20 slugs in the treatment groups with an implanted RFID tag (T + CO₂ + G and T + CO₂) died compared to an average of 0.9±0.3 slugs in each of the treatment groups with no RFID tag inserted (CO₂+, CO₂ and U) (Figure 4.8).

During the 14 days post-insertion, mortality had risen to 8.1±1.1 of the 20 slugs in groups with an RFID tag inserted (T + CO₂ + G and T + CO₂), and 1.3±0.4 of the 20 in those groups without tags (CO₂+, CO₂ and U). After day 15, slug survival was unaffected by the RFID tag insertion. Between day 15 and 28 there was no statistically significant difference in mortality recorded in different treatment groups (F=3.4, d.f.=4,8, p>0.05) irrespective of whether an RFID tag had been implanted. Mortality in both the tagged and untagged treatment groups was low from day 15 to 28, with a mean of 0.26 slugs per day dying in the treatment groups with an implanted RFID tag (T + CO₂ + G and T + CO₂) and 0.09 slugs per day in each of the treatment groups with no RFID tag inserted (CO₂+, CO₂ and U).

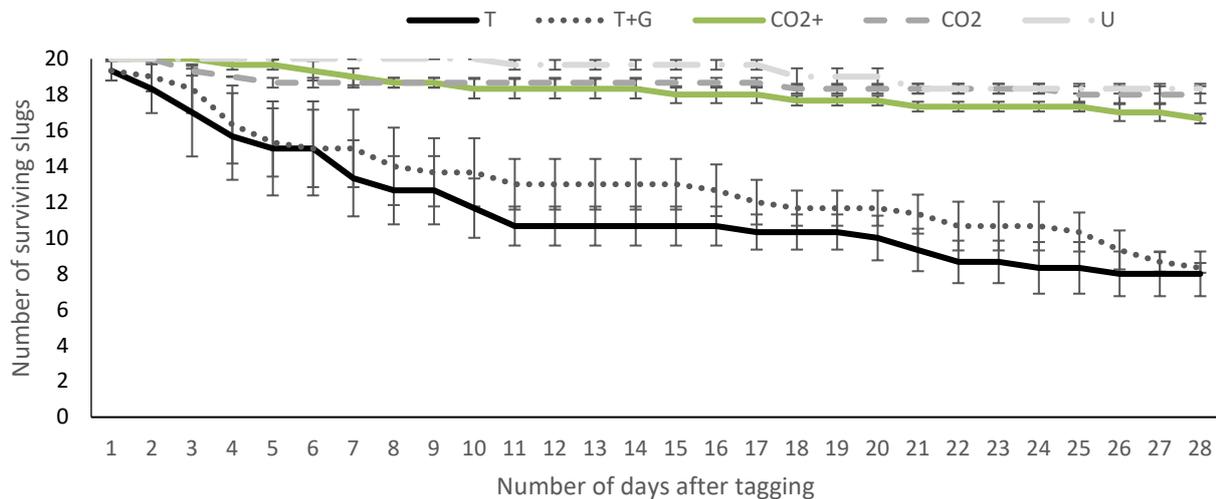


Figure 4.8. The effect of treatments on the survival of *Deroceras reticulatum* over three 28-day periods. Mortality was recorded daily for 28 days post-tag insertion. Points show the mean for the treatment group, bars show ± SE. (T (Tag inserted) + CO₂) Slug anaesthetised using CO₂, a hole made in the body wall using the tag implanter and an RFID tag injected. (T + CO₂ + G (Glue applied to insertion site)) Slug anaesthetised using CO₂ and with an RFID tag injected as in treatment Tag inserted + CO₂, but with the resultant hole in the body sealed with the addition of a small drop of glue (Loctite Precision Max, Loctite, USA). (CO₂+) Slug anaesthetised using CO₂, a hole made in the body wall using the tag implanter but without injection of an RFID tag. (CO₂) Slug anaesthetised using CO₂ only. (U) Untreated slug.

Lettuce consumption

Over the full experimental period a significantly lower daily consumption of lettuce was recorded in treatments in which RFID tags had been implanted into slugs (T + CO₂ + G and T + CO₂) (F=10.1, d.f.=4,1977, p<0.001). During the 7-day period after tag insertion, slugs consumed a mean of 0.03±0.03 g per day in the T + CO₂ treatment group and 0.05±0.03 g for the T + CO₂ + G treatment

group, compared to 0.14 ± 0.02 g for the control group (U), 0.11 ± 0.02 g for the CO₂+ treatment group and 0.11 ± 0.02 g for the CO₂ treatment group).

A significant interaction between treatment group and day was observed ($F=7.3$, $d.f.=4,1977$, $p<0.001$) indicating that the initial effect of treatment reduced over time. From day 15 to day 28 no significant difference in lettuce consumption was recorded between treatment groups irrespective of tagging status ($F=1.2$, $d.f.=4,960$, $p>0.05$), indicating a sustained and full recovery in food consumption rate by those tagged slugs that survived the procedure, occurred after an initial period of reduced intake.

Egg production

There was no statistically significant effect of treatment on the number of batches of eggs laid by slugs surviving the full 28-day experimental period over the first seven days ($F=0.7$, $d.f.=4,66$, $p>0.05$) or across the full 28 days ($F=2.3$, $d.f.=4,66$, $p>0.05$).

Locomotor behaviour

The mean distance travelled in the one-hour observation period by tagged and untagged slugs did not differ significantly in recordings made either 14, 21 or 28 days after tag insertion ($F=0.3$, $d.f.=1$, $p>0.05$; Figure 4.94.9(A)). No significant difference in the mean velocity was observed between tagged and untagged slugs in any of the experimental assessments made at 14, 21 and 28 days after tag insertion ($F=0.001$, $d.f.=1$, $p>0.05$; Figure 4.9 4.9(B)).

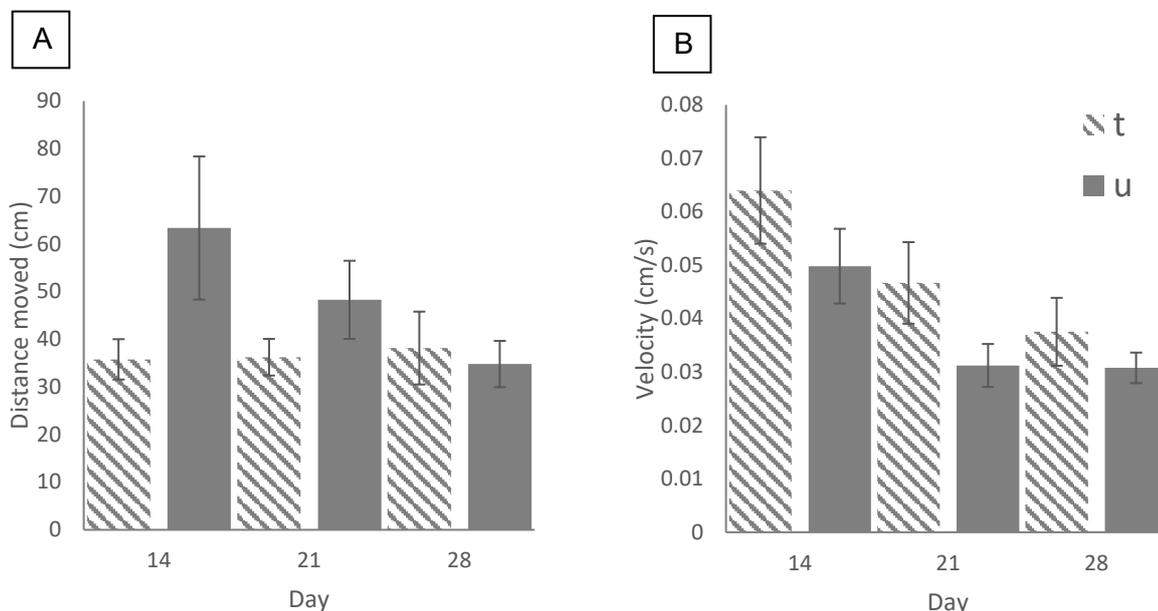


Figure 4.9. (A) Mean distance moved ($\text{cm} \pm \text{SE}$) and (B) mean velocity ($\text{cm s}^{-1} \pm \text{SE}$) around a circular (50 cm diameter) arena by 17 tagged (t) and 17 untagged (u) slugs on day 14, 21 and 28 after tag insertion.

4.4.2. In-field tracking

Following release into the field, slugs were readily detected when both above and below the soil surface. Tracking of slugs released in April 2017 was terminated after 38 days, whilst observations were made for 35 days following November releases.

April 2017

For the first 2 hours after release, eight of the nine slugs remained close (23.5 ± 7.3 cm) to the release point and tagged slugs were observed feeding and moving over the surface. The ninth slug was not detected again after release. The first observed tagged slug feeding occurred 35 minutes after release. Two slugs were no longer visible on soil surface 1-hour post-release with all being detected within the soil horizon during assessments made at 15 hours post-release. Of the nine slugs labelled with RFID tags that were released into the field, five were regularly detected for the duration of the full five-week experimental period. The five slugs monitored throughout that period were all recorded within a short distance of the original release point. The mean linear distance between detection points during the five weeks was 247 ± 31.4 cm, and at the end of the experiment, the mean total displacement from the initial release point was 78.7 ± 33.7 cm.

November 2017

During the three periods of intense monitoring (the night of release and following two nights) all twenty slugs remained close to their release point/first point of detection (43.3 ± 10.2 cm). During the three nights of intense monitoring, slugs were observed feeding, with the first observation occurring 24, 183 and 131 minutes after sunset respectively. In total 10, 5 and 10 slugs were observed feeding on at least one occasion during the respective monitoring periods. Thereafter, of the 20 slugs released, 18 were detected regularly during the five-week experimental period (Figure 4.10). The mean linear distance between detection points during the five weeks was 514.8 ± 72.0 cm, and at the end of the experiment the mean distance from the original release point was 101.9 ± 24.1 cm, with the maximum distance from the original release point being 408.8 cm.

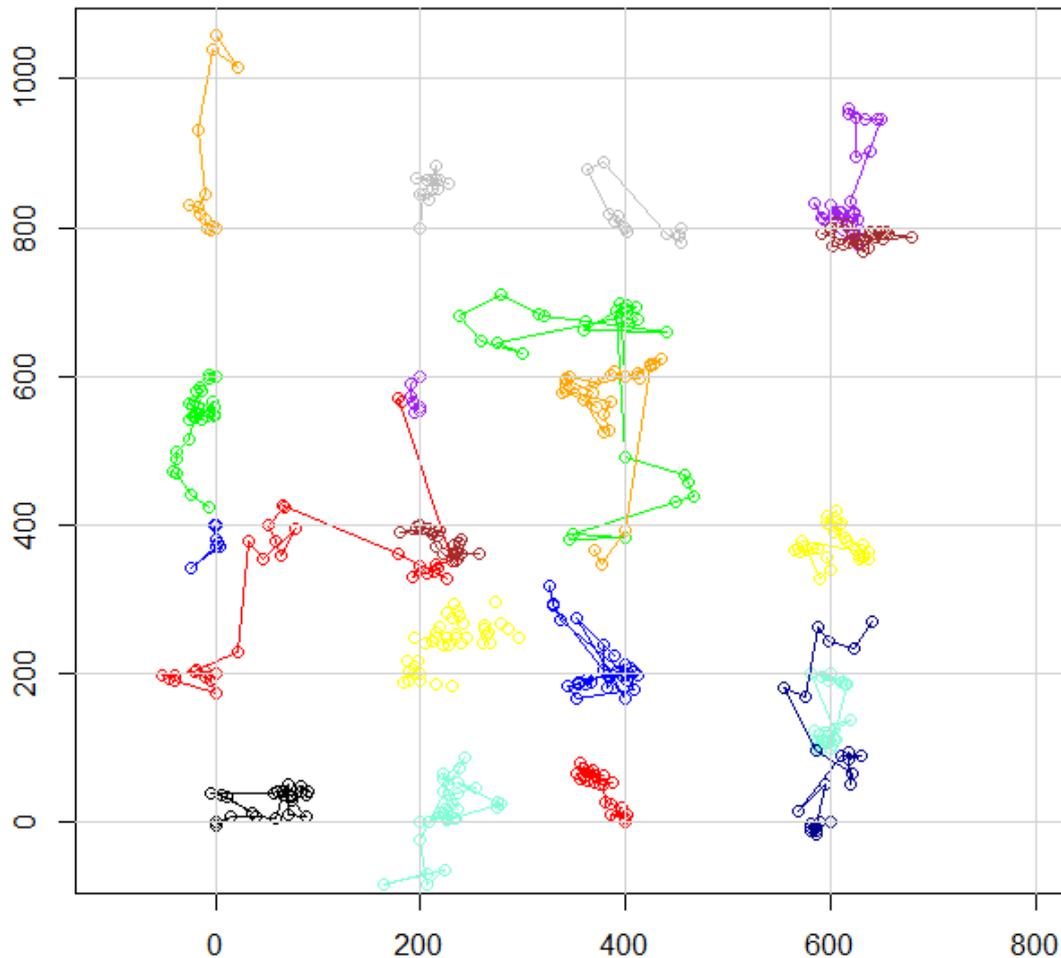


Figure 4.10. Map of *Deroceras reticulatum* movement in an 800 by 1000 cm area of a field in Shropshire over a five-week period from 15th November to 21st December 2017 using RFID technology to track and identify individuals. X and Y axis show distance (cm) from release point one (where the lines cross at 0, 0). Each circle shows a position where the slug was detected, the joining lines connect consecutive points along the slug's path but do not necessarily represent the route taken by the slug between points.

Effect of temperature and rainfall

April/May

Temperatures at the April/May 2017 field site (Figure 4.11 (A)) were 1.2 and 2.6°C higher respectively and rainfall lower by 24.3 and 15.3 mm respectively than the 30-year average (Met Office, 2018). Within the field study period, there were 25 consecutive days with no rainfall (< 1 mm). Slug movement between daily observations showed a significant but weak correlation with temperature (Pearson's correlation; $r=0.4$, $t=2.1$, $d.f.=28$, $p<0.05$, $R^2=0.1$) but no significant correlation with rainfall (Pearson's correlation; $r=-0.2$, $t=-1.1$, $d.f.=28$, $p>0.05$) (Figure 4.11 (A)).

November/December

The temperature (maximum and minimum within 0.6°C) during the two months of November/December (Figure 4.11 (B)) was similar to the 30-year average, rainfall was lower by 9.7 mm in November and higher by 15.7 mm December (Met Office, 2018). The number of slugs

active during the daily scotophase was not significantly correlated with the maximum temperature (Pearson's correlation; $r=0.4$, $t=1.9$, d.f. =21, $p>0.05$) but there was a significant weak correlation with daily rainfall (Pearson's correlation; $r=0.4$, $t=2.2$, d.f.=21, $p<0.05$). There was a period of snowfall, which remained on the ground from 8th – 15th December, coinciding with a period of low and declining slug activity (Figure 4.11(B)).

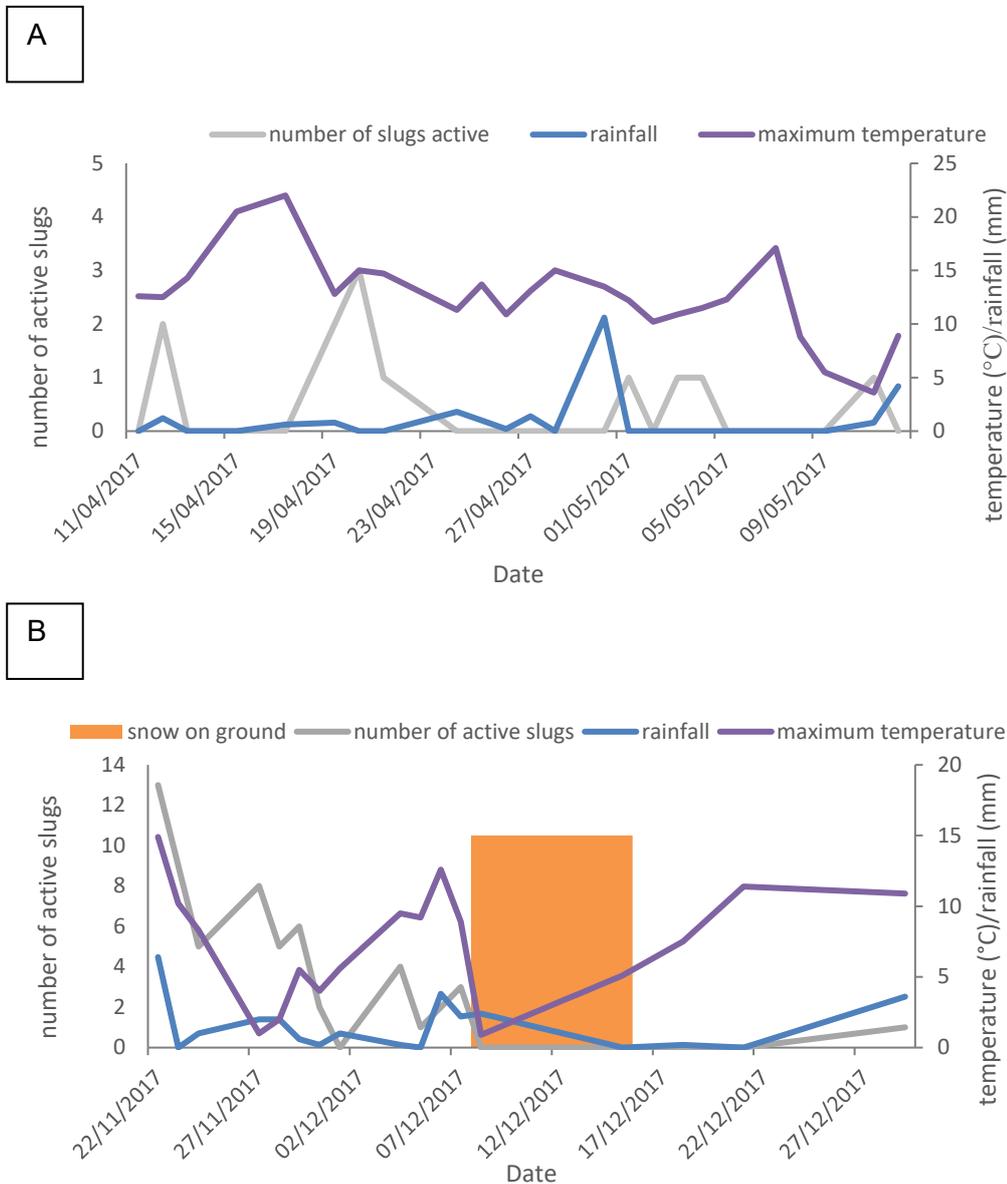


Figure 4.11. The number of active slugs overnight in relation to the maximum daily temperature (°C) and daily rainfall (mm) during (A) the five-week tracking period from 5th April 2017 and (B) the five-week tracking period from 20th November 2017.

5. Discussion

In commercial arable crops, the grey field slug (*Deroceras reticulatum*) is reported to display a discontinuous distribution whereby patches of higher numbers of slugs are distributed within areas of lower slug densities (South, 1992; Bohan *et al.*, 2000a; Archard *et al.*, 2004; Mueller-Warrant *et al.*, 2014). In response to increasing pressure to reduce pesticide usage, this report investigated the potential for targeting molluscicide application to these higher density patches of *D. reticulatum*. This was achieved by determining the temporal and spatial stability of the high slug density patches, assessing the locomotory behaviour of *D. reticulatum* which underlies these discontinuous distributions observed in arable crops, and identifying some physical soil characteristics which could influence patch location. In this chapter the results and their implications are discussed in relation to commercial control of slugs, together with the limitations of the study and future work required before a commercially acceptable procedure can be developed to implement the findings.

5.1.1. Potential for patch application of molluscicides

The discontinuous distribution of slug populations in arable crops has been established in both North America and Europe, but little information is available on the temporal and spatial stability of the resultant patches of higher slug numbers. Mueller-Warrant *et al.* (2014) investigated the distribution of slugs, and reported that where numbers were highest (maximum mean number of slugs assessed using surface refuge traps of between 7.9 and 21.1 per trap) significant aggregations appeared in the same area of the field on different assessment dates, suggesting stable patches occurred. In the two field sites where the highest trap counts were below 3 slugs per trap (2.8 and 2.3), however, no stable areas of higher slug densities were detected (Mueller-Warrant *et al.*, 2014). In the current study, an analogous discontinuous distribution of slugs was observed in all fields in which the slug population was sufficiently large for statistically significant differences between high and low trap counts to be distinguished (approximate mean of 3 slugs per trap). In these fields, the patches that were defined by grid sampling were found to be spatially stable throughout the cropping season. Spatial stability is key if patch application (applying molluscicides only to areas of fields where higher slug densities occur) is to be successful, once identified growers need confidence that the area identified as having a higher density of slugs will remain in the same location throughout the susceptible crop growth stages.

Identification of stable patches was dependant on the number of slugs present suggesting that when using the trapping grid method, a threshold for reliable patch location of approximately 3-4 slugs per trap is required to accurately locate higher density patches. In all fields with a mean trap count of 4 slugs per trap or above, spatially and temporally (across the cropping season) stable patches were identified. The difficulties associated with detecting areas of higher slug densities in the fields with low populations of surface-active slugs (in the region of 3-4 slugs per trap and

below) may not indicate that in these areas they were not present, only that they could not be accurately identified with the methods of refuge trapping used in this study (Petrovskaya *et al.*, 2018). In future research the patch stability detected in both the current work and that of (Müller-Warrant *et al.*, 2014), may reduce difficulties associated with the requirement for a minimum level of slug activity on the soil surface before accurate patch location can be achieved. As patches remained in similar positions for extended periods in the crops studied, mapping may be achieved with as little as one assessment. As weather exerts a significant effect on slug behaviour (South, 1992; Choi *et al.*, 2004), days with optimum conditions could be selected for this assessment. Commercial confidence in such research may benefit from the current AHDB recommended threshold for pellet application in standing cereal crops, a mean of 4 slugs per trap from traps distributed across the field (AHDB, 2016). Future assessment of patch location in research developing patch treatment techniques would be undertaken at population levels close to those at which decisions on pellet application are made.

If patches with higher slug densities are to be used for targeted molluscicide applications, then an understanding of the biological and behavioural mechanisms underpinning their formation and coherence is essential if reliance on spatial correlation (with associated risks) is to be avoided. Investigation of slug locomotory behaviour and dispersion in the field has been hampered by their vertical distribution above and below the soil surface. Various methods have been developed for assessing slug numbers in both locations (surface refuge traps, soil flooding, DATs; South, 1992, dye marking; Hogan and Steele, 1986, UV dye; Foltan and Konvicka, 2007), but movement between these horizons has made collection of detailed data on the locomotory behaviour of individual animals over extended periods of time difficult.

The development of RFID technology offered a new method of identifying individual slugs using tags attached to the body surface or inserted into the body cavity (Grimm, 1996). The first use in the field was in a study of *Arion lusitanicus*, in which tagged slugs were released into grassland, demonstrating the potential for identifying individuals over extended periods of time (Grimm and Paill, 2001). The method was subsequently adopted for the assessment of field survival rates and locomotor activity of *Arion* spp. but in both cases as a mark-recapture technique rather than for tracking individual movement (Ryser *et al.* 2011, Knop *et al.* 2013), it was also concluded that damage caused when attaching tags to smaller species such as *D. reticulatum* may result in significant mortality or behavioural modification in survivors.

The current work extended the use of the technology by developing and testing a process by which tags could be inserted into the body cavity of *D. reticulatum* without affecting subsequent behaviour of survivors selected for use in experimental work. The method was used to investigate behavioural traits that result in the formation and coherence of slug patches in arable fields. RFID tracking in the current study yielded a higher “recapture” rate than reported by Grimm and Paill (2001) for both spring and autumn releases. This higher rate could be due to the larger read range of modern equipment; Grimm and Paill (2001) used a device with a read range up to approximately

15 cm (when there was no a barrier between tag and device) compared to a range of up to 20 cm depth of soil in this study.

Individual slugs were successfully tracked for up to 5 weeks in this study and showed that they did not return to the same refuge each night, confirming the finding of Hommay *et al.* (1998) that slugs were using the same refuge for a maximum of two consecutive nights. Despite this, the lateral dispersion of individual slugs was low. Although 20% of tracked individuals moved away and were lost after release, the mean linear displacement of the remaining 80% was only 78.7 cm (April 2017) and 101.9 cm (November 2017) from the initial release point after the 5-week period. Such a low linear displacement will result in retention of slugs in areas offering favourable soil conditions, supporting formation and cohesion of higher density patches, which in turn will be reinforced by the known slime trail following behaviour displayed by the species (Rollo and Wellington, 1981). The limited dispersion of slugs during the two tracking periods supports the work carried out looking at patch stability, providing improved understanding of a mechanism leading to areas of higher slug density remaining in the same location for extended periods of time.

The low proportion of slugs that quickly moved away from the initial release point in the current field study was similar to the 27 % of slugs reported to have left the study area established in previous work (Grimm and Paill, 2001). Such dispersers may contribute to the inter-change of individuals between the discrete slug patches, and tracking these individuals, may allow conclusions to be drawn on their importance for patch cohesion and possibly the establishment of new slug patches.

5.1.2. Current limitations for patch application of molluscicides

Refuge trapping is a method of assessing surface activity of slugs rather than providing absolute population estimates (Hommay *et al.*, 2003), their use for identifying the location of slug patches in a commercially viable integrated pest management system is therefore limited. Crop damage is caused by slug grazing on plants above the soil surface, and in some crops on the seeds in the soil (Glen *et al.*, 1990; South, 1992). As slug populations are distributed between the surface and the upper horizons of the soil and the proportion in each varies with time due to the effect of a range of environmental factors, trapping methods that focus on surface activity can result in inaccurate assessments of population distribution across arable fields, particularly if reliant on a single or few assessment dates. In addition, based on the size and distribution of patches identified in the current study, models have indicated that the density of trapping points required in arable crops would preclude economic viability (Petrovskaya, 2018).

Two alternative methods for locating the areas of higher slug densities were investigated during this study. Anecdotal evidence from growers (P. Jackson, Pers. Comm.) suggest that parts of fields in which higher crop damage is more regularly observed indicate areas at generally increased risk from slug activity. Identification of such areas allows either the targeting of standard slug pellet

applications to areas where they may have maximum impact, or the view is sometimes expressed by some farmers that they are best applied at a higher rate in these areas. The results from this study and the similarly weak correlation between post emergence plant density and slug numbers reported from North America (Mueller-Warrant *et al.*, 2014) support the conclusion that plant damage may not be a satisfactory assessment method for targeted pesticide applications. Damage from slugs before a crop emerges can prevent germination and reduce plant density (Glen *et al.*, 1993). Leaf shredding during the early growth stages in patches with high numbers of slugs can also result in lower plant densities. A reduction in plant density from slug damage either pre emergence or at early growth stages may potentially result in greater proportional damage to remaining plants in such patches when compared to surrounding areas where feeding has not occurred, combined with the ability of the crop to rapidly produce new leaves when actively growing (AHDB, 2018), the value of crop damage as an indicator of slug activity is limited. There are several possible causes of poor establishment beside slug damage, for example, seed bed conditions or disease (AHDB, 2018). Environmental factors will affect crop growth (AHDB, 2018), slug population size (Port and Port, 1986; Willis *et al.*, 2008) and different rates of feeding (Wareing and Bailey, 1986), which may also lead to variations in visible damage and weaker correlations with slug numbers.

5.1.3. Edaphic factors and areas of higher slug densities

The investigation of the possible use of soil characteristics to identify the locations in which slug patches may form was more successful and shows potential. Several soil characteristics, pH, soil moisture, bulk density (and associated porosity), soil texture and organic matter, were identified in this study as factors which could influence the distribution of slugs in arable fields and will be further investigated during a future AHDB funded project.

The importance of soil moisture for surface activity has been demonstrated by several authors (South, 1992, Shirley *et.al*, 2001; Choi *et.al*, 2004), with slugs showing a preference for damp but not waterlogged soils (Carrick, 1942; Young and Port, 1991; Glen and Symondson, 2003). Laboratory experiments in this study supported these findings, but field experiments resulted in significant differences between areas with high or low slug densities being identified at only a single site. Soil moisture levels are affected by a range of environmental conditions and are highly variable over relatively short periods of time, potentially making direct measurement inappropriate for work relating slug patch location to this factor, soil characteristics that affect water retention may be more suitable candidates for procedures identifying the location of slug patches. Factors affecting water retention include organic matter, soil texture, bulk density and infiltration.

Decomposing plant matter in the soil can affect soil properties in addition to providing a food source for slugs. Higher organic matter content can improve the water holding capacity of the soil (Franzluebbers, 2002) and improve soil structure (Boekel, 1963; Hillel, 2008) both of which are

important for slugs. Slugs are unable to regulate their own body moisture and so rely on water in their environment (South, 1992), therefore, increased water retention in the soil during dry conditions would be favourable for slugs. Increased organic matter can improve soil structure, reducing bulk density of the soil and provide access to refuges within the upper soil horizons, which is important to slugs during adverse environmental conditions or for reducing the risk of predation. The results of earlier studies showing preferences for soils with higher organic matter content were not replicated in laboratory experiments in this work, however, this was possibly a result of the type of organic matter used. Significant effects demonstrated at one of the field sites and the results of other published work supports its inclusion in future work.

Soil texture (percentage of sand, silt and clay) were only assessed at a single site in this study, but a significant difference between the level of each of the three size fractions was found to occur between areas of the field with patches of higher and lower slug densities. Higher clay or silt content and lower sand content in soil will result in increased water retention making them less prone to drying (Rice, 2002; Hillel, 2008). Published evidence suggests that slugs display a preference for heavier soils with a higher clay content, partly because of the higher moisture retention characteristics (Gould, 1961; South, 1992; Ondina *et.al*, 2004; AHDB, 2016). Soil texture remains a candidate for investigation in future research.

Bulk density and associated porosity are linked to soil texture, organic matter content and compaction resulting from weather at the time of cultivation and cultivation method (Franzluebbers, 2002; Nimmo, 2004; Chaudhari *et.al*, 2013; Kalev and Toor, 2018). Soils with higher bulk densities inhibit the ability of slugs to move through the soil profile, with fewer cracks resulting in fewer available refuges and increased slug mortality (Stephenson, 1975; Kozłowski and Pallardy, 1997; Shirley *et.al*, 2001). Field experiments under the current project yielded limited evidence of significant impact of bulk density on the location of patches of higher slug density, but the factor requires further research to fully understand its role.

Infiltration rate was not found to be significantly related to slug patch location in any of the fields investigated in this study. The method used for assessment of this factor (simplified falling head method) may have contributed to this outcome due to the high variation in measurements. The alternative techniques require longer recording time, making infiltration a less suitable characteristic for determining the location of areas of higher slug densities in commercial crops.

No individual soil characteristic was strongly related to the location of slug patches in the six fields studied in this work programme. With the exception of soil pH, those factors for which a significant relationship was established with the distribution of slugs in at least one field site may affect soil moisture or the ability of slugs to move into and through the soil profile (potentially providing them a refuge from adverse environmental conditions of predators). The lack of a single factor that was related to patch location in all or most of the fields studied suggests, however, that such relationships are complex and it is likely that a combination of edaphic factors will influence the location of higher density slug patches.

5.1.4. Potential for development/uptake of targeted application for slug control

New technology for precision farming is emerging onto the market, for example, Terramap, a system that maps up to 21 different soil characteristics in arable fields has recently been released (Hutchinsons Ltd, 2016). These characteristics include several which were identified in this study as potential candidates for locating areas of higher slug densities, including pH, texture, organic matter and moisture. The system currently produces maps using 800 sampling points per hectare, a density that would create a finer grid than used in this study and so has potential for adaptation for the purpose of slug patch location. The evidence suggests that the use of precision agriculture techniques is increasing in England, a survey of over 2800 farmers in 2012 reported an increase in the use of GPS (14 to 22 %), soil mapping (14 to 20 %), variable rate applications (13 to 16 %) and yield mapping (7 to 11 %; Defra, 2013). The trend of increased uptake of precision agriculture practices continued in the 2018 survey (>2500 farmers), with 17 % of farmers having introduced new precision agriculture technologies in the previous 12 months and 7 % of farmers intending to adopt further new techniques in the following 12 months (Defra, 2019). The adoption of soil maps for the purposes of pest management has already been used for targeting nematicides for potato cyst nematode control (Perry *et al.*, 2006), PCN are a relatively immobile pest compared to many insect pests, such as aphids and beetles facilitating targeted control (Godwin and Miller, 2003). Given the adoption of precision agriculture is increasing, soil characteristics identified in this report are currently being mapped for other purposes (offering potential for cost sharing between different on-farm tasks) and sufficient stability of areas of higher slug densities has been confirmed, a well-researched approach for slug control may be of interest to the industry.

5.1.5. Future work

The development of a new IPM system for slug control relies initially on an improved understanding of the interactions between the various candidate factors identified in the current study, their relationship with and impact on slug biology and behaviour, and the establishment of a combination of stable soil characteristics that strongly identifies the location of patches of higher slug density.

The work in this report has primarily concentrated on wheat and OSR crops, however, the combination of soil characteristics which determine the location of the higher slug densities may also apply to other crops, for example, potatoes, brassicas, lettuce, asparagus and strawberries where slug damage is also economically important (Speiser *et al.*, 2001; The Andersons Centre, 2014). Further work would be required to confirm this hypothesis. In potato crops, for example, the creation of ridges for planting alters the field environment (Stalham and Allison, 2015). Cultivation method and seed bed conditions are known to affect the number of slugs (Glen and Symondson, 2003), by creating a non-uniform surface, drier ridges with fine soil tilth and furrows with a higher

moisture content. These differences would need to be investigated in potato crops in relation to slug distribution and movement to confirm whether the same method of locating patches can be used.

Between-season stability was not confirmed in this study, with limited data for multiple field seasons due to the low number of slugs detected in some fields. The data available makes a clear conclusion on patch stability between seasons difficult. There are also several alternative explanations for this which require further investigation, such as cultivation method, effect of compaction, natural changes in the soil properties or changing distribution of natural enemies. Understanding the inter-season stability of the areas of higher slug densities remains an important area for future work.

5.1.6. Potential for pesticide reduction

Following the removal of methiocarb (HSE, 2014) from the European market and the uncertain future of metaldehyde (Appleby, 2019; Pickstone, 2019) it is increasingly important that the remaining active ingredient, ferric phosphate (Defra, 2018) is used as sustainably as possible. Sustainable use can be promoted by optimising pesticide use through targeted application, bringing potential environmental benefits and possibly some direct savings to the grower. In addition to reducing the environmental effects of active ingredients it may also improve the cost effectiveness of alternative options such as the use of nematodes. Currently nematodes are not considered to be a viable option in many crops such as wheat and oilseed rape. Although unlikely to solve this issue in isolation, targeted application of the products may improve cost-benefit calculations.

The size of the slug patches detected, using a 100 m by 100 m grid, in this study varied between fields from 300 to 7000 m², in order to fully establish the mean size and number of these patches in arable fields, and so potential pesticide reductions, further work would need to be done.

6. References

AHDB. 2010. *Slug control*. [Online] AHDB. Available at:

<http://www.ahdb.org.uk/projects/slugcontrol.aspx> [Accessed 9th October 2015]

AHDB. 2014. *Encyclopaedia of pests and natural enemies in field crops*. [ONLINE] AHDB.

Available at: <https://ahdb.org.uk/knowledge-library/encyclopaedia-of-pests-and-natural-enemies>

[Accessed 9th October 2015]

- AHDB. 2016. *Integrated slug control*. [ONLINE] AHDB. Available at: <https://cereals.ahdb.org.uk/publications/2016/april/18/integrated-slug-control.aspx> [Accessed 27th July 2019]
- AHDB. 2018. *Wheat growth guide*. [ONLINE] AHDB. Available at: <https://cereals.ahdb.org.uk/media/185687/g66-wheat-growth-guide.pdf> [Accessed 27th July 2019]
- Appleby, M. 2019. *Government withdraws metaldehyde slug killer ban after "concerns raised about decision-making process"*. [ONLINE] Horticulture Week. Available at: <https://www.hortweek.com/government-withdraws-metaldehyde-slug-killer-ban-concerns-raised-decision-making-process/ornamentals/article/1592601> [Accessed 24th August 2019]
- Archard, G. A., Bohan, D. A., Hughes, L and Wiltshire, C. W. 2004. Spatial sampling to detect slug abundance in an arable field. *Annals of Applied Biology*, 145, pp.165-173.
- Bagarello, V., Iovino, M. and Elrick, D. 2004. A simplified falling-head technique for rapid determination of field-saturated hydraulic conductivity. *Soil Science Society of America Journal*, 68(1), pp.66-73.
- Boekel, P. 1963. The effect of organic matter on the structure of clay soils. *Netherlands Journal of Agricultural Science*, 11, pp.250-263.
- Bohan, D., Glen, D., Wiltshire, C. and Hughes, L. 2000a. Parametric intensity and spatial arrangement of the terrestrial mollusc herbivores *Deroceras reticulatum* and *Arion intermedius*. *Journal of Animal Ecology*, 69, pp.1031–1046.
- Bohan, D., Bohan, A., Glen, D., Symondson, W., Wiltshire, C. and Hughes, L. 2000b. Spatial dynamics of predation by carabid beetles on slugs. *Journal of Animal Ecology*, 69, pp.367–379.
- Carlile, B. 2006. *Pesticide selectivity, health and the environment*. Cambridge University Press.
- Carrick, R. 1942. The grey field slug *Agriolimax agrestis* L., and its environment. *Annals of Applied Biology*, 29(1), pp.43-55.
- Chaudhari, P.R., Ahire, D.V., Ahire, V.D., Chkravarty, M. and Maity, S. 2013. Soil bulk density as related to soil texture, organic matter content and available total nutrients of Coimbatore soil. *International Journal of Scientific and Research Publications*, 3(2), pp.1-8.

Choi Y, Bohan D, Powers S, Wiltshire C, Glen D, Semenov M. 2004. Modelling *Deroceras reticulatum* (Gastropoda) population dynamics based on daily temperature and rainfall. *Agriculture, Ecosystems and Environment*, 103, pp.519–525.

Choi, Y. H., Bohan, D. A, Potting, R. P. J., Semenov, M. A. and Glen, D. M. 2006. Individual based model of slug population and spatial dynamics. *Ecological Modelling*, 190, pp.336-350.

Clark, P.J. and Evans, F.C. 1954. Distance to nearest neighbour as a measure of spatial relationships in populations. *Ecology*, 35(4), pp.445-453.

Clarke, A. 2014. *EU votes to ban methiocarb slug pellets*. [Online] Farmers Weekly. Available at: <http://www.fwi.co.uk/arable/eu-votes-to-ban-methiocarb-slug-pellets.htm> [Accessed 12 October 2015]

Cook, R.T. 2004. The tolerance of the field slug *Deroceras reticulatum* to freezing temperatures. *Cryo Letters*, 25(3), pp.187-194.

Council Directive 91/414/EEC of 15 July 1991 concerning the placement of plant protection products on the market. [Online] *Official Journal of the European Union* L 230/1. Available at: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0414&from=en> [Accessed 9th June 2016]

Council Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. [Online] *Official Journal of the European Union* L 327/1. Available at: http://eur-lex.europa.eu/resource.html?uri=cellar:5c835afb-2ec6-4577-bdf8-756d3d694eeb.0004.02/DOC_1&format=PDF [Accessed 9th June 2016]

Council Directive 2006/118/EC of the of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration. [ONLINE] *Official Journal of the European Union* L 372/19. Available at: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:372:0019:0031:EN:PDF> [Accessed 3rd September 2019]

Council Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for community action to achieve the sustainable use of pesticides. [Online] *Official Journal of the European Union* L 309/71. Available at: <http://eur->

lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02009L0128-20091125&from=EN
[Accessed 27th August 2016]

Defra. 2013. *Farm Practices Survey Autumn 2012 – England*. [ONLINE] National Statistics
Available at:

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/181719/defra-stats-foodfarm-environ-fps-statsrelease-autumn2012edition-130328.pdf [Accessed 9th August 2019]

Defra. 2018. *Restrictions on the use of metaldehyde to protect wildlife*. [Online] UK Government.
Available at: <https://www.gov.uk/government/news/restrictions-on-the-use-of-metaldehyde-to-protect-wildlife> [Accessed 2nd May 2019]

Defra. 2019. *Farm Practices Survey 2018 – England*. [ONLINE] National Statistics. Available at:
https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/792165/fps-general-statsnotice-4apr19.pdf [Accessed 9th August 2019]

Foltan, P. and Konvicka, M. 2008. A new method for marking slugs by ultraviolet-fluorescent dye. *Journal of Molluscan Studies*, 74, pp.293–297.

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, pp.89-96.

Franzluebbers, A. 2002. Water infiltration and soil structure related to organic matter and its stratification with depth. *Soil and Tillage Research*, 66(2), pp.197–205.

Garthwaite, D., Barker, I., Ridley, L., Mace, A., Parrish, G., MacArthur, R. and Lu, Y. 2018. *Pesticide Usage Survey Report 271; Arable crops in the UK 2016*. [ONLINE] Fera. Available at:
<https://secure.fera.defra.gov.uk/pusstats/surveys/documents/arable2016-v9.pdf> [Accessed 20th April 2019]

Getz, L. L. 1959. Notes on the Ecology of Slugs: *Arion circumscriptus*, *Deroceras reticulatum*, and *D. laeve*. *American Midland Naturalist*, 61(2), p.485.

Glen, D.M., Milsom, N.F. and Wiltshire, C.W. 1990. Effect of seed depth on slug damage to winter wheat. *Annals of Applied Biology*, 117(3), pp.693-701.

Glen, D.M., Spaul, A., Mowat, D., Green, D. and Jackson, A. 1993. Crop monitoring to assess the risk of slug damage to winter wheat in the United Kingdom. *Annals of Applied Biology*, 122 (1), pp. 161-172.

Glen, D.M., El Titi, A., Katur, K., Buchs, W., Kreye, H., Ulber, B., Worz, M. 2003. *Further development of farmer-friendly methods for estimating slug infestation incidence in soil and damage risk to oilseed rape. Final Project Report.* [Online] UFOP. Available at: <https://www.ufop.de/files/9013/3922/7287/FurthrFarmerFriendly.pdf> [Accessed 12th May 2019]

Glen, D.M. and Symondson, W.O. 2003. Influence of soil tillage on slugs and their natural enemies. In: El Titi, ed. *A Soil tillage in agroecosystems*. Florida: CRC Press. Pp.207-227.

Godwin, R.J. and Miller, P.C.H. 2003. A review of the technologies for mapping within-field variability. *Biosystems Engineering*, 84(4), pp.393-407.

Gould, H. J. 1961. Observations on slug damage to winter wheat in East Anglia, 1957-1959. *Plant Pathology*, 10(4), pp.142–146.

Grimm, B. 1996. A new method for individually marking slugs (*Arion lusitanicus* (Mabille)) by magnetic transponders. *Journal of Molluscan Studies*, 62, pp.477-482.

Grimm, B. and Paill, W. 2001. Spatial distribution and home-range of the pest slug *Arion lusitanicus* (Mollusca: Pulmonata). *Acta Oecologica*, 22(4), pp.219-227.

Health and Safety Executive. 2014. *Withdrawal of Plant protection Products in the United Kingdom; Plant Protection Products Regulation (EC) no 1107/2009; Step 1 withdrawal notice – methiocarb.* [Online] UK Government. Available at: <https://secure.pesticides.gov.uk/pestreg/getfile.asp?documentid=23563> [Accessed 20th September 2017]

Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J., Aupinel, P., Aptel, J., Tchamitchian, S. and Decourtye, A. 2012. A common pesticide decreases foraging success and survival in honey bees. *Science*, 336, pp.348-350.

Hillel, D. 2008. *Soil in the Environment*. London: Elsevier.

Hillocks, R. 2012. Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, 31, pp.85-93.

Hogan, J. and Steele, G. 1986. Dye-marking slugs. *Journal of Molluscan Studies*, 52(2), pp.138-143.

Hommay, G., Lorvelec, O. and Jacky, F. 1998. Daily activity rhythm and use of shelter in the slugs *Deroceras reticulatum* and *Arion distinctus* under laboratory conditions. *Annals of Applied Biology*, 132, pp.167-185.

Hommay, G., Kienlen, J.C., Jacky, F. and Gertz, C. 2003. Daily variation in the number of slugs under refuge traps. *Annals of Applied Biology*, 142(3), pp.333-339.

Hutchinsons Ltd. 2016. *TerraMap*. [ONLINE] Hutchinsons Ltd. Available at: <https://www.omniaprecision.co.uk/terramap/> [Accessed 8th August 2019]

Jessop, H. 1969. The effects of simulated slug damage on the yield of winter wheat. *Plant Pathology*, 18(4), pp.172-175.

Kalev, S and Toor, G. 2018. The Composition of Soils and Sediments. In: Torok, B and Dransfield, T. ed. *Green Chemistry: an inclusive approach*. Massachusetts: Elsevier. Pp.339-357.

Kennedy, T.F., Connery, J., Fortune, T., Forristal, D. and Grant, J. 2013. A comparison of the effects of minimum-till and conventional-till methods, with and without straw incorporation, on slugs, slug damage, earthworms and carabid beetles in autumn-sown cereals. *Journal of Agricultural Science*, 151(5), pp.605-629.

Knop, E., Rindlisbacher, N., Ryser, S. and Gruebler, M. 2013. Locomotor activity of two sympatric slugs: implications for the invasion success of terrestrial invertebrates, *Ecosphere*. 4: article 92.

Kozlowski, T.T. and Pallardy, S.G. 1997. *Growth control in woody plants*. Elsevier. Pp.277-279.

Met Office. 2018. *Newport (Salop) Climate*. [Online] MetOffice. Available at: <https://www.metoffice.gov.uk/public/weather/climate/gcck28u76> [Accessed 6th November 2018]

Meir, E. and Fagan, W.F. 2000. Will observation error and biases ruin the use of simple extinction models? *Conservation Biology*, 14(1), pp.148-154.

Ministry of Agriculture. 1986. *The Analysis of Agricultural Materials*. Third edition. London: Her Majesty's Stationary Office.

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, pp.4–13

Nimmo, J.R. 2004. *Porosity and pore size distribution*. In: Hillel, D. ed., *Encyclopedia of Soils in the Environment*, vol. 3. London: Elsevier. Pp.295-303.

Ondina, P., Hermida, J., Outeiro, A. and Mato, S. 2004. Relationships between terrestrial gastropod distribution and soil properties in Galicia (NW Spain). *Applied Soil Ecology*, 26(1), pp.1-9.

Perry, C., Vellidis, G., Sullivan, D.G., Rucker, K. and Kemerait, R. 2006, January. Predicting nematode hotspots using soil EC data. In *Proc. 2006 Beltwide Cotton Conference*. National Cotton Council San Antonio, TX.

Petrovskaya, N., Petrovskii, S. and Murchie, A.K. 2012. Challenges of ecological monitoring: estimating population abundance from sparse trap counts. *Journal of The Royal Society Interface*, 9(68), pp.420-435.

Petrovskaya, N.B., Forbes, E., Petrovskii, S.V., Walters, K.F.A. 2018. Towards the development of a more accurate monitoring procedure for invertebrate populations, in the presence of an unknown spatial pattern of population distribution in the field. *Insects*, 9, p.29

Pickstone, S. 2018. *DEFRA to reconsider future use of metaldehyde after legal challenge*. [ONLINE] Haymarket Media Group Ltd. Available at: <https://www.endsreport.com/article/1592611/defra-reconsider-future-use-metaldehyde-legal-challenge> [Accessed 24th August 2019]

Pope, T., Gundalai, E., Elliott, L., Blackshaw, R., Hough G., Wood, A., Bennison, J., Prince, G. and Chandler, D. 2015. Recording the movement of adult vine weevil within a strawberry crop using radio frequency identification tags. *Journal of Berry Research*, 5, pp.197-206.

Port, C.M., and Port, G. R. 1986. The biology and behaviour of slugs in relation to crop damage and control. *Agricultural Zoology Reviews*, 1, pp.255-299.

R Core Team. 2013. *R: A language and environment for statistical computing*. R Foundation for statistical computing, Vienna, Austria. [ONLINE] The R Foundation. Available at: <http://www.R-project.org/> [Accessed 23rd August 2019]

Ramsden, M., Kendall, S.L., Ellis, S.A. and Berry, P.M. 2017. A review of economic thresholds for invertebrate pests in UK arable crops. *Crop Protection*. 96, pp.30-43.

Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing council directives 79/117/EEC and 91/414/EEC. [ONLINE] *Official Journal of the European Union* L 309/1. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32009R1107> [Accessed 3rd September 2019]

Regulation (EC) No.1185/2009 of the European Parliament and of the Council of 25 November 2009 concerning statistics on pesticides. [ONLINE] *Official Journal of the European Union* L 324/1. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32009R1185> [Accessed 3rd September 2019]

Regulation (EC) No. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. [ONLINE] *Official Journal of the European Union* L 353/1. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008R1272> [Accessed 3rd September 2019]

Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. [ONLINE] *Official Journal of the European Union* L 70/1. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0396&from=EN> [Accessed 3rd September 2019]

Rice, T. 2002. Importance of soil texture to vineyard management. *Practical Winery and Vineyard Journal*, March/April 2002, [ONLINE] Practical Winery. Available at: https://digitalcommons.calpoly.edu/cgi/viewcontent.cgi?referer=https://scholar.google.co.uk/&httpsredir=1&article=1031&context=ersc_fac [Accessed 12th April 2019]

Rollo, D. and Wellington, W. 1981. Environmental orientation by terrestrial mollusca with particular reference to homing behaviour. *Canadian Journal of Zoology*, 59, pp.225-2339.

- Roussel, J., Haro, A. and Cunjak, R.A. 2000. Field test of a new method for tracking small fishes in shallow rivers using passive integrated transponder (PIT) technology. *Canadian Journal of Fisheries and Aquatic Sciences*, 57, pp.1326-1329.
- Ryser, S., Rindlisbacher, N., Gruebler, M. and Knop, E. 2011. Differential survival rates in a declining and invasive farmland gastropod species. *Agriculture, Ecosystems and Environment*, 144, pp.302-307.
- Shirley, M.D., Rushton, S.P., Young, A.G. and Port, G.R. 2001. Simulating the long-term dynamics of slug populations: a process-based modelling approach for pest control. *Journal of Applied Ecology*, 38(2), pp.401-411.
- Speiser, B. 1999. Molluscicidal and slug-repellent properties of anaerobically digested organic matter. *Annals of Applied Biology*, 135(1), pp.449-455.
- Speiser, B., Glen, D., Piggott, S., Ester, A., Davies, K., Castillejo, J. and Coupland, J. 2001. *Slug damage and control of slugs in horticultural crops*. [ONLINE] CORE. Available at: <https://core.ac.uk/download/pdf/10919097.pdf> [Accessed 6th August 2019]
- South, A. 1965. Biology and ecology of *Agriolimax reticulatus* (Müller) and other slugs: spatial distribution. *Journal of Animal Ecology*. 34(2), pp.403-417.
- South, A. 1992. *Terrestrial slugs: Biology, Ecology and Control*. London: Chapman & Hall.
- Stalham, M and Allison, M. 2015. *Improving cultivation practices in potatoes to increase window of workability and soil structural stability. Report no. 2015/6*. [ONLINE] AHDB. Available at: https://potatoes.ahdb.org.uk/sites/default/files/publication_upload/201211%20Soil%20Workability%20R452_0.pdf [Accessed 6th August 2019]
- Stephenson, J.W. 1975. Laboratory observations on the effect of soil compaction on slug damage to winter wheat. *Plant Pathology*, 24(1), pp.9-11.
- Tan, K. 2005. *Soil sampling, preparation, and analysis*. Second edition. Florida: CRC Press.
- Taylor, L.R. 1961. Aggregation, variance and the mean. *Nature*, 189(4766), p.732.

The Andersons Centre. 2014. *Crop production technology; the effect of the loss of plant protection products on UK agriculture and horticulture and the wider economy*. [ONLINE] NFU. Available at: <https://www.nfuonline.com/andersons-final-report/> [Accessed 7th February 2016]

The Crop Residues (Burning) Regulations 1993. SI 1993/1366.

Triebkorn, R.A., Henderson, I.F. and Martin, A.P. 1999. Detection of iron in tissues from slugs (*Deroceras reticulatum* (Müller)) after ingestion of iron chelates, by means of energy-filtering transmission electron microscopy (EFTEM). *Pesticide Science*, 55(1), pp.55-61.

Want, R. 2006. An introduction to RFID technology. *IEEE Pervasive Computing*, 1, pp.25-33.

Wäreborn, I. 1970. Environmental factors influencing the distribution of land molluscs of an oligotrophic area in southern Sweden. *Oikos*, 21(2), pp.285-291.

Wareing, D. and Bailey, S. 1985. The effects of steady and cycling temperatures on the activity of the slug *Deroceras reticulatum*. *Journal of Molluscan Studies*, 51, pp.257-266.

Willis, J. C., Bohan, D. A., Powers, S. J., Choi, Y. H. and Gussin, E. 2008. The importance of temperature and moisture to the egg-laying behaviour of a pest slug, *Deroceras reticulatum*. *Annals of Applied Biology*, 153, pp.105-115.

Wood, C. 2006. *Countryside Survey 2007(Soils) Preparatory Phase II; Soil bulk density sampling*. [ONLINE] NERC. Available at: <http://nora.nerc.ac.uk/id/eprint/503786/> [Accessed 10th March 2019]

Young, A.G. 1990. Assessment of slugs using bran-baited traps. *Crop Protection*, 9, pp.355-358.

Young, A.G. and Port, G.R. 1991. The influence of soil moisture on the activity of *Deroceras reticulatum* (Müller). *Journal of Molluscan Studies*, 57(1), pp.138-140.