Locomotor behaviour promotes stability of the patchy distribution of slugs in arable fields

Forbes, Emily; Back, Matthew; Brooks, Andrew; Petrovskaya, Natalia; Petrovskii, Sergei ; Pope, Tom; Walters, Keith

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Complete List of Authors:	Forbes, Emily; Harper Adams University, Crops & Environment Back, Matthew; Harper Adams University, Crops & Environment Science Brooks, Andrew ; Harper Adams University, Crops & Environment Petrovskaya, Natalia; University of Birmingham, School of Mathematics Petrovskii, Sergei; University of Leicester, Department of Mathematics Pope, Tom; Harper Adams University, Crop & Environment Sciences Walters, Keith; Harper Adams University, Crops & Environment
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Authors: Emily Forbes[1], Matthew A. Back[1], Andrew Brooks[1], Natalia B. Petrovskaya[2], Sergei V. Petrovskii[3], Tom W. Pope[1] and Keith F A Walters[1]

Affiliations:

[1]Centre for Integrated Pest Management, Harper Adams University, Newport, Shropshire TF10 8NB, UK

[2]School of Mathematics, University of Birmingham, Birmingham B15 2TT, UK

[3]Department of Mathematics, University of Leicester, Leicester LE1 7RH, UK

Email addresses: eforbes@harper-adams.ac.uk (EF); mback@harper-adams.ac.uk (MB); Andrew Brooks: asbrooks@harper-adams.ac.uk (AB); n.b.petrovskaya@bham.ac.uk (NP); sp237@le.ac.uk (SP); *tpope@harper-adams.ac.uk* (TP); kwalters@harper-adams.ac.uk (KW)

Corresponding author: Keith F A Walters, Centre for Integrated Pest Management, Harper Adams University, Newport, Shropshire TF10 8NB, UK. Email: kwalters@harper-adams.ac.uk; Tel: 07552 689357

Abstract:

BACKGROUND: The distribution of the grey field slug (*Deroceras reticulatum* Müller) in arable fields is characterised by patches containing higher slug densities dispersed within areas of lower densities. Behavioural responses that lead to the spatial/temporal stability of these patches are poorly understood, thus this study investigated behavioural mechanisms underpinning slug distribution using a new method for long-term tracking of individual slug movement in the field.

RESULTS: A technique for implanting Radio Frequency Identification (RFID) tags (each with a unique identification code) beneath the body wall of slugs was developed. Laboratory tests indicated no consistent detrimental effect on survival, feeding, egg-laying or locomotor behaviour (velocity, distance travelled). Movement of individual slugs above and below the soil surface was recorded for >5 weeks (in spring and autumn) in winter wheat fields. Most (~80%) foraged within a limited area; and at the end of the observation period were located at a mean distance of 78.7 ±33.7 cm (spring) or 101.9 ±24.1 cm (autumn) from their release point. The maximum detected distance from the release point was 408.8 cm. The remaining slugs (~20%) moved further away and ultimately were lost.

CONCLUSIONS: RFID tagging allowed continuous tracking of individual slugs, even below the soil surface. Localised movement of 80% of tracked slugs over 5-weeks offers a mechanism promoting stable slug patches in arable crops. Rapid dispersal of the remaining slugs facilitates exchange of individuals between patches. Precision targeting of pesticides at such stable slug patches may facilitate reduced usage.

Key words: grey field slug, patchy distribution, in-field tracking, RFID tags, slug locomotory behaviour, slug patch stability

1. INTRODUCTION

The grey field slug, *Deroceras reticulatum* Müller is the most economically important slug pest in Europe and also damages a wide range of agricultural crops in Asia and USA[1, 2]. *Deroceras reticulatum* is reported to display a discontinuous distribution in arable fields characterised by patches containing higher slug densities dispersed within areas of lower slug densities[3-5]. Few studies have investigated the behavioural responses that influence the formation of these areas of higher slug densities (patches) or their spatial or temporal stability. Difficulties associated with studying and effectively tracking individual *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[6], 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[7]. Various techniques have been developed to assess populations, including surface searching, refuge traps, hand sorting of soil, soil flooding, defined area traps and capture-recapture approaches[6], 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, however, constrain our understanding of the mechanisms underpinning the formation, stability or location of higher density patches[8].

Previous studies of the behaviour of *D. reticulatum* have attempted to track the movement of individuals using approaches such as freeze-marking (marks made on the mantle using hot copper wire irons)[9], dye injected into the slug[10], UV dye[11] and radioactive isotopes[12]. A common problem with these methods is the requirement for the slug to be on the soil surface for it to be located and individually identified. In addition, the markers can be short-lived or difficult to distinguish in the field making the identification of individuals difficult. For example, the radioactive isotopes used by Hakvoort and Schmidt[12] could only be identified for approximately 10 days after the radioactive feed source was removed. Moreover, the freeze-marks applied to the slug's mantle by Richter[9] only lasted for up to 2 months on mature *D. reticulatum* while the injected dyes used by Hogan and Steele[10] were difficult to detect on darker individuals making them hard to distinguish in the field.

Radio Frequency Identification (RFID) tags have been used to track movement in a range of vertebrate and invertebrate species, including fish[13], honeybees entering and exiting hives[14] and vine weevils[15]. The technology allows individuals to be uniquely identified, and RFID tags buried in the upper horizons of the soil have been detected at least 20 cm below the surface. Whilst the technique has a high potential for tracking slug movement and behaviour, regardless of their position in the soil profile, few studies have attempted to develop its use. Grimm[16] injected RFID tags into the foot of *Arion lusitanicus* (Mabille), a much larger (<13cm long) slug species than *D. reticulatum* (<5cm), 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*.[17] to assess field survival rates of *A. lusitanicus* and *Arion rufus* (Linnaeus, 1758) and by Knop *et.al*.[18] to investigate the 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.

This study investigated behavioural characteristics of *D. reticulatum* which can contribute to the cohesion of the observed slug patches in arable fields. In order to be effective when tracking individual slugs, the method used to mark them must not affect key aspects of biology and behaviour, must allow for differentiation between individuals, and be sufficiently long-lasting.

The work therefore had two objectives. Firstly, to develop a method of implanting RFID tags into the smaller slug species *D. reticulatum* and investigate subsequent survival, feeding and possible sub-lethal effects on locomotor behaviour. Secondly, to use the technique to investigate the locomotor behaviour of individual slugs, with a particular focus on the mechanisms underpinning the stability of slug patches.

2. MATERIALS AND METHODS

2.1 Laboratory studies

Deroceras reticulatum were collected using surface refuge traps baited with approximately 75g chicken feed pellets[19] 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 more than 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 5ml 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 photophase and 10°C during scotophase, to reflect UK conditions in autumn and spring, and allowed a 48 hour acclimatisation period before being used in experiments.

2.1.1 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. CO_2 was gently released through the hole into the container using a Corkmaster CO_2 dispenser and 8 g CO_2 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 ¾ 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.

2.1.2 Treatments

Five treatments, with 20 slugs per treatment, were used to assess the effect of different aspects of the tagging process on slug survival, feeding and reproduction:

(i) Tagged (T) + CO_2 + Glue (G):– slugs were anaesthetised using CO_2, an RFID tag inserted and glue (Loctite Precision Max, Loctite, USA) applied over the insertion site to seal the wound.

(ii) Tagged (T) + CO_2:- slugs were anaesthetised using CO_2 and an RFID tag was inserted.

(iii) CO_2+:- slugs were anaesthetised and the implanter needle was inserted through the body wall but no tag was injected.

(iv) CO_2:- slugs were anaesthetised with CO_2 only.

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

2.1.3 Effect of implanting RFID tags on 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 repeated three times in consecutive 28 day runs using different cohorts of slugs.

2.1.4 Effect of implanting RFID tags on 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.

2.1.5 Effect of implanting RFID tags on 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-day period following treatment.

2.1.6 Effect of implanting RFID tags on locomotor behaviour

Slugs were maintained in the laboratory for a 48 hour acclimatisation period under the conditions described above, before being 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 + CO_2; 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). A video-camera (SONY HDR-CX240E Handycam, SONY, Japan) was positioned at 100cm 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. The recordings took place between 2 and 8 hours after the lights came on in the controlled environment rooms, with the order of slug treatments being randomised on each recording occasion. 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.

2.2 Field studies

2.2.1 Locomotor behaviour of *D. reticulatum* in winter wheat

The behaviour of the slugs was investigated in commercial winter wheat crops in Shropshire, UK (52°46’01.26’N 002°34’50.14’W), in spring (April; 9 slugs) and autumn (November; 20 slugs). A 4 x 5 grid of refuge traps (unbaited refuge traps consisting of upturned terracotta plant pot saucer 18 cm diameter; LBS Horticulture Supplies, Lancashire, UK) was established in the study area, with 2 metres 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 + CO_2; 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 metre 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 (waypoint marker) 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 the spring and for 8 hours post-release in the autumn. In autumn, 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 35 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 peg in relation to the original release point (marked by the flexi-cane on the grid node) were determined before the distance between sequential waypoint markers 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)air temperature was recorded at 30 minute intervals using data loggers (iButton DS1921G-F5 thermochrons, Maxim integrated Products, USA). Rainfall records were obtained from the Harper Adams University weather station which was situated within 0.5 miles of the study field.

2.3 Statistical analyses

All statistical analyses were conducted using R 3.4.2. (R core Team[20]).

2.3.1 Effect of implanting RFID tags on survival, feeding, production of egg batches and locomotor behaviour in the laboratory

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, and on mean velocity and total distance moved using ANOVA.

2.3.2 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.

3. RESULTS

3.1 Laboratory studies

3.1.1 Slug 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 + CO_2 + G and T + CO_2) ($F=45.8$, d.f.=4,10, $p<0.001$; Fig. 1). During the 7 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 + CO_2 + G and T + CO_2) died compared to an average of 0.9±0.3 slugs in each of the treatment groups with no RFID tag inserted (CO_2+, CO_2 and U) (Fig. 1). During the 14-day post-insertion, mortality had risen to 8.1±1.1of the 20 slugs in groups with an RFID tag inserted (T + CO_2 + G and T + CO_2), and 1.3±0.4 of the 20 in those groups without tags (CO_2+, CO_2 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 + CO_2 + G and T + CO_2) and 0.09 slugs per day in each of the treatment groups with no RFID tag inserted (CO_2+, CO_2 and U).

3.1.2 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 + CO_2 + G and T + CO_2) (F=10.1, d.f.=4,1977, p<0.001; Fig. 2). During the 7-day period after tag insertion, slugs consumed a mean of 0.03±0.03 g per day in the T + CO_2 treatment group and 0.05±0.03 g for the T + CO_2 + G treatment group, compared to 0.14±0.02 g for the control group (U), 0.11±0.02 g for the CO_2+ treatment group and 0.11±0.02 g for the CO_2 treatment group (Fig. 2). 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.

3.1.3 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, either during the first 7 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; Fig. 2).

3.1.4 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) (Fig. 3). 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) (Fig. 3).

3.2 In-field tracking of slugs with implanted RFID tags

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

3.2.1 Slugs released in spring

For the first 2 hours after release, eight of the nine slugs remained close (23.5±7.3 cm) to the release point and were subsequently tracked (Fig. 4), and all tracked slugs were observed feeding and moving over the soil surface or on crop plants. The ninth slug was not detected again after release. The first observation of a tagged slug feeding occurred 35 minutes post-release. Two tracked slugs were no longer visible on the soil surface one hour post-release with all the remaining six also being detected below the soil surface 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 mean displacement from the initial release point at the end of this period was only 78.7± 33.7 cm (Fig. 4). However, the mean distance between sequential slug locations recorded at weekly intervals after release was 165.2 ±32.4 cm. Thus although they will travel longer distances while foraging, many may return to or remain within the "local area", thus contributing to patch cohesion.

3.2.2 Slugs released in autumn

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 also 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 (Fig. 5), 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 being 408.8 cm. The mean distance between sequential slug locations recorded at weekly intervals after release was 203.3 ±30.9 cm, but their tracks recorded over the five week period indicated that despite the length of their foraging trips, frequent changes in direction (turns) resulted in most remaining within the same "local area", thus contributing to patch cohesion.

3.2.3 Effect of temperature and rainfall

Slug activity on the soil surface can be affected by temperature or surface moisture (which in turn can be affected by recent rainfall events or cummulative precipitation over longer periods).

If either rainfall or temperature were abnormally high or low then the data collected on slug locomotion may have been affected. Spring (April/May): Temperatures at the April/May 2017 field site (Fig. 6(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 normal range of variability. 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, $p>0.05$, R^2=0.04; Fig. 6(A).

Autumn (November/December): The temperature during the two months of November/December (Fig. 6(B)) was similar (both maximum and minimum within 0.6 °C) to the 30 year average. Rainfall was lower by 9.7 mm in November and higher by 15.7 mm in December[21], also within normal range of variability. 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, $p>0.05$, R2=0.1) but there was a significant weak correlation with daily rainfall (Pearson's correlation; r=0.4, t=2.2, d.f.=21, $p<0.05$, R2=0.2. 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 (Fig. 6(B)).

4. DISCUSSION AND CONCLUSIONS

This study confirms the potential for using RFID technology to investigate the behaviour of the slug *D. reticulatum* in the field. Unlike other techniques[9-12] it does not rely on slugs being active on the soil surface but can be used to track movement beneath the surface for extensive periods.

Although RFID tags have been inserted into a larger, *Arion* species[16], it was previously thought that the technology could not be used in smaller slug species such as *D. reticulatum* as the relative size of RFID tags and fully grown adults would result in lethal damage being caused to the body during implanting[11]. Whilst still large in comparison to body size, advances in technology have meant smaller RFID tags are available; 8 mm tags were used in this study, compared to the 11 mm tags used by Grimm[16]. By elimination of the other components of the insertion procedure, it can be concluded that the initial effect on survival and feeding detected in the current study during the first two week post-implantation period were due to the RFID tag itself. Slugs into which tags were implanted suffered initial increases in mortality unlike the larger *Arion* spp. tagged by Grimm[16] and it is probable that their size made them more susceptible to damage to internal organs caused by the process, as proposed by Foltan and Konvicka[11].

The digestive gland and crop are found on the left lateral side of a slug body[6], and damage to these organs would result in mortality occurring due to starvation over a period of days. The heart, kidney and reproductive organs are located on the right lateral side and so are less vulnerable to damage caused by tag insertion using the procedure developed for this study, damage to these organs would be likely to lead to faster mortality than that observed in the laboratory experiments. There were no instances of immediate mortality at the time of tag insertion but increased slug mortality was recorded during a period of up to 14 days post implantation. Damage to the digestive system may explain these observations and is supported by the finding that feeding in tagged slugs was also negatively affected. Damage to the digestive system may limit feeding rate by reducing the capacity of the crop, leading to progressive starvation over a period of time. The ultimate recovery to normal feeding levels observed in some slugs suggests that they are able to adapt to the tag so long as the initial implantation did not cause excessive damage to internal organs or obstruct feeding to the point of starvation. No effect on egg production was identified (reflecting Grimm[16]), supporting the proposition that organs on the right lateral side of the body are less likely to be affected by the procedure.

RFID tags were used successfully by Knop *et al.*[18] as a method of marking slugs in mark-recapture experiments investigating the locomotor activity of a native and invasive species. The current study extends this work, by showing that their insertion had no significant effect on slug locomotion, including the distance moved or velocity of tagged slugs. In addition, it was confirmed that the technique could be used to effectively track and record detailed behavioural characteristics pertaining to the dispersion of individual slugs in the field over a sustained period of time. The latter is a critical observation that allows the technique to be used in the field to investigate the impact of *D. reticulatum* behaviour on slug patch stability.

Visual observations made during periods of intense monitoring in the field experiments indicated that the emergence and resumption of activity on the soil surface of naturally occurring *D. reticulatum* as dusk approached, coincided with the time of appearance of the first tagged slugs in the same area of the field, increasing confidence in the validity of the technique. Similarly, tagged slugs were found to actively feed and move over the soil surface during the night, whereas during the day they were not visible on the soil surface, consistent with published reports that slugs are more active during hours of darkness and find refuge during the day[6, 22, 23].

The impact of both temperature and rainfall on tagged slug activity in the current study were also consistent with published findings. Mean temperatures in the April/May field experiment were close to the optimum for slug activity (movement: 17 °C; feeding: 14 °C[22]). However, rainfall was low, which meant that soil conditions were dry throughout the experimental period, and the resultant large cracks that developed facilitated slug movement deeper into the soil than under less dry conditions[6]. Significantly reduced surface activity is known to occur after extended periods of low rainfall[7] and this was reflected in there being little lateral slug movement recorded, and individual slugs not being detected at every monitoring visit during the April/May period (the latter mirroring qualitative observations of low surface activity of naturally occurring slugs in the same field). The lack of detection, although not confirmed in this study, would suggest slugs moving vertically down the soil profile, where the temperature and moisture remains more constant, to a depth greater than the read range of the antennae. In the November/December monitoring period, the effect of rainfall was again consistent with published findings[7, 22], with increases in the number of active slugs coinciding with periods of rainfall. Although a period of snowfall coincided with a reduction in slug activity, some movement was still detected supporting the findings of Mellanby (1961) who found *D. reticulatum* active at temperatures as low as 0.8 °C.

Results from both of the experimental tracking periods show that the majority (80%) of the slugs followed had remained within a relatively short distance from their original release position at the end of the experimental periods (with extended observations of some indivduals confirming this after 47 days). This suggests that longer distance dispersal of slugs within arable fields is limited, at least in established wheat crops, which may be a contributory mechanism leading to temporally and spatially stable slug patches. Cohesion of the patches may also be reinforced by behavioural responses that result in slugs following slime trails left by others when encountered[25]. Whilst several species of slugs are known to follow trails for mating and homing, this is much less frequent (8% of trails) in *D. reticulatum*, according with other observations that suggest that the species only exhibit trail following when reproductively active, a small part of their lifetime. Thus although trail following may re-inforce patch cohesion it will only be of minor significance.

Collectively, however, such mechanisms may result in the majority of slugs in winter wheat fields existing in semi-discreet groups. However, the low proportion of more active individuals that either rapidly dispersed from the initial release point or were otherwise found to have moved away (further than the 5 m intensive search area around the release points) from the main slug patch at the end of the observation period (~20% –6 out of 29 slugs released in spring and autumn), would lead to regular exchange of individuals between patches.

The method developed under this study of utilising RFID tags for the investigation of slug behaviour in the field, facilitates the establishment of a more complete mechanistic understanding of the distribution of slug populations in the field. Current concerns about the impact of agricultural and horticultural practices on the natural environment have resulted in widespread recognition of the need to optimise or minimise the use of agro-chemicals in crop production[26]. Whilst maintaining effective control, substantial reduction in the amount of active ingredient used to manage slug populations could be achieved by use of precision application technology to target treatments at slug patches, whilst leaving inter-patch areas untreated[8]. Before such an approach can be developed, however, clear evidence of the spatial and temporal stability of these patches is required, together with development of a commercially viable method of identifying their location and dimensions. This study has indicated that the small spatial range of individual *D. reticulatum*, may be a major contributory factor to spatial cohesion, in conjunction with slime trail following behaviour[25]. Both a modelling study investigating the contribution of these and other behavioural characteristics to patch formation, and empirical research to quantify both the temporal period over which patches remain cohesive and identify factors that define the locations in which they form, are ongoing.

In summary, RFID tagging meets the primary requirements of an effective method of tracking individual slugs, which can be identified from the unique tag numbers even when below the soil surface and tracked over periods of at least five weeks under field conditions.

The data on individual slug movement collected using this technique provides evidence for a potential mechanism leading to the stability of higher density slug patches in arable crops. As the pressure to reduce pesticide usage increases, improved understanding of the behaviour of *D. reticulatum* will potentially have significant economic and environmental benefits by facilitating research into commercially viable methods for precision application of pesticides.

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Figure Legends

Figure 1. The effect of treatments on the survival of *Deroceras reticulatum* over 28 day periods. Points show the mean (of three replicates) ± SE. T + CO_2 = Slug anaesthetised using CO_2 and RFID tag injected; T + CO_2 + G = Slug anaesthetised using CO_2, RFID tag injected and the resultant hole in the body wall sealed with glue; CO_2+ = Slug anaesthetised using CO_2, a hole made in the body wall using the tag implanter but without injection of an RFID tag; CO_2 = Slug anaesthetised using $CO_{2;}$ U = Untreated slug; n=20 for each treatment group in each replicate.

Figure 2. The effect of the RFID tagging process on (A) mean food consumption (g ± SE) of *Deroceras reticulatum* in four successive 7-day periods after treatment and(B) mean number of egg batches ±SE produced by *D. reticulatum* over the course of the 28-day period following treatment . T + CO_2) = Slug anaesthetised using CO_2 and RFID tag injected; T + CO_2 + G = Slug anaesthetised using CO_2, RFID tag injected and the resultant hole in the body wall sealed with glue; CO_2+ = Slug anaesthetised using CO_2, a hole made in the body wall using the tag implanter but without injection of an RFID tag; CO_2 = Slug anaesthetised using $CO_{2;}$ U = Untreated slug, n=20 for each treatment group.

Figure 3. (A) Mean distance moved in a 1 hour observation period (cm ± SE) and (B) mean velocity (cms^{-1} ± 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.

Figure 4. Map of *Deroceras reticulatum* movement in a winter wheat field in Shropshire over (A) a two-hour period and (B) a five week period during spring (5 April - 12 May) using RFID technology to track and identify individual slugs. X and Y-axes indicate 2-dimentional distance

(cm) from the intial release point (a grid node in each case). Each circle shows a position where the slug was detected, circles joined by straight lines indicate consecutive detection points along the slug’s path but do not necessarily represent the precise route of travel taken between points.

Figure 5. Map of *Deroceras reticulatum* movement in an 800 by 1000 cm area of a winter wheat field in Shropshire over a five week period in autumn (15^{th} November to 21^{st} December) using RFID technology to track and identify individuals. X and Y-axes show distance (cm) in 2-dimensions from the intial release point (a grid node in each case). Each circle shows a position where the slug was detected, circles joined by straight lines indicate consecutive detection points along the slug’s path but do not necessarily represent the precise route of travel taken between points.

Figure 6. The number of slugs that were active slugs overnight in relation to the maximum daily temperature (°C) and daily rainfall (mm) during (A) the five-week spring tracking period and (B) the five-week autumn tracking period.

Figure 1.

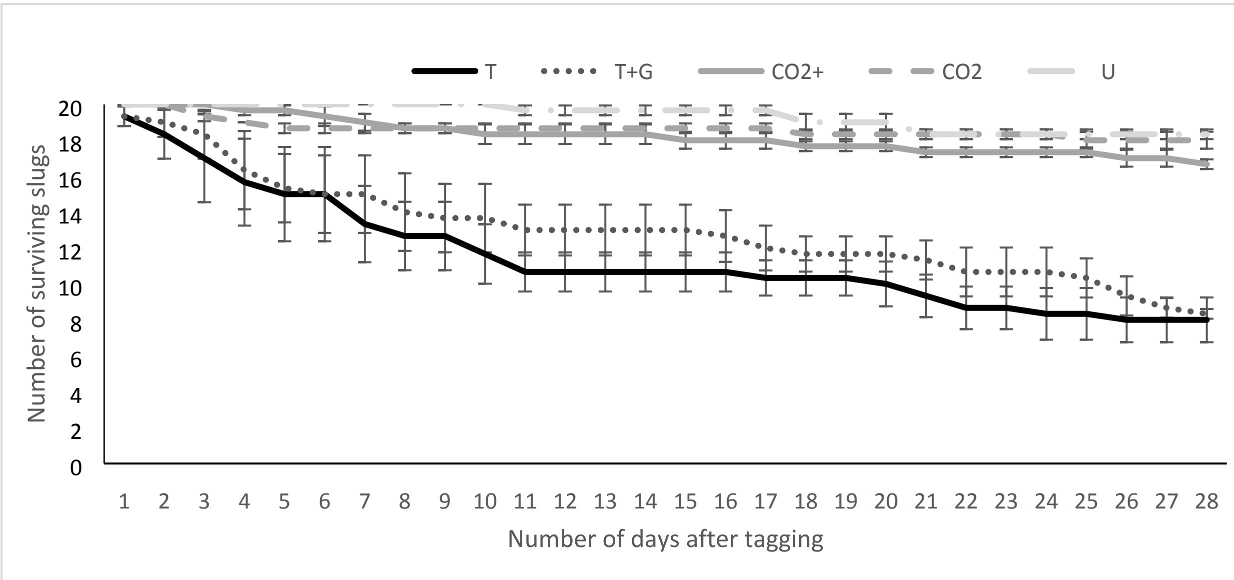
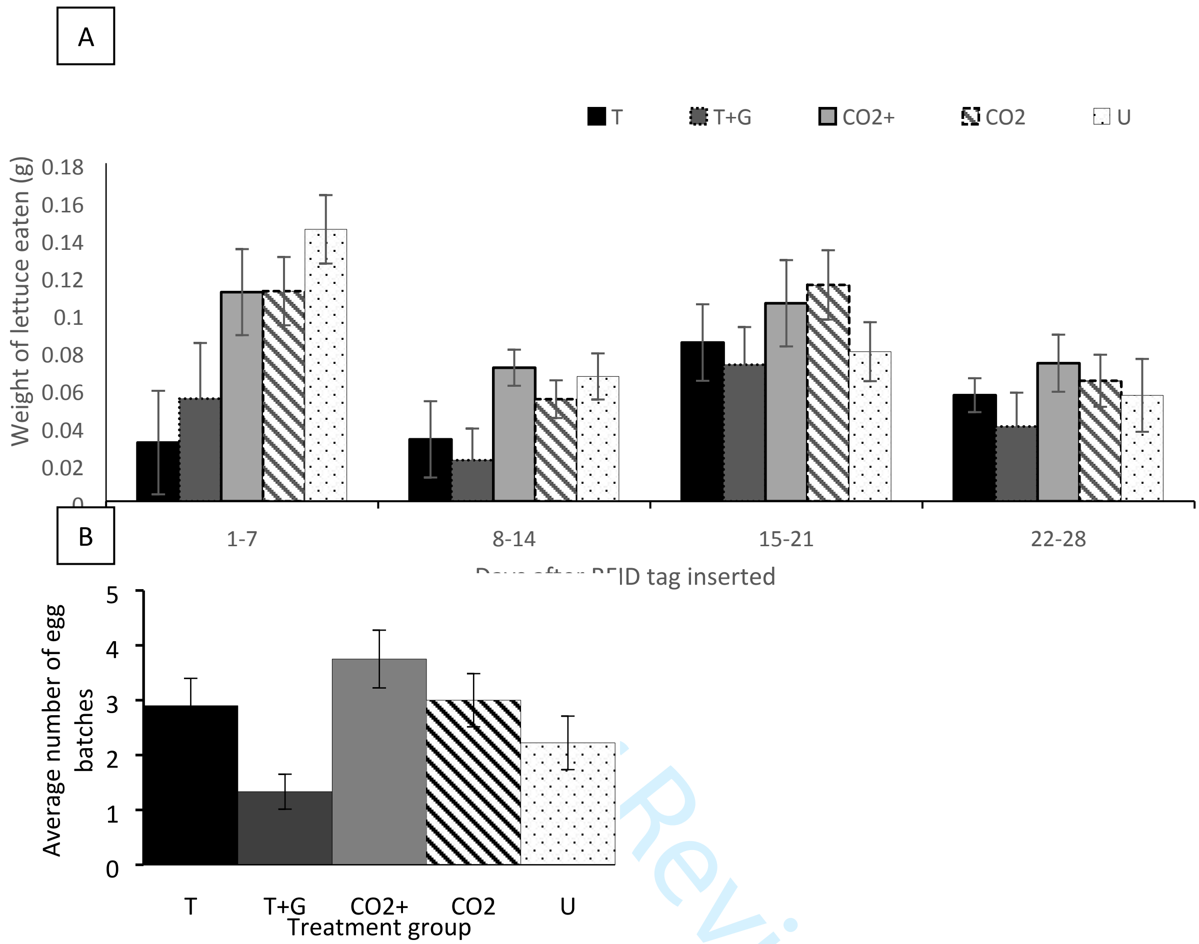

T
T+G
CO2+
CO2
U
Number of surviving slugs
0
2
4
6
8
10
12
14
16
18
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
Number of days after tagging

Figure 2


A
T
T+G
CO2+
CO2
U
Weight of lettuce eaten (g)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
1-7
8-14
15-21
22-28
B
Average number of egg batches
5
4
3
2
1
0
T
T+G
CO2+
CO2
U
Treatment group

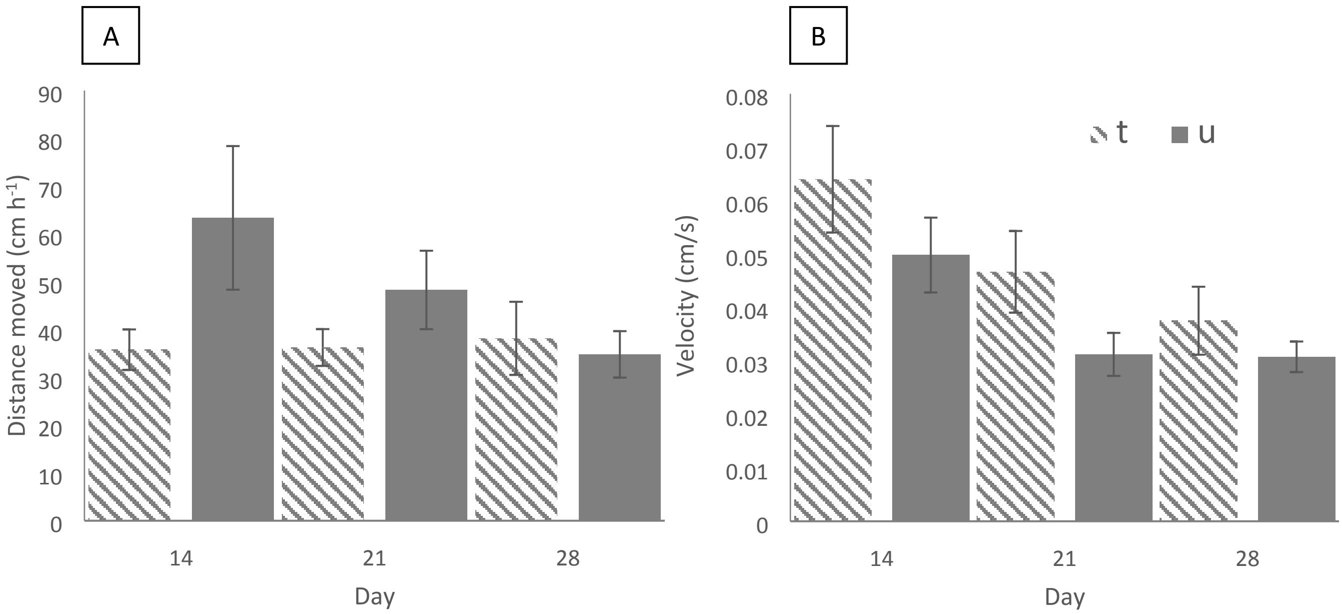

A
B
90
80
70
60
50
40
30
20
10
0
Distance moved (cm h-1)
14
21
28
Day
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Velocity (cm/s)
t
u

Figure 4

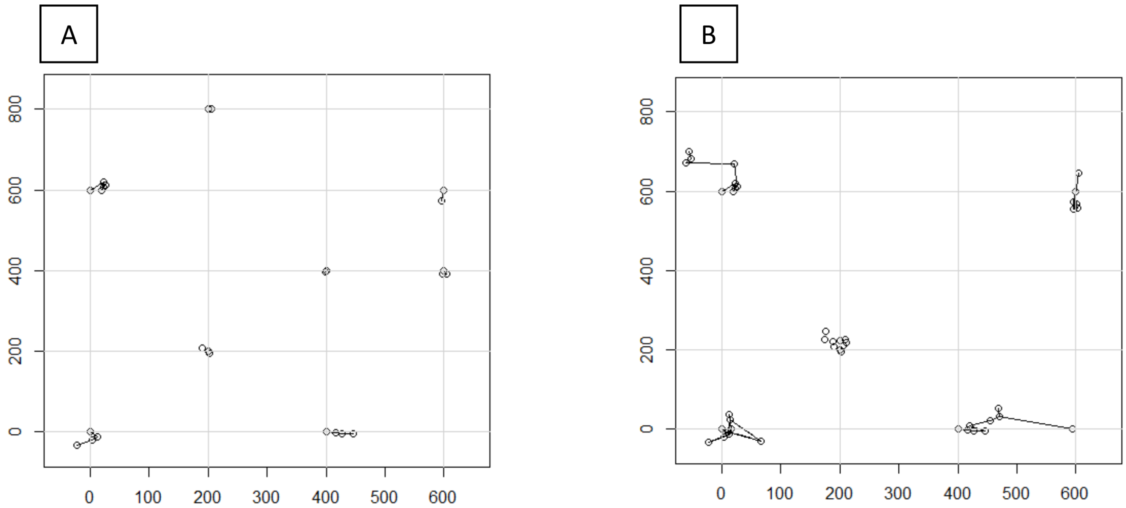

A
800
600
400
200
0
0
100
200
300
400
500
600
B
800
600
400
200
0
0
100
200
300
400
500
600

Figure 5

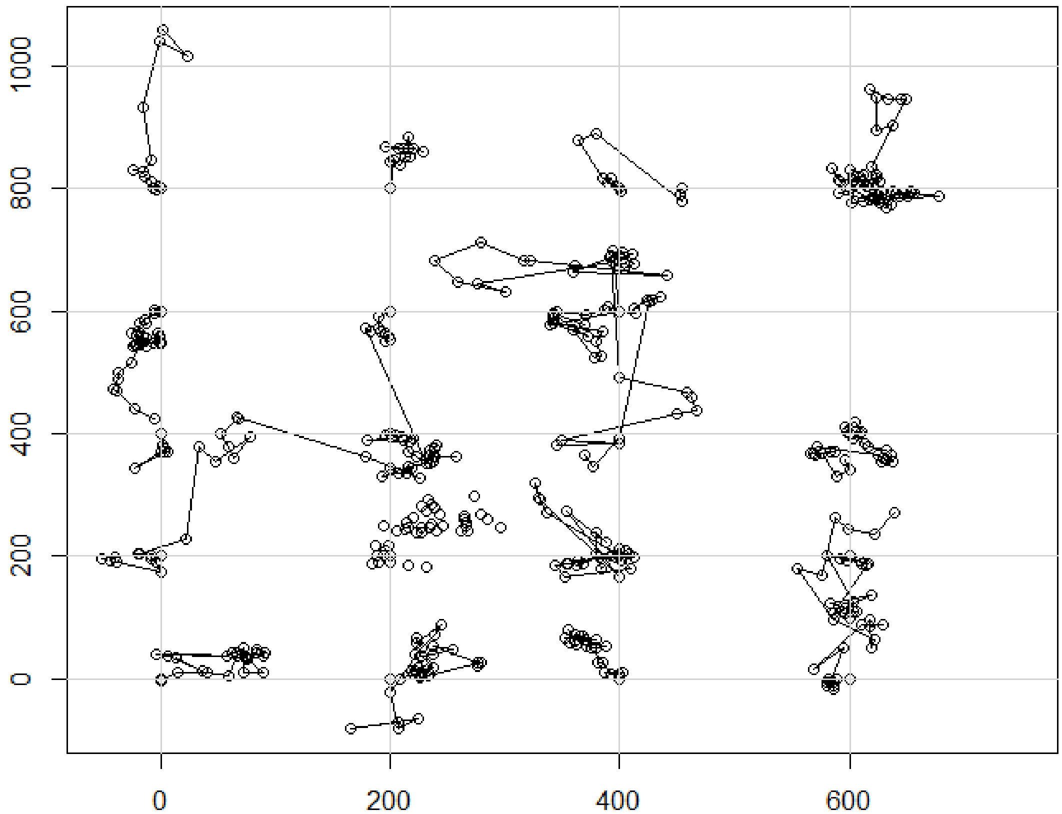
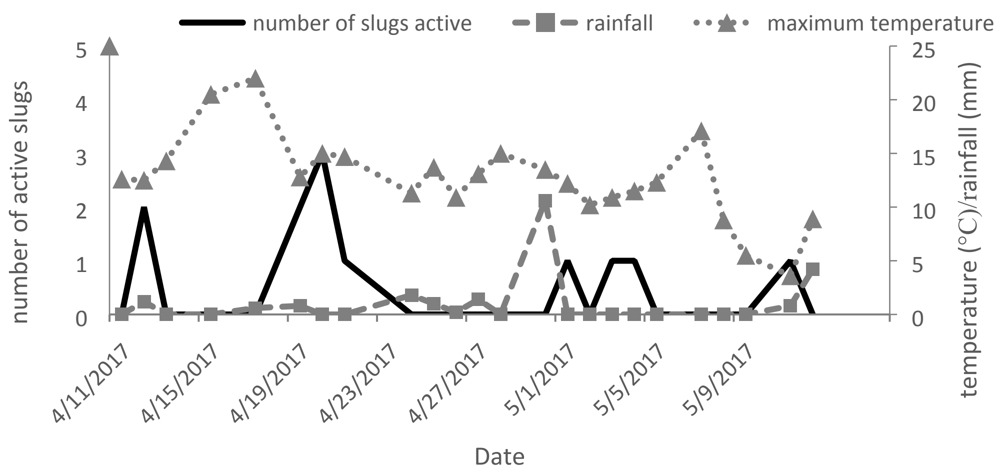
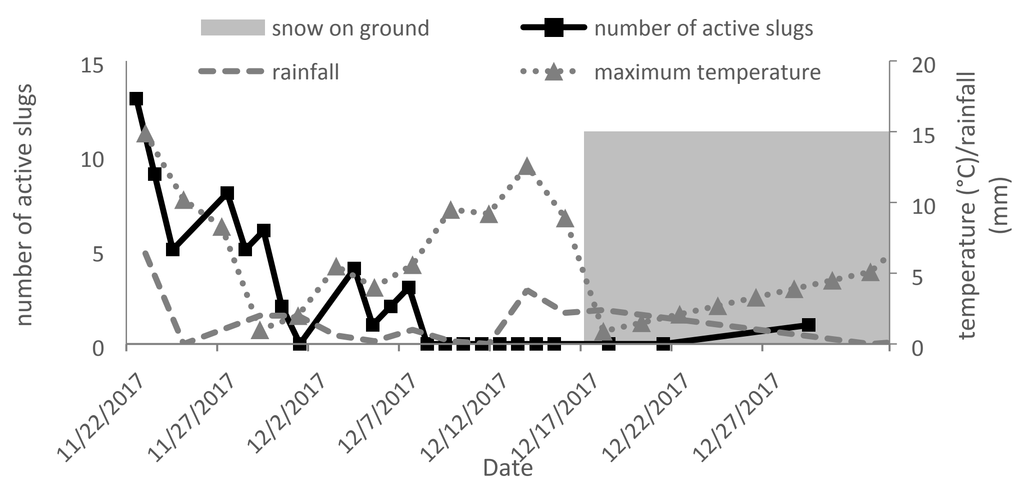


Figure 6

A


number of slugs active
rainfall
maximum temperature
number of active slugs
temperature (°C)/rainfall (mm)
0
1
2
3
4
5
0
5
10
15
20
25
4/11/2017
4/15/2017
4/19/2017
4/23/2017
4/27/2017
5/1/2017
5/5/2017
5/9/2017
Date

B


snow on ground
number of active slugs
rainfall
maximum temperature
number of active slugs
temperature (°C)/rainfall (mm)
0
5
10
15
0
5
10
15
20
11/22/2017
11/27/2017
12/2/2017
12/7/2017
12/12/2017
12/17/2017
12/22/2017
12/27/2017
Date