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Integrated slug control in arable crops: Risk assessment, trapping, agronomy and chemical control

by

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ABSTRACT

Slugs are major pests and often cause serious damage to winter wheat and oilseed rape at establishment. Because molluscicides are a limited market, products based on new chemistry are unlikely to be available for some years. This project was targeted by industry to improve pest management strategies using existing crop protection chemicals, based on a system of risk assessment and integrated control as summarized below.

Slug damage risk. Slugs (especially *Deroceras reticulatum*, but also *Arion*, *Milax* and *Tandonia* spp.) are most damaging when they feed on wheat seeds because each slug can kill up to about 50 seeds in the first week after sowing. Seed kill increases at a decreasing rate with slug body weight, thus, weight-for-weight, smaller slugs kill more seeds than larger slugs. Feeding on shoots and leaves of wheat can also be important. Oilseed rape seedlings are highly vulnerable to feeding by slugs but seeds are not attacked.

Assessing slug risk. Slug populations in autumn can only be predicted for one or two weeks at most, as slugs respond rapidly to weather changes. Put slug traps out before cultivation, when the soil surface is visibly moist and the weather mild (5-25^{o}C). Traps consist of a cover about 25cm across, with a small heap (20ml or 2 heaped teaspoonfuls) of chicken layers' mash (NOT slug pellets) beneath. In each field nine traps (13 in fields larger than 20ha) should be set out in a 'W' pattern. Concentrate on areas known to suffer damage. Leave traps overnight and examine early next morning. Trapping between drilling and emergence can be worthwhile in wet weather. Threshold trap catches and decision trees for control are described in more detail in HGCA Topic sheets 84 (winter wheat) and 85 (oilseed rape).

Reducing risk. A fine and consolidated seedbed protects seeds and germinating seedlings. Shallow cultivation to incorporate crop residues after harvest reduces slug numbers especially in dry conditions. Slugs are denied access to cereal seeds if seeds are drilled at 3cm depth in a fine consolidated seedbed. In cloddy seedbeds, increase sowing depth to 4-5cm. Monitoring crops regularly for slug damage from sowing to first tillering (cereals) or the four-true-leaf stage (oilseed rape) is worthwhile. Damage after this stage is less likely to result in further plant loss, but monitoring should continue through the winter.

Applying slug pellets. The greatest benefit is generally achieved from an application just after drilling (and after rolling if this is done). Soil from rain splash on pellets does not significantly affect the time taken for slugs to feed, so do not wait until after rain; slugs start to kill wheat seeds almost immediately after drilling. Slugs are killed more quickly by broadcast pellets compared to pellets drilled with seeds. Broadcasting gives more consistent slug control, particularly in combination with fine, firm seedbeds that help protect seeds and seedlings. Admixed pellets will be ineffective in fine seedbeds because both seeds and pellets are unavailable to slugs, which survive to attack seedlings. Pellet admixtures with wheat seeds can be effective when direct-drilling, or in open cloddy seedbed. Earlier treatments (on stubble) or later treatments (at emergence) are likely to be less effective, especially when conditions favour slug activity. Further treatment is justified where slug activity is high and crop growth is slow. See also HGCA Topic Sheet 88.

SUMMARY

Slugs are major pests of arable crops and often cause serious damage to winter cereals and oilseed rape at establishment. Control is often expensive and relies on molluscicides formulated as baits, which can damage other wildlife. Growers who have had recent experience of slugs are inclined to use pellets prophylactically. There is a generally agreed need to improve the efficiency and targeting of slug control methods to minimise their adverse effects and reduce costs to farmers.

This project was targeted by industry to improve control strategies in winter wheat and oilseed rape. Because, globally, molluscicides are a restricted market, products based on new chemistry are unlikely to be available for some time. The project aimed to provide best advice on slug control, which will be more effective and benign for the environment. In all experimental work, high quality slug pellets (metaldehyde (De Sangosse UK) or methiocarb (Bayer CropScience)) were used so best possible control was achieved.

Aims and objectives

The aim was to devise a rational risk assessment system for the integrated control of slugs in arable crops, appropriate for incorporation into integrated crop management (ICM) guidelines. Within this overall aim there were four primary scientific objectives: -

1. Quantify interrelationships between slug populations and key environmental factors such as soil, weather and agronomic conditions, including the timing and method of pellet application, and control efficacy.
2. Quantify relationships between slug populations and conditions in the previous crop to evaluate their use as a damage indicator in the succeeding crop.
3. Develop a reliable predictor of crop damage based on these relationships.
4. Predict the need for and timing of slug pellets as part of an integrated control strategy, whilst reducing unnecessary use.

This summary first highlights the most important findings for industry, followed by an outline of the investigations and results for each objective, with the main messages underlined.

Most important findings for industry

≠Development and validation of novel trapping systems and firm guidance on the use of traps that are not reliant on piles of slug pellets for risk assessment in winter wheat and oilseed rape, including the limitations on the usefulness of traps and their use together with:

≠New Decision Trees for guidance on slug control in winter wheat and oilseed rape.

≠Clear recommendations for slug pellet timing to achieve optimum control based on our important finding that slug pellet application just after drilling (just before damage is expected) is of critical importance in wet autumns. Slug pellets are effective in killing slugs active on the surface but the large reservoir of slugs in the soil is largely unaffected and, in wet autumns, slugs reproduce and grow rapidly, so that new slugs become surface-active as the autumn progresses. Pellet timing is not critical in dry autumns – applications to stubble 6 weeks before drilling can be effective - but slug pellets are less likely to be needed and it is not possible to make an informed risk assessment several weeks before drilling. Therefore, even in dry autumns

it is best to wait until just after drilling, when the best decision can be made on whether to treat or not. Further treatment with slug pellets may be necessary in fields where there is high population pressure. It is therefore essential to continue to monitor crop damage throughout the winter.

- Analysis of the effect of rain splash on pellet efficacy. We asked the question: is heavy rainfall just after pellet application likely to make slug pellets unavailable to slugs? Although initial studies showed that pellets can be covered by soil from rain splash or moved, time-lapse video investigations of slug behaviour revealed that the presence of soil from rain splash on pellets did not affect the acceptability of pellets to slugs. We conclude that it is best to apply pellets at the right time in relation to crop vulnerability, rather than wait until after heavy rainfall events, because slugs start to feed on wheat seeds almost immediately after drilling and a single slug can kill up to 50 wheat seeds in the week following drilling.
- Time-lapse video recordings show that slugs take longer to find pellets drilled with seeds compared to broadcast pellets. This supports field studies where slug pellets drilled with the seeds were less effective than broadcast pellets.
- New understanding that our ability to predict slug populations can only be short term (a few weeks rather than months) because slug populations respond rapidly to favourable weather. We encountered contrasting situations each year. In 2002, slug numbers were high and building in wet weather in June to early August, posing a strong potential threat, but populations collapsed in dry conditions from August onwards. In 2003, dry weather resulted in little reproduction and low numbers of *Deroceras reticulatum*. Large numbers of juvenile *Arion distinctus* were present in early summer but were killed by the dry conditions from August onwards. In 2004, slug populations were low until early August then built rapidly in wet weather, giving severe slug damage. We have also demonstrated the threat posed by small slugs: weight-for-weight they can kill more wheat seeds than larger mature slugs. Thus, it is important to monitor the influence of weather on slug population development especially August, because August is, on average, one of the wettest as well as warmest months, thus conditions are often suitable for rapid slug reproduction and growth.
- Cultivation (even non-inversion tillage) in a dry autumn is enough to hold slug populations in check, but in a wet autumn, cultivation is less effective because slug populations are able to recover.
- Clear demonstration of the benefits of integrated control in winter wheat, with slug pellets applied pre-emergence providing initial protection and seedbed consolidation providing later protection.

Objective 1: Quantify interrelationships between slug populations and key environmental factors, including timing and method of pellet application, and control efficacy.

Objective 1.1 Importance of egg hatch and growth of juvenile slugs

Studies of the rate of growth of juvenile field slugs, *Deroceras reticulatum*, revealed large differences in growth rates between individuals, even from the same egg batch. There were also considerable differences in the growth rate between juveniles of this species hatching in the spring and autumn. Despite this, age and body weight were found to be significant indicators of maturity and an equation was derived to predict the probability of maturity based on weight. These predictions were validated for *D. reticulatum* in the field.

Studies of populations of *D. reticulatum* and other pest species in arable fields from 2002 to 2004 showed large differences in numbers of juveniles and their survival within and between years. The presence of large numbers of juveniles demonstrated successful breeding by *D. reticulatum* in generally wet weather from May to early August 2002. However, numbers declined in dry weather from then to late October. In 2003, numbers of adult *D. reticulatum* were similar to 2002, but juveniles were virtually absent in the dry conditions. In contrast, *Arion distinctus* bred well in spring 2003. In 2004, juvenile and adult *D. reticulatum* were present in small numbers up to early August, then juvenile numbers increased rapidly as a result of successful breeding in the wet weather from early August onwards.

These findings highlight the considerable capacity for rapid increase in numbers and biomass of *D. reticulatum* in arable fields, as a result of its high fecundity, ability to breed whenever conditions are suitable and rapid growth rate of juveniles, together with differences in breeding success of different species.

The capacity of juvenile slugs to damage winter wheat was investigated in the laboratory by measuring the numbers of wheat seeds killed in the first week after sowing in relation to slug body weight, for three common pest species: *D. reticulatum, A. distinctus* and *Milax gagates*. Individual slugs killed up to about 50 seeds during this period. For *D. reticulatum* and *M. gagates*, the number of seeds killed increased with slug body weight but at a declining rate towards an asymptote. Thus, weight for weight, juveniles of these species killed more wheat seeds than older individuals. One reason for this was that juveniles ate less of each seed, but always killed the seeds because they always took the embryo. For *A. distinctus*, the number of wheat seeds killed also increased with slug body but, weight for weight, this slug killed fewer seeds than the other species, partly because it took more from each seed than did the other species.

Objective 1.2 Patterns of vertical movement of slugs in soil following cultivation

Soil samples were taken at two depths (0-10 cm and 10-20 cm) from a replicated field experiment in autumn 2001 with plots ploughed or uncultivated. In the uncultivated plots, more than 99% of the slug population was found in the upper 0-10 cm layer of soil, where numbers and weight of slugs were significantly greater than on ploughed plots. Initially, following ploughing, numbers in the upper 10 cm layer were reduced by 96% compared to unploughed plots and on later dates numbers were reduced by 62% - 86%. The surviving slugs moved back to the surface layers from about 15 days after ploughing. Thus, if sufficient slugs survive ploughing to be a risk to the following crop, there is the potential for a substantial proportion of them to live at a depth where they do not come into contact with slug pellets applied at drilling, then move back to the surface to damage emerging crops after slug pellets applied around drilling time have ceased to be effective.

Objective 1.3 Patterns of surface activity

The activity of individual slugs (*D. reticulatum*) was recorded, using time-lapse, infra-red video techniques, on coarse seedbeds and fine seedbeds, either without slug pellets or with pellets broadcast on the soil surface. When no slug pellets were present, there were no significant differences in slug behaviour between seedbeds, measured as the time before onset of activity, the distance travelled and the period of activity before sunrise. With slug pellets present, there were no significant differences between seedbeds in the total distance

travelled, or in the distance travelled or time before the first feed on a slug pellet. The majority of slugs fed on the first pellet they encountered. Of those slugs that did not feed, more were observed on coarse than on fine seedbeds, but this difference was not statistically significant. We conclude that the success of slug control measures should not normally be influenced by seedbed texture.

Objective 1.4 Method of slug pellet application (broadcasting on soil surface or drilling with seeds)

The activity of individual slugs (*D. reticulatum*) was recorded, using time-lapse, infra-red video techniques, on seedbeds with slug pellets either broadcast on the soil surface or drilled with wheat seeds. Slugs took significantly and substantially more time before their first feed on pellets drilled with seeds compared to pellets broadcast on the surface, indicating that it was more difficult for slugs to find pellets drilled with wheat seeds than pellets broadcast on the surface. This finding supports published studies showing that slug pellets drilled with the seeds are sometimes less effective than pellets broadcast on the soil surface. Pellets drilled with seeds can be completely unavailable to slugs if the seeds are drilled into fine seedbeds that prevent slugs from feeding on the seeds. Pellets drilled with seeds are only effective in seedbeds where slugs can gain ready access to the seeds (e.g. where direct drilling has left open slots or in cloddy seedbeds). They will be more effective than broadcast pellets only if slugs do not come to the surface. However, in cloddy seedbeds pellets fall down between clods and come to rest in the regions where slugs are feeding.

Objective 1.5 Influence of weather, at and after time of application, on efficacy of slug pellets.

We investigated the effects of heavy rainfall on the visibility and distribution of slug pellets based on durum wheat, which are highly resistant to rainfall. Pellets were placed on the surface of coarse or fine seedbeds at field capacity in trays and left for 6 hours, so that the pellets could become well hydrated, then exposed for 30 min to heavy splashy rainfall from an indoor rain-tower. Pellets were less likely to remain visible on coarse than on fine soil. However, although pellets on fine textured soil remained more visible, they were more likely to be moved from their original positions because of soil flooding and surface run-off.

In order to assess the influence of soil contamination on the attractiveness of pellets to slugs, we recorded the activity of individual slugs (*D. reticulatum*), using time-lapse, infra-red video techniques, on seedbeds with slug pellets that were either clean or contaminated with soil. There were no significant differences in the mean time to the first pellet feed between clean and soil-contaminated pellets or between types of pellet. This indicates that although pellets may be contaminated with soil following heavy rainfall, this does not affect the ability of slugs to find the pellets.

Because of the importance of protecting wheat seeds, the normal recommendation for slug pellet application to winter wheat is just before drilling (if the soil can be left undisturbed for a few days after application) or immediately after drilling and rolling, to kill slugs before they can feed on seeds. However, the weather may be unsuitable for pellet activity at the time of drilling, e.g. very dry windy conditions, and it is sometimes considered that it may be better to apply the pellets to the stubble of the previous crop, when the weather is suitable for slug activity and also suitable for maximum pellet efficacy. Such pre-emptive treatment could also have the benefit that slugs are killed before they are turned under by ploughing and

might, thus, prevent slugs moving back to the soil surface after pellets applied at around the time of drilling have ceased to be effective. We, therefore, established a series of experiments in winter wheat and oilseed rape over three years to compare the performance of slug pellets broadcast on stubble up to 6 weeks before drilling, with pellets broadcast on seedbeds soon after drilling and rolling. In total, there were six experiments in winter wheat and two experiments in oilseed rape. Each experiment consisted of a randomised Latin Square with five replicates of five treatments, including untreated plots.

In 2002 and 2003, there were no significant differences in the protection provided at crop establishment by slug pellets applied to stubble compared to pellets applied after drilling and rolling. Dry weather in the late summer and early autumn of these years was unfavourable for slug activity, reproduction and growth and slug populations were unable to recover from stubble treatments during the period when crops were at risk. In autumn 2004, however, wet weather from early August onward was highly favourable to slugs. Under these conditions, slug pellets applied to stubble reduced slug surface activity in the stubble but soil sampling showed that the large reservoir population in the soil was not significantly reduced by slug pellet treatment. By the time that winter wheat crops were at risk, slugs had resumed surface activity following stubble treatments and such treatments were much less effective, over a shorter period, than pellets applied after drilling and rolling. These findings show clearly the role of autumn weather and the importance of applying pellets just before damage is expected in a wet autumn. Application of pellets just after drilling allows a proper assessment of damage risk to be made based on soil and weather conditions at drilling, as outlined under Objective 4, whereas this is not possible for pellets applications to stubble, which, thus, may be made unnecessarily. In essence, stubble treatments are most effective and give 6 weeks protection when they may not really be needed, but they are relatively ineffective when long-lasting protection is important. It is best to assess soil & weather conditions at and after drilling, then treat if necessary.

Objective 1.6 Influence of tillage, sowing date, seed treatment, growth stage of the crop etc

A long-term replicated factorial field experiment was established on clay soil at ADAS, Boxworth, Cambridgeshire in 2001, to compare slug activity and slug damage on consolidated seedbeds and loose seedbeds, with slug pellets applied pre-emergence, post emergence or both pre- and post-emergence. Winter wheat was sown in autumn 2001 and 2002, followed by oilseed rape in autumn 2003 and winter wheat in autumn 2004. Imidacloprid seed treatment (Sibutol Secur, Bayer CropScience) was also included as a factor in autumn 2001. Stubble-applied treatments of slug pellets were compared in autumn 2002, 2003 and 2004.

For winter wheat, drilled on 19 October 2001, slug pellets applied pre-emergence or both pre- and post-emergence significantly reduced the percentage of plants damaged by slugs in December 2001. The reduction in damage after the post-emergence treatment was not significant compared with the untreated mean (40%). Seedbed consolidation had no significant effect in December, but slug activity, predominantly *D. reticulatum*, continued during the late-winter period peaking in January. On three dates in November and December, slug activity was significantly higher on the consolidated compared with the loose seedbed but in January and March, no differences were recorded. On 7 March, the mean percentage of plants damaged by slugs was significantly lower on the consolidated seedbed compared with the loose seedbed (means of 5.9%

and 15.5%, respectively). The effects of imidacloprid seed treatment were not significant compared with the non-insecticide treated seed (Sibutol).

In autumn 2002, significant reductions in slug activity were recorded during November for the pre-emergence treatment applied after drilling winter wheat on 24 October. However, there was considerably less slug damage than in 2001 and no significant reductions in slug damage were obtained from any treatments in autumn/early winter 2002. During November, significant reductions in slug activity were obtained from stubble-applied pellets. On two dates in November 2002, there was significantly greater slug surface activity on consolidated than on loose seedbeds but, by March 2003, this difference was reversed and slug damage was significantly lower on consolidated than on loose seedbeds. Thus, as in the previous year there was initially greater surface activity on consolidated seedbeds, which probably reflected the difficulty that slugs had in moving through the soil and was not a predictor of slug damage. This supports the view that, after crop emergence, crop damage is a better indicator of the need for control measures than trap catches. In both 2001-02 and 2002-03, the reduction in damage from seedbed consolidation extended beyond the period of protection obtained from molluscicide, demonstrating the value of consolidation for controlling slug damage. The results for 2001-02 also clearly demonstrate the benefits of integrated control, with initial protection provided by slug pellets and later protection from consolidation.

Winter oilseed rape was drilled on 3 September 2003 but establishment was initially poor in dry conditions. Slug activity increased during late October and was significantly lower on ploughed compared with direct-drilled plots. Slug activity was significantly reduced on 5 November by pre-emergence, post-emergence and pre + post-emergence pellet applications compared with the untreated (0.8 slugs/three traps). Significant reductions were also obtained on 6 January from pellets applied post-emergence or pre + post-emergence compared with the untreated mean (0.7 slugs/three traps). Although the incidence of damage was slight, the percentage of oilseed rape plants damaged was significantly lower where plots were established by ploughing compared with direct drilling. These results demonstrated a lower incidence of slug activity after ploughing and emphasised a possible benefit from reduction of slug damage to oilseed rape.

Winter wheat drilling was delayed by wet weather until 13 November 2004. Pre-drilling slug activity was higher than in autumn 2001-2003 and was significantly lower on plots that had been established in the previous year by ploughing compared with direct drilling. A stubble application of slug pellets on 21 October provided significant reductions in slug activity compared with untreated plots on 25 October and 1 November. Post drilling slug activity declined and no significant differences were recorded for post-drilling applications of slug pellets or for mean plant populations or percentages of wheat plants damaged by slugs.

Objective 2. Quantify relationships between slug populations and conditions in the previous crop to evaluate their use as a damage indicator in the succeeding crop.

Individual-based simulation models have been recently developed, which incorporate, for the first time, all the major factors thought to affect the population dynamics of *Deroceras reticulatum*. Simulation models are particularly valuable for this species because of its flexible life-cycle (it can breed at any time of year when

conditions are suitable), its rapid rate of growth and high reproductive capacity. The predictions of the *D. reticulatum* population models were compared with the dynamics of slug populations in arable fields.

The predictions of the model of Shirley *et al.* (2001) over a series of 6-week periods (considered to be sufficient for practical pest forecasting) were compared with the numbers of slugs recorded in defined area traps on an arable field in set-aside in north-east England. When the model was run using the daily weather for the period of study, the model predictions closely matched the measured populations. However, when the model was run using average weather, as would need to be done for damage forecasting, the model predictions were sometimes very different from measured populations. This finding emphasises the difficulty of predicting slug populations when future weather cannot be accurately forecast for more than a few days.

The predictions of the model of Shirley *et al.* (2001) were also compared in the same way with data on *D. reticulatum* populations from arable fields in south west England, obtained by soil sampling, using field-collected data as the starting point. Even when the model was run using the daily weather for the period of study, the model predictions diverged from the measured population in three out of four six-week periods. Possible reasons for these discrepancies were examined and it was concluded that incorrect estimation of the egg bank by the model was the most likely explanation. When the model was run for adult slugs (>200mg) only, the correspondence between modelled and actual populations was much better. This was expected because the model had been calibrated using data for active slug populations from traps.

The simulation model of Choi *et al.* (2005) has been constructed from a model of the dynamics of slug populations in continuous wheat in relation to daily temperature and rainfall. This simulation model was compared with data on *D. reticulatum* populations in cereal fields going into oilseed rape in south west England. Once again lack of knowledge of the size of the egg bank made it necessary to estimate this. However, by adjusting the egg densities in March, it was possible to obtain good predictions of the densities of juvenile and adults until mid August. From then, the model overestimated the slug population. We believe that the reason for this is that the study fields were cultivated for oilseed rape in late August and we know that, following cultivation, the *D. reticulatum* population was reduced to about 10-15% of its previous level.

We conclude that the population models of Shirley et al. (2001) and Choi et al. (2005) can be used, together with assessments of slug populations to provide useful estimates of *D. reticulatum* activity and population development, up to the time of autumn cultivation, provided that the models are run using actual daily weather. These models need further development to be used as decision support tools (see Objective 4).

Objective 3. Develop a reliable predictor of crop damage based on these relationships.

Trapping to assess slug activity

Earlier projects funded by HGCA have shown that (1) the number of slugs active on the soil surface and recorded in traps is dependent on soil moisture and temperature and (2) the number of slugs recorded in traps before cultivation is the best predictor of the severity of damage to winter wheat. A catch of four or more slugs per trap baited with slug pellets, after three nights, in the period before cultivation indicates a potential risk to winter wheat. By using simple thresholds for air temperature and soil moisture it is possible to

identify periods when slug activity will be high and suitable for trapping or for control with molluscicides. These thresholds have been combined in a decision support model, called the Trap or Treat model.

We compared the numbers and sizes of slugs found in traps in arable fields with the numbers/m^2 and sizes of slugs recorded from soil samples taken at the same time. The relationship between trap numbers and slug density in soil varied. Under suitable conditions for slug activity (moist soil surface and mild moist weather), the number of slugs (Ó100 mg) per trap was approximately equivalent to the density of slugs of this size class per m^2 of soil. However, small slugs (<100 mg) were under-recorded in traps compared to their densities in soil. This is a disadvantage of trapping, as we have shown that, weight-for-weight, small *D. reticulatum* and *M. gagates* can kill more wheat seeds than larger slugs under laboratory conditions.

In order to investigate the reasons for this difference in numbers of large and small slugs recorded in traps, we conducted time-lapse infra-red video studies to compare the behaviour of large (>500 mg) and small *D. reticulatum* (<100 mg) entering and leaving special traps which are opaque when viewed in daylight but are transparent to infra-red illumination. Recordings were made overnight both indoors and outdoors. Significantly more slugs of both size classes entered traps during the night than were present at dawn. However, similar numbers of both size classes entered the traps during the night and the reduction in numbers at dawn was similar for both size classes. Thus, these observations do not explain why slugs <100 mg are under-recorded in traps in the field.

Although traps baited with slug pellets have been used for monitoring slug activity, slug pellets are undesirable in traps because in concentrated amounts they are a potential hazard to pets and wildlife. Chicken layers' mash is known to be an effective non-toxic bait for slug traps. In order to use traps with this bait for damage prediction, we compared the numbers of slugs in traps baited with slug pellets with the numbers in traps baited with layers' mash, under weather conditions suitable for slug surface activity. The numbers recorded after one night in traps baited with layers' mash were strongly correlated with numbers recorded after three nights in pellet-baited traps. When mean numbers were at the threshold value of four slugs per trap in the pellet-baited traps after three nights, this was equivalent to a catch of about four slugs overnight in mash-baited traps overnight. We conclude that a threshold catch of four slugs per trap baited with chicken layers mash overnight indicates a potential risk to a following crop of winter wheat.

Objective 4. Predict the need for and timing of slug pellets as part of an integrated control strategy, whilst reducing unnecessary use.

System of risk assessment and integrated control for winter wheat

Damage to winter wheat seeds and seedlings before emergence directly affects yield. This damage is highly dependent on agronomic and weather conditions as well as slug activity, so that treatment is necessary when trap catch has exceeded the threshold and agronomic and weather conditions are suitable for slug damage.

We have developed a system to assess the risk of slug damage, which is closely interlinked with cultural control measures. There are four main elements:-

1. Trap to assess slug activity during the period before cultivation and possibly after drilling.
2. Use trap catches *together with other information* to assess the risk of slug damage

3. Reduce the risk of slug attack by cultivations and adjustment of drilling depth
4. Monitor crops throughout the early susceptible growth stages

1. Trap to assess slug activity during period before cultivation and possibly after drilling.

Trapping should be done during the period before cultivation. It is essential to take advantage of suitable weather for trapping and put traps in place only when the soil surface is moist and temperatures are favourable for slug activity (minimum night temperature greater than 5^{o}C, maximum daytime temperature less than 25 ^{o}C). After cultivation, trapping may under-estimate the true slug population as surface activity is reduced. However, trapping between drilling and emergence is valuable if wet weather persists, because under these conditions increasing slug populations may pose a threat to emerging wheat.

Nine traps baited with layers' mash should be laid out in a 'W' pattern in each field (13 traps if the field is larger than 20 ha). If certain areas of the field are known to suffer from slug damage, traps should be concentrated in these areas. It is a distinct advantage that traps with this bait are examined after one night. However, slugs can leave traps if they become too hot after exposure to sun, so that early morning examination may be required. Slugs will remain in traps if the sky remains overcast or if the weather is cool.

2. Use trap catches together with other information *to assess the risk of slug damage*

For winter wheat, seedbed conditions are particularly important in influencing the vulnerability of seeds to slug damage and probably explain much of the variation in the relationship between slug numbers in traps and the percentage of wheat seeds killed. An average of four or more slugs per trap will justify slug pellet treatment, provided that favourable conditions for slug activity (and control) continue and provided that: -

- the field is drilled during a period of generally wet weather
- wet weather delays sowing in a prepared seedbed
- the seedbed tilth is coarse and cloddy, and further consolidation is not possible following sowing
- wet weather continues after drilling and further trapping shows evidence of high slug activity on the seedbed
- the crop is slow to emerge or to grow through the early vulnerable stages and symptoms of slug damage are seen.

3. Reduce the risk of slug attack by cultivations and adjustment of drilling depth

The best approach to prevent early damage is sowing at sufficient depth (3 cm) in a fine, consolidated seedbed to deny access to the seeds by slugs and provide suitable conditions for rapid germination. In cloddy seedbeds, seeds should be sown a little deeper (4-5 cm), where they are better protected.

The more cultivations and the more intensive they are, the greater the likelihood that slug numbers will be reduced, especially in dry weather. However, whatever the method of cultivation, it is important that the seedbed is fine and firm to protect seeds and young seedlings. Thus, although reduced tillage can allow more slugs to survive compared with ploughing, it can have advantages for slug control if it produces finer seedbeds compared with ploughing. Moreover, surviving slugs are not buried at depth by reduced tillage, as

we have shown happens after ploughing, so slugs are more likely to be surface-active following reduced tillage and therefore vulnerable to slug pellets. Reduced tillage also retains seedbed moisture for germination under dry conditions, which helps the crop to grow rapidly through the early vulnerable stages.

4. Monitor crops throughout the early susceptible growth stages

Crops should be examined regularly for slug damage. Slug trapping is not normally necessary after emergence except if there is any doubt about whether the damage is caused by slugs. Cereal crops are most susceptible to damage from sowing to first tillering (GS 21). After this growth stage is reached, further damage is less likely to result in additional loss of plants. However it is important to continue to monitor crops throughout the winter and be ready to treat if there is evidence of fresh damage to young leaves and plants show signs of being set back by slug damage.

Basis for system of risk assessment for winter wheat using slug population models

As described under Objective 2, population models can be used to provide an estimate of *D. reticulatum* populations, up to the time of cultivation in autumn, provided that (1) the models are initially calibrated with slug populations in the soil and (2) the population predictions are updated using actual daily weather. This can be done up to the date of cultivation, then the effect of cultivation can be estimated (based on studies in this project and earlier published information), to provide an estimate of the likely size of the population following cultivation. If the field is drilled immediately after cultivation, there is no need to run the population model again, but if drilling is delayed, the model can then be run again to estimate slug population size at drilling. The potential percentage seed killed by slug can then be estimated using slug biomass, the % fine soil aggregates in the seedbed and seed depth.

Risk assessment and integrated control for oilseed rape

There is little published information on the relationship between slug numbers and damage to oilseed rape. For this reason, from 2002 to 2004 we investigated whether it would be possible to assess the risk of slug damage based on the numbers of slugs trapped in cereal crops and stubble prior to drilling winter oilseed rape. There was a significant correlation between the numbers of slugs trapped when conditions in cereal stubble were suitable, and the percentage of oilseed rape seedlings damaged by slugs up to the two-leaf stage. The upper limit of this relationship is currently our best indication of slug damage risk to oilseed rape seedlings when weather and crop conditions favour slug survival and feeding. The relationship between the numbers of slugs trapped in cereals before harvest and damage to oilseed rape seedlings was less good. However, because weather during the short period between harvesting cereals and drilling winter oilseed rape may be unsuitable for trapping, it may be worthwhile to trap in standing cereals up to 10 days before harvest, particularly if oilseed rape seeds are to be broadcast into standing cereals or stubble (eg Autocast).

As a guideline originating from this project, a catch of four or more slugs/trap in standing cereals, or one or more slugs/trap in cereal stubble indicates a potential damage risk if other conditions are met, as described in the Decision Tree.

PAPER 1 - *Objective 1.1*

Factors Influencing the Batch Size, Development and Hatching Rate of *Deroceras reticulatum* Eggs

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Abstract

The effects of laying season (spring or autumn) and incubation temperature on early stages in the lifecycle of *Deroceras reticulatum* (Müller) were investigated. Eggs laid by wild-caught parents were kept at ambient temperature, constant 12°C or constant 15°C. Egg batch size was positively associated with parental weight in spring, but not in autumn. Batch size, but not laying season or incubation temperature, was a significant predictor of whether any of the eggs in a batch hatched. Development time was inversely related to incubation temperature and this was modified by the laying season. Hatching rate, however, did not vary with temperature or laying season, and the overall mean value (± S.E.) was 65.9 ± 5.8 %.

Introduction

Underpinning effective slug control in arable crops is the need to predict when and where particular increases in populations are likely to occur so that steps can be taken in advance to minimise the economic loss that would otherwise result (Schley & Bees, 2003). Various models have been proposed in recent years to forecast changes in slug population dynamics as a consequence of environmental factors and their impact on growth and mortality (e.g. Bohan *et al.*, 1997; Shirley *et al.*, 2001; Choi *et al.*, 2004). Due to the complexity of slug behaviour and limited data on some aspects of their fundamental biology, however, these are often based on a number of assumptions about lifecycle parameters and have, to a greater or lesser extent, only been able to predict confidently part of the overall picture. There is a need, therefore, for studies to bridge these gaps in the knowledge of *Deroceras reticulatum* biology so that models can give more accurate predictions.

The potential of a slug population to attain pest proportions is largely determined by its initial size and the speed with which it can complete its lifecycle whilst favourable conditions prevail. The initial population size in short-lived, annual species is effectively dependent upon the number of eggs laid, and how many of them develop and hatch to give rise to the following generation. A number of early studies have shown that temperature affects hatching, and that under natural conditions, hatching takes longer in autumn as eggs over-winter (e.g. Carrick, 1942; Arias & Crowell, 1963; Pinder, 1969). There is nothing published, however, that assesses the effect on development time and hatching rate of rearing eggs laid in different seasons at the same temperature; this may be an important first step in beginning to refine predictions to take into account

the impact of unseasonable weather conditions on the next cohort, such as the exceptionally mild autumns or winters experienced in recent years. Furthermore, there is little that addresses the question of whether parental slug weight influences batch size. These aspects are investigated in the studies presented in this paper. Whilst this paper is concerned with early stages in the lifecycle of *D. reticulatum*, focusing on the period from egg laying to hatching, the work forms part of an extensive study into the development of *D. reticulatum*. After hatching, individuals entered into the next stage of the study which assessed growth and survival rates (*Paper 2*).

Materials and Methods

The eggs used in each of the spring and autumn experiments were laid by 50 adult *D. reticulatum*. The slugs were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). After collection slugs were weighed, using a Mettler MT5 balance, and placed in individual Petri dishes lined with moist laboratory tissue. They were fed with Chinese cabbage and carrot *ad libitum.* Cuttle fish bone was provided as a source of calcium. The slugs were maintained at a constant temperature of 20 ± 2°C (mean ± S.E.) in a Sanyo MIR-253 incubator with a constant photoperiod of 16L:8D and were cleaned weekly by transferring them to a clean dish with fresh food. Over a two week period a total of 60 egg batches were collected. Each batch was placed on fine grade netting and rinsed with distilled water to remove any soiling before being transferred into another Petri dish lined with moist laboratory tissue. The number of eggs per batch was recorded.

In each season the 60 egg batches were allocated equally and at random to three temperature treatments; two constant (12 ± 2°C and 15 ± 2°C) (mean ± S.E.) and one fluctuating (ambient). The mean ambient temperature (± S.E.) during the hatching period was 13.3 ± 0.1°C (range 4.3-24.1°C) in spring and 5.7 ± 0.1°C (range -3.0-21.8°C) in autumn. Constant temperatures were maintained in Sanyo MIR-235 incubators with a photoperiod of 16L:8D, provided by two fluorescent tubes (1 x 15 Watt and 1 x 13 Watt). The temperatures were monitored using Tinytalk® data loggers (Gemini Data Loggers, UK). For the ambient treatment Petri dishes containing egg batches were placed in a plastic tank and housed outside the Ridley Building, University of Newcastle upon Tyne. The temperature inside and outside the tank was also recorded using a Tinytalk® data logger and there was no additional light (i.e. natural photoperiod).

Egg batches were prevented from drying out by remoistening with distilled water as required. Hatching was checked weekly. Regular monitoring continued until two full weeks had elapsed since the last slug hatched. On each occasion any offspring were removed from the Petri dishes and entered into the next stage of the study (*Paper 2*). The development time and number of slugs hatching per batch were recorded.

Regression was used to analyse variables between which a cause-effect relationship was postulated; for binomial data binary logistic regression was applied, otherwise linear regression was used. Comparisons between regression lines were made according to the procedure detailed in Zar (1999).

Continuous data were tested for normality and transformed if necessary. Percentages were arcsine transformed. In all cases this resulted in parametric data which were then analysed using analysis of variance (ANOVA) followed by Tukey (equal variances assumed) or Dunnett (equal variances not assumed) post-hoc tests as appropriate.

Results

Parents laying eggs in spring were significantly heavier than those laying eggs in autumn (ANOVA: $F_{1, 67} = 6.266$, $P < 0.001$). The mean parental weight (± S.E.) in spring was 585.43 ± 22.73 mg and in autumn it was 469.10 ± 18.31 mg. The mean batch size did not differ between treatments either between or within seasons (*Table 1.1*).

Table 1.1: Results of two-way analysis of variance (ANOVA) to compare mean batch size of Deroceras reticulatum *eggs between incubation treatments within and between seasons.*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	2	61.22	30.61	0.43	*n.s*
Season	1	6.08	6.08	0.09	*n.s*
Interaction	2	33.95	16.98	0.24	*n.s*
Error	114	8155.75	71.54		

For eggs laid in spring there was a significant relationship between parental weight and both the size of the first batch laid (linear regression: N = 31, $R^2 = 0.280$, $P < 0.01$) and the mean batch size during the collection period (linear regression: N = 31, $R^2 = 0.142$, $P < 0.05$) (*Fig. 1.1 (a) & (b)*). Although the P-values are both below the critical 5% level, however, the R^2 value which indicates the amount of variation in one variable that is explained by the other, is relatively small. There was no significant relationship between parental weight and either the size of the first batch laid (linear regression: N = 38, $R^2 = 0.094$, *n.s.*) or the mean batch size during the collection period (linear regression: N = 38, $R^2 = 0.011$, *n.s.*) for the eggs laid in autumn (*Fig. 1.1 (c) & (d)*). In *Fig. 1.1*, the regression lines are shown for eggs laid in spring and were set to intercept at the origin since the number of eggs must equal zero when parental weight equals zero.

The analyses presented to investigate the relationship between hatching and batch size were confined to constant temperature treatments only since heavy rain in spring 2002 resulted in flooding of a number of ambient treatment batches and the loss of some eggs. Batch size, laying season and incubation temperature were assessed as potential predictors of whether any of the eggs in a batch hatched. Batch size had a significant influence; the larger the batch size the more likely it was that at least one of the eggs would hatch (Binary logistic regression: N = 76, Z = 2.65, $P < 0.01$, percentage concordant pairs = 78.5%) (*Fig. 1.2*). Specifically, the odds ratio of 1.18 indicated that the chances of at least one egg in a batch hatching increased

1.18 times with each successive single egg increase in batch size (i.e. approximately 20%). Incubation temperature and laying season had no effect.

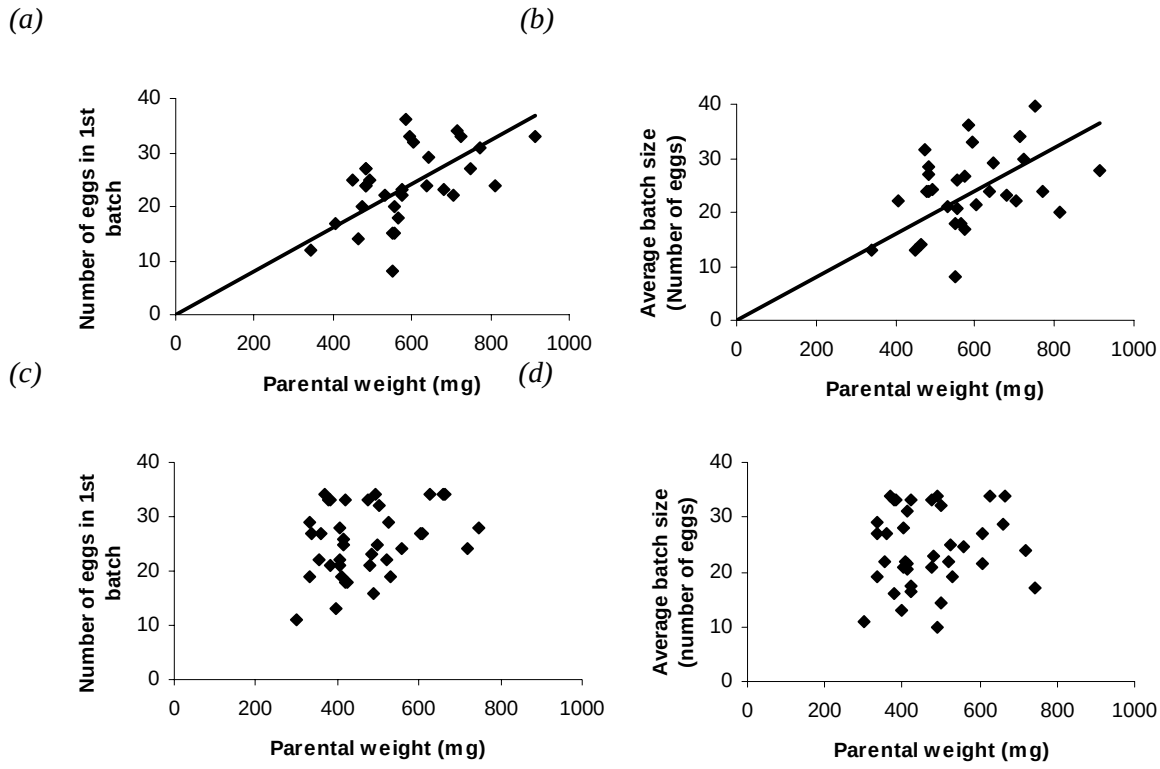

Figure 1.1: Scatterplots of the number of Deroceras reticulatum *eggs in the first batch and the mean batch size of eggs laid in spring (a) & (b) and autumn (c) & (d) against parental weight. Regression equation for (a): y = 0.040x; (b) y = 0.040x.*

The total number of eggs hatching was related to batch size in a linear fashion (*Fig. 1.3*). For every combination of laying season and incubation temperature the number of eggs hatching increased with batch size (linear regression: Spring: 15°C: N = 20, $R^2 = 0.61$, $P < 0.001$; 12°C: N = 20, $R^2 = 0.52$, $P < 0.001$; Autumn: 15°C: N = 20, $R^2 = 0.64$, $P < 0.001$; 12°C: N = 16, $R^2 = 0.36$, $P < 0.01$). Comparisons between the four regressions using analysis of covariance showed that there were no significant differences in their slopes or elevations, i.e. the four regressions are coincident and, therefore, at least at the constant temperatures studied here, the number of eggs hatching per batch is unaffected by laying season or incubation temperature (ANCOVA: $F_{6,68} = 0.41$, *n.s.*). The regression lines were set to intercept at the origin since the number hatching must equal zero when batch size equals zero.

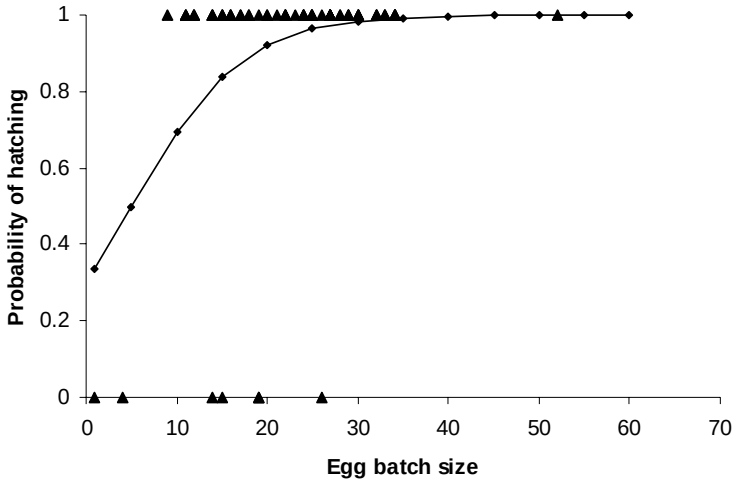

Figure 1.2: Results of binary logistic regression analysis to investigate the relationship between batch size and whether any eggs hatched for Deroceras reticulatum. *Binary logistic regression equation: Probability of hatching = (exp (-0.842 + 0.1659 x batch size))/(1 + exp (-0.842 + 0.1659 x batch size)). (Binary response indicated by black triangles; probability of hatching predicted by binary logistic regression indicated by solid line).*

For the purposes of comparison with eggs incubated at field temperatures, regression analysis was performed on the data from autumn laid eggs incubated at ambient temperature, which were not affected by flooding. The trend observed at constant temperatures was not confirmed; there was no significant relationship between batch size and the number of eggs hatching for autumn laid eggs reared at ambient temperature (linear regression: Autumn: ambient: N = 20, $R^2 = 0.08$, *n.s.*).

The analysis of the influence of incubation temperature and laying season on development time is based on data from all three incubation temperatures. Evidence suggests that *D. reticulatum* eggs can withstand complete immersion in water for a number of days with no effect on development (Arias & Crowell, 1963; Rollo & Shibata, 1991). It is therefore assumed that the brief period of flooding described above did not have any detrimental effect on remaining eggs. Incubation temperature, laying season and an interaction between these two factors all influenced egg development time (*Table 1.2*).

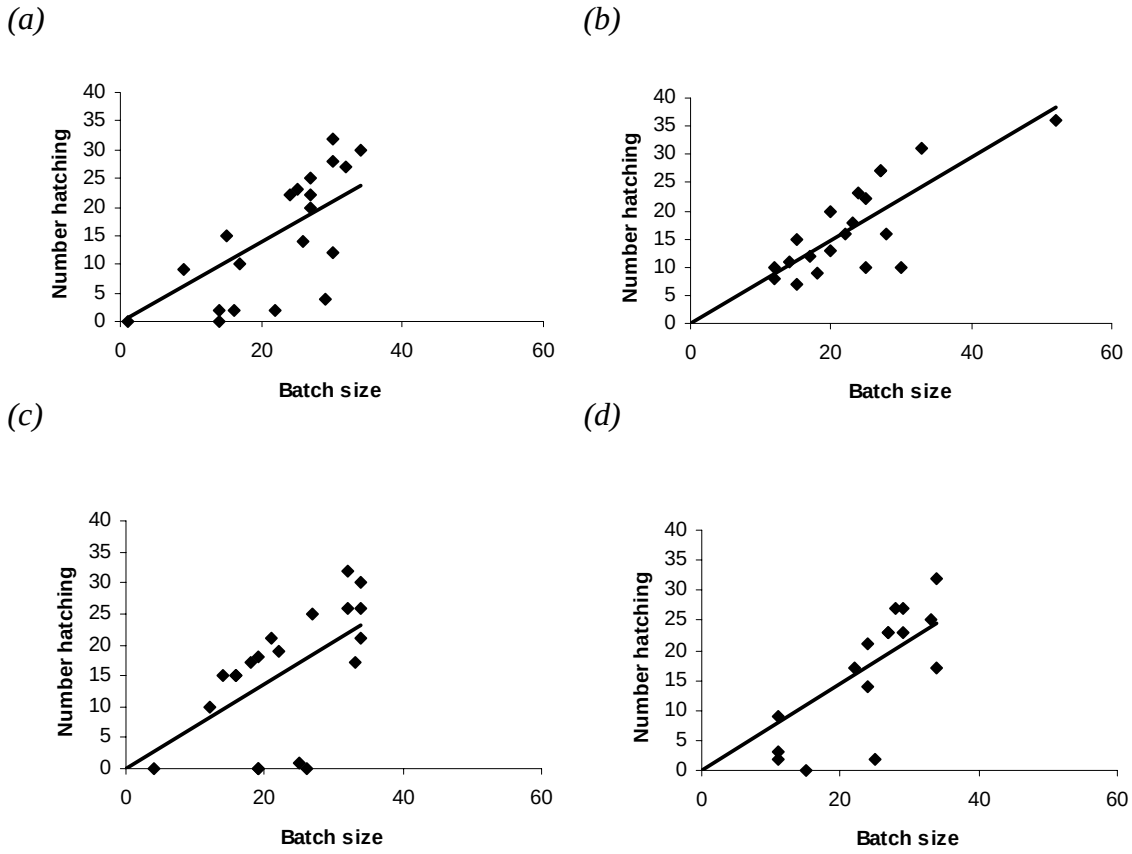

Figure 1.3: Scatterplots of the number of Deroceras reticulatum *eggs hatching and the batch size for eggs laid in spring at (a) 12°C & (b) 15°C and in autumn at (c)12°C & (d) 15°C. Regression equation for (a) y = 0.700x; (b) y = 0.738x; (c) y = 0.680x; (d) y = 0.723x.*

Table 1.2: Results of two-way analysis of variance (ANOVA) to compare the development time of Deroceras reticulatum *eggs at three incubation temperatures (ambient, 12°C and 15°C) in each of two seasons (spring and autumn).*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	2	1.35	0.67	148.47	*< 0.001*
Season	1	0.34	0.34	75.12	*< 0.001*
Interaction	2	0.43	0.21	47.10	*< 0.001*
Error	101	0.46	4.53×10^{-3}		

Inspection of the mean values for each combination of incubation temperature and laying season (*Fig. 1.4*) indicated that for eggs laid in spring those incubated at 15°C developed sooner than those at 12°C or ambient temperature. There was little difference between these latter two temperatures (mean spring ambient temperature during hatching (± S.E.) = 13.3 ± 0.1°C). The mean development time of eggs laid in autumn differed between all three incubation temperatures with hatching occurring sooner the higher the temperature (mean autumn ambient temperature during hatching (± S.E.) = 5.7 ± 0.1°C).

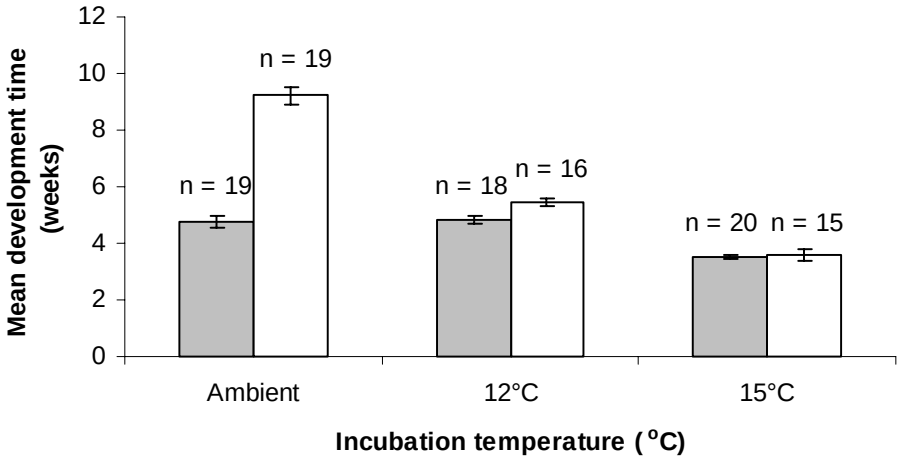

Figure 1.4: Mean development time for Deroceras reticulatum *eggs laid in spring and autumn, incubated at ambient temperature, 12°C and 15°C (± S.E.) (grey bars = spring; white bars = autumn).*

Since there is a much greater seasonal difference in development time at ambient temperature than at either constant temperature, the analysis was repeated for constant temperatures only, to see whether the interaction was still apparent *(Table 1.3).* Although the significance level for season and the interaction term were reduced in this second analysis the overall results were unaltered. As there is virtually no seasonal difference in the development time at 15°C it can be inferred that the interaction is due mainly to the 12°C treatment for the constant incubation temperatures.

Table 1.3: Results of two-way analysis of variance (ANOVA) to compare the development time of Deroceras reticulatum *eggs between two constant incubation temperatures (12°C and 15°C) in each of two seasons (spring and autumn).*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	1	42.22	42.22	131.53	*< 0.001*
Season	1	2.03	2.03	6.32	*< 0.05*
Interaction	1	1.40	1.40	4.35	*< 0.05*
Error	65	20.86	0.32		

To investigate the influence of incubation temperature and laying season on hatching rate, hatching rate is expressed as the percentage of the total egg batch that hatched. Since the fate of eggs washed away by flooding in the spring 2002 ambient treatment was unknown these results pertain only to the constant incubation temperatures. *Table 1.4* shows the mean hatching rate (± S.E.) for each laying season and incubation temperature. There were no significant differences between treatments (*Table 1.5*). For the purposes of comparison the hatching rate of slugs laid in autumn and reared at ambient conditions, which

were not affected by flooding, was 66.0 ± 7.11%. The results from constant incubation temperatures in the laboratory therefore give a good indication of what is observed at field temperatures.

Table 1.4: Mean hatching rate (± S.E.) of Deroceras reticulatum *eggs at each of two laying seasons and incubation temperatures.*

Laying Season	*Incubation Temperature (°C)*	*n*	*Mean Hatching Rate ± SE (%)*
Spring	12	20	60.0 ± 8.55
	15	20	74.5 ± 4.72
Autumn	12	20	65.1 ± 8.94
	15	16	63.6 ± 8.17

Table 1.5: Results of two-way analysis of variance (ANOVA) to compare the hatching rate of Deroceras reticulatum *eggs between two constant incubation temperatures (12°C and 15°C) in each of two seasons (spring and autumn).*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	1	462.08	462.08	0.65	*n.s*
Season	1	301.55	301.55	0.42	*n.s*
Interaction	1	698.06	698.06	0.98	*n.s*
Error	72	51153.12	710.46		

Discussion

It is difficult to study the relationship between parental weight and batch size in wild-caught slugs because we do not know their laying history prior to being brought into the laboratory. There is evidence from work on the snail, *Helix aspersa* (Müller), that the number of batches previously laid affects batch size (Madec *et al.*, 2000). These authors showed that for wild-caught individuals brought into the laboratory towards the end of hibernation there were significantly more eggs in the first batch laid than in subsequent batches. Since slugs do not hibernate we do not have a similar 'start-point' to indicate whether a batch is the first that an individual has laid, or another in a long sequence of batches. Furthermore, it has been reported in 'higher' molluscs that the nutritional status of the parent affects batch size (Steer *et al.*, 2004). This may also be of importance in slugs, but again, it is an unknown factor in field collected specimens. The results presented here, therefore, need to be interpreted with caution particularly in view of the low, albeit significant, R^2 values. They are offered as a preliminary exploration of this relationship that could be investigated more comprehensively in future studies.

That there is a link between parental weight and number of eggs produced has been reported in a number of species, for example in the molluscs *Loligo vulgaris* (Lamarck) (Coelho *et al.*, 1994) and *Helix aspersa* (Madec & Daguzan, 1993) as well as crustaceans (Runge, 1984; Austin, 1998) and fish (Coward & Bromage, 1999). In the studies detailed here both the mean batch size for a given parent during the collection period and the size of the first batch laid in the laboratory were positively associated with parental weight of slugs collected in spring, but not in autumn. There are a number of potential explanations for this observation. Firstly, slugs laying eggs in spring have over-wintered as eggs or juveniles themselves and consequently have developed slowly over a longer period of time than those laying eggs in autumn which probably hatched in spring of the same year and reached maturity more rapidly. This difference in development time may result in spring laying parents being a more homogeneous group than the autumn parents, with a more stable reproductive physiology. A study using *Helix aspersa* found that parents with slower growth contributed more to offspring production than faster growing parents and suggest that this may be due to differences in reproductive ability (Dupont-Nivet *et al.*, 2000). This may also be the case for *D. reticulatum.*

A second explanation could be that this seasonal difference is related to temperature conditions experienced by slugs prior to being brought into the laboratory. Under natural conditions slugs laying eggs in spring have been adapting to gradually rising temperatures following winter. Although the incubation temperature used in these experiments is, at 20°C, higher than ambient conditions for spring in the North East of England, it is in the same direction that the slugs have been acclimatising to, i.e. warmer than in winter. Slugs laying eggs in autumn would, conversely, have been acclimatising to gradually cooler temperatures towards winter and the incubation temperature is, therefore, in the opposite direction to natural conditions for the time of year. Perhaps this resulted in 'thermal shock' which disrupted egg laying. It has been shown that the mean supercooling point of *D. reticulatum* rises in the winter, i.e. slugs freeze at higher temperatures in the winter, although they are able to survive this freezing for almost twice as long compared to the summer (Cook, 2004). Evidently there is a shift in thermal tolerance during autumn and slugs may be more sensitive to unseasonably high temperatures at this time of transition.

A third possibility is that the lack of an association between batch size and parental weight in autumn is due to egg laying becoming less coordinated towards the end of the reproductive period. By the time the parents were collected from the field in autumn they may have been near the end of their peak in egg production whereas those collected in spring were more likely to be at the start of this process. This may explain why the association was stronger earlier in the year.

There is a scarcity of studies in the literature that investigate the relationship between hatching and batch size. In the experiments presented here, the larger the batch, the greater was the chance that at least one of the eggs would hatch. The incremental rise in the odds of hatching with each additional egg in the batch seemed, initially, to be relatively high at just under 20%. There is nothing in the literature, however, that

details the baseline chance of hatching, i.e. the absolute probability that a single egg will hatch. This increase in the odds of hatching may actually be 20% of a very small number. Unfortunately, it was not possible to estimate this from the current study since there was only one batch where a single egg was laid.

The relationship between number of eggs hatching and batch size was more or less linear at constant incubation temperatures, which follows from the previous result. The comparison of regression lines for this relationship indicated that, at least at 12°C and 15°C, the number of eggs hatching was unaffected by the laying season or rearing temperature. This supports the previous analysis of factors influencing whether or not at least one egg in a batch hatches; laying season and incubation temperature were found to have no effect here either.

Interestingly, however, for the eggs laid in autumn and incubated at ambient temperature, there was no relationship between batch size and the number of eggs hatching. There were no significant differences in batch size between temperature treatments and since egg batches were allocated to the treatments randomly any differences in egg quality would be expected to affect hatching equally. It seems improbable that any insulation effect, with eggs in the middle of batches being more protected from very cold temperatures by eggs on the edges of the batches, would be strong enough to alter hatching dynamics either; temperature differences would not be great enough. A more likely explanation of this difference between constant and field temperatures is that the assessment period favoured the former. Assessment ceased when two full weeks had elapsed since the last egg hatched. This period was chosen for reasons of practicality as the next stage of the experiment was underway and was very labour intensive (*Paper 2*). The results from the study of development times, however, show that this is faster and there is less variation at constant incubation temperatures, i.e. the standard errors are smaller. If hatching had been monitored for longer than two weeks after the last slug hatched then perhaps continued sporadic hatching would have been observed at ambient conditions and a pattern between number of eggs hatching and batch size similar to that observed at the constant temperatures may have been apparent.

It was observed that egg development time was generally inversely related to temperature. This is in agreement with the findings from other studies (e.g. Carrick, 1942; Judge, 1972; South, 1989b; Rollo & Shibata, 1991). It was shown by Rollo and Shibata (1991) that the development time for *Deroceras laeve* (Müller) eggs was determined by the mean of fluctuating temperature regimes rather than the magnitude of the temperature range. This could explain why the development times for *D. reticulatum* in the spring experiments presented here did not differ significantly between the ambient and the constant 12°C treatments. Although the mean ambient spring temperature of 13.3 ± 0.1°C was slightly higher than the constant 12°C this was not sufficient to result in a significant difference in development time between these treatments. Development times at both of these temperatures, however, were longer than at 15°C. In autumn, mean ambient temperature during the development period was considerably lower than either

constant temperature, at 5.7 ± 0.1°C and the inverse relationship between temperature and development time was clear (*Fig. 1.4*).

The effect of season on development time was very interesting. The ambient treatments effectively acted as a control to confirm that eggs were developing normally for the given time of year. The development time here was, as expected, more prolonged in cooler autumn conditions compared to spring. The contrast between seasonal development times at each of the constant temperatures was, however, surprising. If temperature was the main factor influencing development time, given uniform moisture, humidity and day light regime, then we would expect that it would take the same length of time at a given constant temperature, regardless of the laying season, i.e. we would expect day-degrees to determine the development time. There was, however, a significant interaction between temperature and season for constant temperature treatments, meaning that the development times at a given temperature are not the same in both seasons, i.e. day-degrees alone do not fully account for differences in development times. The results indicate that at 15°C the development times were almost the same in spring and autumn (*Fig. 1.4*) implying that the interaction is due mainly to the 12°C treatment. This suggests that there may be a temperature threshold below which laying season influences development time, but above which the increased temperature 'over-rides' the effect of season. These findings are supported by a study of the freshwater snail, *Lymnaea auricularia* (L.), where differences were observed in the development time of spring and summer laid eggs incubated at the same constant temperatures, although a threshold was not postulated (Salih *et al.*, 1981).

In the North East, 12°C corresponds to a warm spring/cool autumn whereas 15°C would be an above-average autumn temperature. Perhaps autumn laid eggs have something inherently different about them that means development is delayed unless temperatures are consistently above the threshold. The adaptive significance of this is that development of autumn laid eggs would not be accelerated as a result of brief periods of mild weather following which a return to progressively cooler conditions might cause considerable mortality among hatchlings. To explore this idea further development times of *D. reticulatum* eggs at temperatures in between 12°C and 15°C, along with switches between temperatures, could be studied to see whether it is possible to pinpoint more accurately a putative 'threshold' value. It would also be necessary to repeat the experiment at temperatures above 15°C to confirm that season continues to have no effect on development time.

Temperature and laying season had no effect on hatching rate under the conditions tested in these experiments. The overall mean hatching rate was 65.9 ± 5.8 %. Although Judge (1972) found similar hatching rates for *D. reticulatum* at temperatures within the range examined here, he found that at considerably higher incubation temperatures the hatching rate decreased, for example, to 20-37% at 27°C. Such high temperatures would not normally be reached during the peak period of egg laying in the UK. Furthermore, *D. reticulatum* usually lays eggs in crevices in the soil or under organic matter on the soil surface such as leaves or logs and they are therefore likely to be buffered from extremes of temperature to a

certain extent. It would seem, therefore, that under natural temperature conditions experienced around peak egg-laying, hatching rate is reasonably uniform. For the 'background' level of egg laying that continues throughout the year in this species, however, particularly in summer when short-term temperatures can approach Judge's experimental conditions, this may have a significant effect on percentage hatching. That temperature in lower ranges has no effect on hatching rate is supported by work on a related slug species, *Deroceras panormitanum* (Lessona and Pollenera), a pest of hardy nursery stock (Schüder, 2004). Schüder found that slug eggs incubated at 12°C, 15°C and 20°C hatched at similar rates, although the percentage hatching, at 84-89%, was higher than observed in *D. reticulatum*. This may be because *D. panormitanum*, which was originally a Mediterranean species (Kerney & Cameron, 1979), is well adapted to the warmer and less variable ambient conditions found in horticultural greenhouses.

In addition to providing useful insights into the basic biology of *D. reticulatum*, the results of these experiments also have a practical application in informing aspects of its control. In an ideal control strategy, a solid understanding of the pest's biology underpins confident predictions of how it will respond to environmental changes such as annual weather fluctuations leading to effective prevention of economically important levels of damage. In a highly adaptable generalist herbivore, such as *D. reticulatum*, this ideal is not easily attained. By an improved appreciation of how factors like season and temperature, acting independently or in combination, impact on the early stages of its lifecycle, population dynamics models can be refined. The more reliable the input of such models, the more confidence we can have in their later predictions. Although the results in this paper require further testing before they can usefully help to parameterise models they indicate areas where adjustments could be valuable.

The data presented in this paper were collected as part of an extensive study of the developmental biology of *D. reticulatum* under different conditions. The investigations described were necessarily restricted in scope by the need to continue later stages of the study. It is, therefore, acknowledged that data collection may have been carried out in different ways had the work been done in isolation; for example, hatching may have been monitored daily and a wider range of temperatures could have been investigated. Nevertheless, the results provide support for other studies in this area of slug biology and indicate some avenues for future research.

In conclusion it was found that:

1. Laying season influenced the association between parental weight and batch size and also modified the relationship between incubation temperature and development time.
2. Incubation temperature was inversely related to development time.
3. Batch size was a significant predictor of whether any of the eggs in a batch hatched.
4. Hatching rate did not vary with temperature or laying season.

As noted in the discussion, there are a number of ways in which this work could be expanded in future studies. Repeating the experiments at a wider range of temperatures would help to provide a more complete

picture of early stages in the lifecycle by showing whether trends at the temperatures tested here are maintained over a wider range. Whilst temperature effects on development and hatching of slug species are relatively widely reported in the literature there is a marked scarcity of studies into seasonal effects on these processes, or indeed combined effects of temperature and season. This would be an obvious area to develop. It would allow further investigation of the idea that there may be a threshold above which laying season fails to influence development time. In addition, it would be valuable to study in more detail the influence of parental weight on batch size. If this were to be a strong relationship when tested more stringently, in combination with the results on batch size, hatching and development times it could be a very useful 'rough and ready' guide as to the potential population size in the following year.

Acknowledgements

The work described here is part of a Defra-sponsored project in the Sustainable Arable LINK Programme. The industry partners are Bayer CropScience Ltd, CropTech, De Sangosse UK, Godfrey Farms Ltd, the Home-Grown Cereals Authority and Lonza Ltd. The academic partners are ADAS UK Ltd, Rothamsted Research and the University of Newcastle. DEFRA is the government sponsor. We thank all the partners in this project, together with A Craig who helped with field work.

PAPER 2 - *Objective 1.1*

Seasonal and Temperature Effects on the Growth Rate and Survival of *Deroceras reticulatum*

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Abstract

The growth and survival of *Deroceras reticulatum* (Müller) hatching in different seasons (spring and autumn), but reared at the same temperatures (ambient, 12°C and 15°C) were investigated. There was considerable variation in growth under identical conditions. Growth was influenced by hatching season at all rearing temperatures; at ambient temperature it was faster in spring, but at the two constant temperatures it was faster in autumn. The association between temperature and growth was negative in spring and positive in autumn. Survival was influenced by hatching season at ambient temperature and 15°C, but not 12°C; at ambient temperature slugs survived longer in autumn than spring whereas at 15°C they survived longer in spring than autumn. Within a season survival differed between ambient and each constant rearing temperature, but was similar between the two constant temperatures; in spring ambient survival was lower than at 12°C and 15°C, but in autumn it was higher.

Introduction

It is well-established that temperature influences many aspects of the biology of terrestrial slugs, including growth rate (e.g. Runham & Hunter, 1970; Godan, 1983; South, 1992). For *Deroceras reticulatum* most studies have shown that the relationship between growth rate and temperature is approximately hyperbolic; there is a positive association up to an optimum of 17-19°C after which higher temperatures have a detrimental effect on development (e.g. Dainton, 1954a; Arias & Crowell, 1963; Dmitrieva, 1969; South, 1982). An exception to this is the work of Judge (1972) whose data indicate that growth is faster at cooler temperatures. All of these studies are based on slugs hatching at one particular point in time, i.e. a single season, although none actually state which season this is. *D. reticulatum* is capable of breeding throughout the year if conditions are favourable, but there are peaks in spring and autumn (Bett, 1960; Hunter, 1968b; Runham & Laryea, 1968). The autumn population have to over-winter either as eggs or recently hatched juveniles and it is postulated that there may be something inherently different in the physiology of these slugs compared to the spring population that adapts them for development and survival at lower seasonal temperatures. If so, it might be expected that the growth trajectories of spring and autumn hatching slugs

will differ when reared under identical conditions. This may explain the discrepancy between the work of Judge (1972) and other authors.

There are no published data that directly assess the effect of hatching season on slug growth. The experiments presented in this paper were, therefore, designed to investigate this. The growth trajectories of slugs hatching in spring and autumn were compared at three rearing temperatures, ambient, 12°C and 15°C, along with their survival. It was also possible to contrast growth and survival between temperatures within a given season to see how trends compare with published studies. These experiments continue the work described in *Paper 1* on factors affecting development and hatching rate. Similarly, in addition to finding out more about the biology of *D. reticulatum*, the results of this paper may be of practical application in refining population dynamics models used in risk assessments for the control of slugs in arable crops.

Materials and Methods

Newly hatched *D. reticulatum* from the eggs studied and described in *Paper 1* were used in these experiments. The eggs had been laid in the laboratory by field collected adults in spring or autumn 2002 and were incubated at one of three temperatures (ambient, 12°C or 15°C). Slugs were fed on a mixed diet of Chinese cabbage and carrot. Cuttlefish bone was provided as a source of calcium.

The experimental treatments described in *Paper 1* were maintained in this second phase of the study, i.e. in each of two hatching seasons (spring or autumn) slugs were reared at one of three temperatures, ambient (fluctuating), 12°C or 15°C (both constant), corresponding to the temperature at which they hatched. Methods of temperature control and photoperiod remained unchanged. At each of the three rearing temperatures for a given season a total of 200 individuals were initially monitored. Due to very low mortality rates in all treatments, this number was subsequently reduced to 100 individuals per treatment in order to make the experiment more manageable. Throughout the experiment slugs were handled using a square-ended paintbrush. Hatchlings were gently removed from the Petri dishes in which egg batches had been incubated. Each individual was placed into a separate 9 cm diameter Petri dish lined with blue laboratory tissue moistened with distilled water. Food was provided *ad libitum*. The slug was then returned to the temperature treatment at which it hatched. Dishes were cleaned weekly when food was replaced. During cleaning the slug was transferred to the lid of the Petri dish. The moist laboratory tissue was then replaced and any soiling was wiped from the surfaces of the dish. Fresh food was added and the slug was transferred back into the dish. Mortality was recorded weekly.

Slugs were weighed at hatching (week 0) and fortnightly thereafter using a Mettler MT 5 balance to an accuracy of 0.01 mg, allowing a brief settling period for the reading to stabilise. A small plastic dish (3.5 cm diameter) was used as a weighing receptacle and the balance was tared to zero before the slug was added. Slugs were transferred from the Petri dish to the weighing receptacle as quickly as possible in order to minimise the amount of mucus lost in weighing. Slugs hatching from eggs laid in spring were monitored for

a total of 20 weeks, by which time autumn laid eggs began hatching and it was not practicable to maintain both sets of slugs simultaneously. It was, however, possible to monitor the autumn hatching slugs for a longer period as there were no further individuals entering the experiment. This set was monitored for a total of 34 weeks during which time some began to lay eggs. If batches of five or more were laid they were collected for the next stage of the experiment (*Paper 3*) and the monitoring of the individual that laid them ceased. When monitoring ceased, slugs were kept for future dissection in the final part of the experiment (*Paper 4*). They were individually preserved in 70% ethanol in glass tubes measuring 5 x 1.2 cm. The ethanol was changed two weeks after slugs were first preserved to ensure that it did not become diluted by diffusion of body fluids.

All comparisons of growth and survival between spring and autumn hatching slugs are based on weeks 0-20, since this is the period of time observed that is common to slugs from both hatching seasons. Additional analyses on the longer observation period for autumn alone concern data from weeks 0-34. All analyses exclude data from individuals removed from the experiment when numbers were reduced to 100 slugs per treatment. For those analyses concerning growth, results relate to the subset of slugs that were alive for the full monitoring period only. Continuous weight data were analysed using a repeated measures analysis of variance (ANOVA). To account for the non-parametric nature of the data, as confirmed by Mauchley's test of sphericity and Box's M-test, the ANOVA was adjusted by applying the lower-bound epsilon correction. Tukey post-hoc tests were carried out as appropriate. Survival data were analysed with the Kaplan-Meier procedure, using the Breslow test to compare between treatments. Hazard ratios were calculated.

Results

Table 2.1 shows the numbers of slugs alive for the full 20 week monitoring period in each treatment. All analyses comparing spring and autumn hatching are based on these slugs.

Table 2.1: Numbers of Deroceras reticulatum *alive for the full 20 week monitoring period in each experimental treatment.*

Hatching	*Rearing Temperature (°C)*			
Season	*Ambient*	*12*	*15*	*Total*
Spring	60	97	94	251
Autumn	94	97	96	287
Total	154	194	190	538

The mean (± S.E.) ambient temperature during the 0-20 week growth period was 12.7 ± 0.1°C (absolute range: 1.2-24.1°C; mean daily range: ± 4.9°C) in spring and 5.6 ± 0.1°C (absolute range:-3.0-14.0°C; mean daily range: 2.8°C) in autumn.

Considerable variation in weight was observed in all treatments, even within groups of slugs reared under identical conditions. An example of this is illustrated in *Fig. 2.1* which shows two spring hatching slugs of

the same age reared under identical conditions (15°C). Taking into account this within treatment variation, there were still significant differences in growth between treatments.

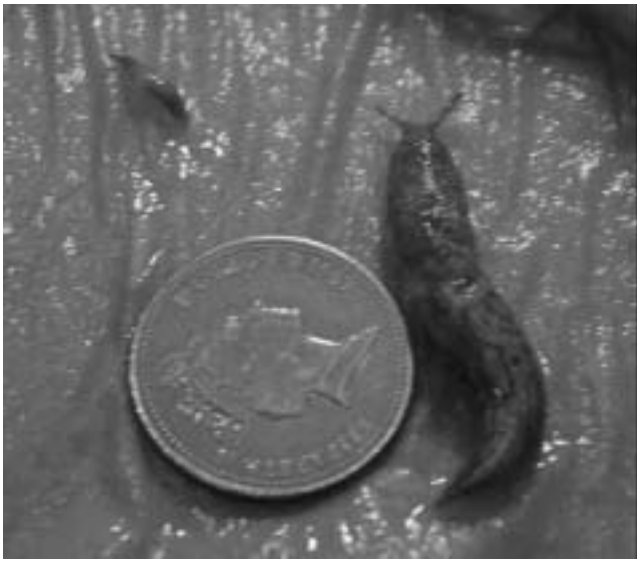


Figure 2.1: Weight variation in Deroceras reticulatum *hatching in spring and reared at 15°C. Both slugs are 20 weeks of age. Scale is indicated by a 1 pence piece.*

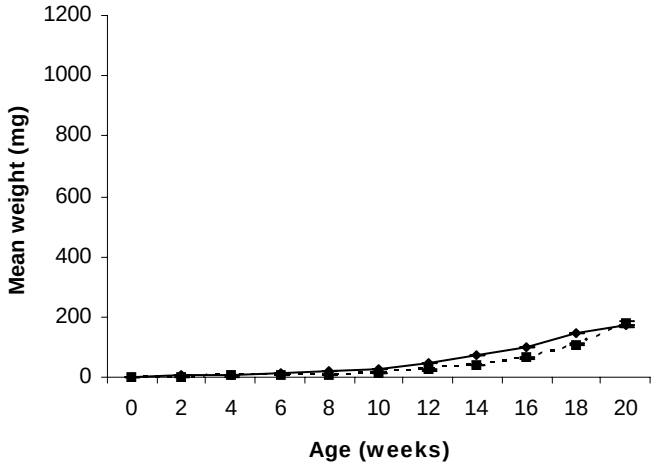
At each of the three rearing temperatures the hatching season had a significant effect on growth rate (*Table 2.2*).

Table 2.2: Results of repeated measures analysis of variance (ANOVA) to compare the growth rate of Deroceras reticulatum *hatching in spring and autumn, reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

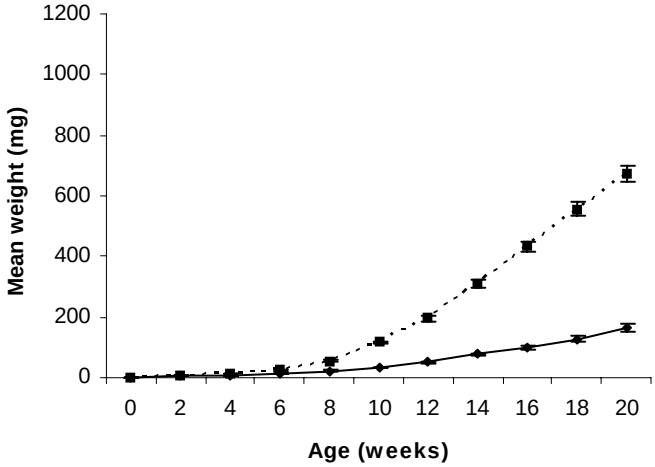
Rearing Temperature (°C)	*N*	*df*	*F*	*P-value*
Ambient	154	1	12.964	*< 0.001*
12	194	1	316.249	*< 0.001*
15	190	1	419.232	*< 0.001*

At all rearing temperatures the growth rate from hatching until week 4-5 was similar in both seasons, but then began to diverge, and this became more marked with time. At ambient temperature slugs hatching in spring grew faster than those hatching in autumn for most of the 20 week monitoring period, although those hatching in autumn began to catch up and overtake in weeks 19-20. At the two constant temperatures, however, the reverse was observed; growth was considerably faster for autumn hatching slugs throughout the monitoring period. By week 20, autumn hatching slugs were on average four times larger than spring hatching slugs at 12°C, rising to eight times larger at 15°C (*Fig. 2.2 (a)-(c)*).

(a)



(b)



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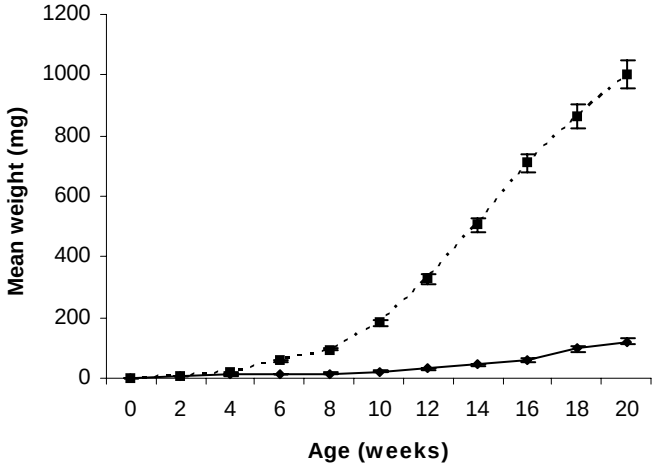

Figure 2.2: Mean weight (± S.E.) (mg) of Deroceras reticulatum *reared at (a) ambient temperature, (b) 12°C and (c) 15°C in spring (solid line) and autumn (dotted line).*

The absolute differences in mean weight between hatching seasons at week 20 were greatest for slugs reared at 15°C and smallest for those reared under ambient conditions (*Table 2.3*).

Table 2.3: Mean weight (± S.E.) (mg) at week 20 of Deroceras reticulatum *hatching in spring and autumn, reared at ambient temperature, 12°C or 15°C.*

Hatching	*Rearing Temperature (°C)*		
Season	*Ambient*	*12*	*15*
Spring	170.05 ± 13.62	163.04 ± 16.64	121.55 ± 12.53
Autumn	180.37 ± 7.41	671.56 ± 25.52	1002.58 ± 45.00

Further inspection of *Table 2.3* shows that the marked differences in growth between seasons at a given rearing temperature are also apparent between some rearing temperatures in a single season, most notably in autumn. There are opposite trends between the seasons in the rearing temperatures at which maximum and minimum mean growth is reached; in spring the highest mean weight is reached in ambient conditions and the lowest at 15°C whereas in autumn the reverse is observed.

Within a given season growth rate was significantly influenced by rearing temperature (ANOVA: spring: $F_{2, 248} = 7.333$, $P < 0.01$; autumn: $F_{2, 284} = 241.343$, $P < 0.001$). Tukey post-hoc tests showed that in spring growth at 15°C was significantly slower than at 12°C ($P < 0.01$) and ambient conditions ($P < 0.01$), but the differences between these latter two temperatures did not reach statistical significance. Indeed, it can be seen that there is a very close correspondence in growth at 12°C and ambient temperature throughout the monitoring period (*Fig. 2.3*). In contrast, autumn hatching slugs reared at 15°C grew considerably faster than at both of the other two rearing temperatures; those at ambient conditions grew slowest. The differences between all three rearing temperatures were significant at $P < 0.001$ (*Fig. 2.4*).

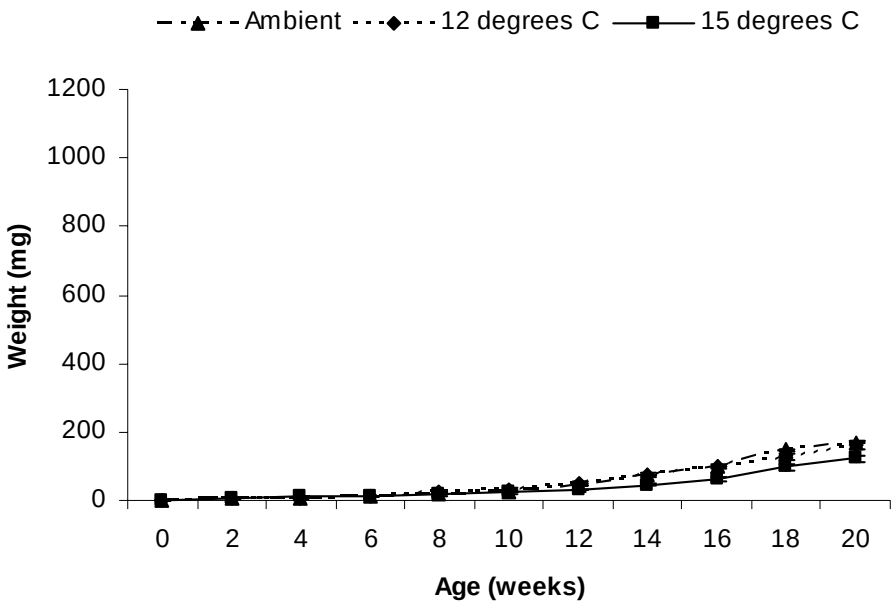

Figure 2.3: Mean weight (± S.E.) (mg) of Deroceras reticulatum *hatching in spring and reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

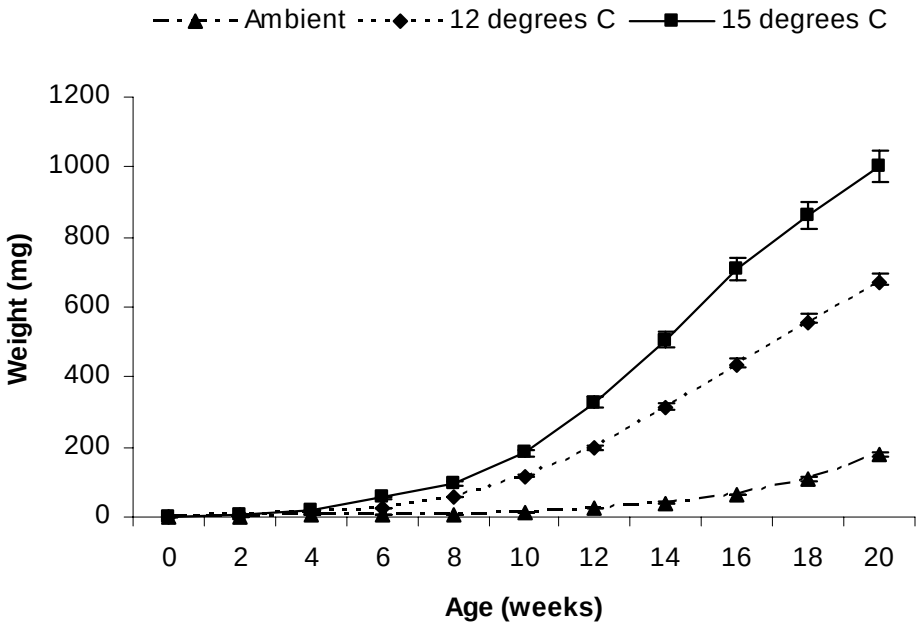

Figure 2.4: Mean weight (± S.E.) (mg) of Deroceras reticulatum *hatching in autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

Table 2.4 shows the numbers of slugs in the experiment up to week 20, excluding those that were removed when numbers were reduced to 100 per treatment. All analyses are based on these slugs.

Table 2.4: Numbers of Deroceras reticulatum *in the experiment up to week 20, excluding those that were removed when numbers were reduced to 100 per treatment.*

Hatching	*Rearing Temperature (°C)*			
Season	*Ambient*	*12*	*15*	*Total*
Spring	141	116	114	371
Autumn	105	121	135	361
Total	246	237	249	732

The Kaplan-Meier procedure was used to analyse survival. In this procedure the cumulative chance of survival between each time interval, *S(t)*, is calculated. This value is called the Kaplan-Meier estimator and it is plotted against survival time to give a classic survival curve known as the Kaplan-Meier estimator plot such as in *Figs. 2.5 & 2.6*. Treatments are compared using the Breslow test.

Fig. 2.5 (a)-(c) compares seasonal differences in survival at each of the three rearing temperatures. The survival rate differed significantly between spring and autumn hatching slugs reared at ambient temperature and 15°C, but not for those reared at 12°C (Kaplan-Meier (Breslow test): Ambient: N = 246, df = 1, G^2 = 49.33, $P < 0.001$; 12°C: N = 237, df = 1, G^2 = 0.63, *n.s.*; 15°C: N = 249, df = 1, G^2 = 5.06, $P < 0.05$). *Table 2.5* shows the mean survival times of slugs at each combination of hatching season and rearing temperature.

Table 2.5: Mean survival times (± S.E.) (weeks) for Deroceras reticulatum *hatching in spring and autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-20)* .

Hatching Season	*Rearing Temperature (°C)*		
	Ambient	*12*	*15*
Spring	13.50 ± 0.63	17.48 ± 0.55	17.36 ± 0.55
Autumn	18.39 ± 0.47	16.74 ± 0.61	15.36 ± 0.65

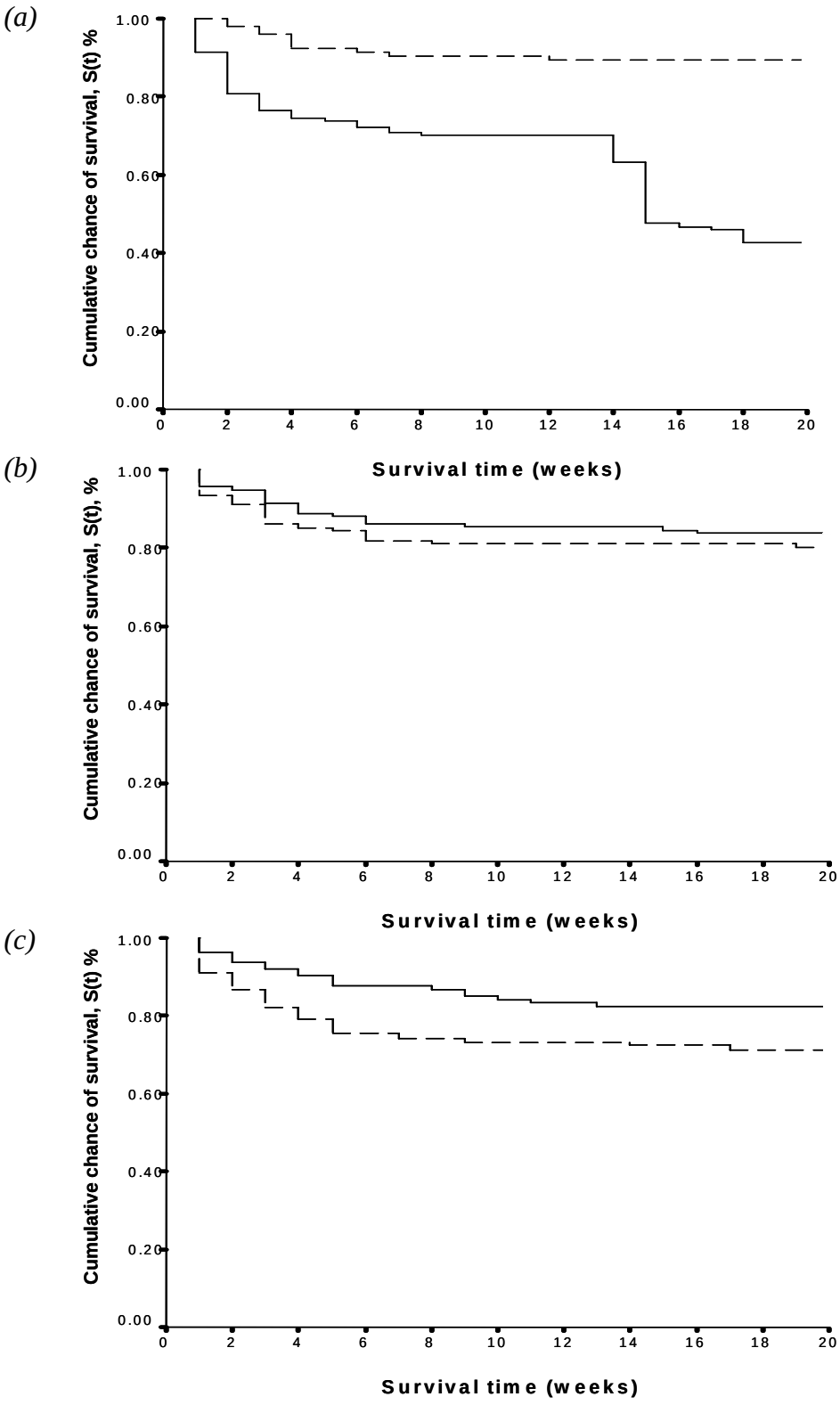

Figure 2.5:Kaplan-Meier estimators for Deroceras reticulatum *reared at (a) ambient temperature, (b) 12°C and (c) 15°C in spring (solid line) and autumn (dashed line).*

Hazard ratios indicate how much the survival rate differs between two groups of individuals. At ambient conditions, slugs hatching in spring had lower survival rates than those hatching in autumn (hazard ratio = 0.13), whereas the converse was seen in slugs reared at 15°C; survival was greater for spring hatching than autumn hatching slugs (hazard ratio = 1.83).

Within a given hatching season survival rates between the two constant rearing temperatures did not differ significantly. There were, however, significant differences in survival of slugs reared at each of these temperatures and ambient conditions (*Table 2.6*). For spring hatching slugs, those reared at ambient temperature had a lower survival rate than at 12°C (hazard ratio = 0.21) and 15°C (hazard ratio = 0.23) whereas for autumn hatching slugs the reverse was observed; those reared at ambient temperatures had a higher survival rate than at 12°C (hazard ratio = 2.07) and 15°C (hazard ratio = 3.20) (*Fig. 2.6 (a)* & *(b)*).

At the end of the longer monitoring period for autumn hatching slugs, the number of individuals per treatment that were alive for the entire 34 weeks was considerably less compared with those alive for the 20 week period which was contrasted with spring hatching slugs. This was particularly so in the constant temperature treatments (ambient: n = 67; 12°C: n = 18; 15°C: n = 13) and is due to the removal from the experiment of slugs laying batches of five or more eggs, in addition to natural mortality; slugs began to lay eggs much sooner under constant conditions. Consequently, statistical analysis of this data needs to be interpreted with extreme caution as the test is highly unbalanced. The results are therefore exploratory in nature and to confirm initial trends further experiments would be required.

Table 2.6: Results of Kaplan-Meier survival analysis (with Breslow test) to compare differences in survival rates between Deroceras reticulatum *hatching in the same season (spring or autumn) and reared at different temperatures (ambient, 12°C or 15°C).*

Laying season	*Pairwise comparison between rearing temperatures (°C)*	*N*	*df*	*Breslow statistic (G2)*	*P-value*
Spring	Ambient vs 15°C	255	1	32.82	*< 0.001*
	Ambient vs 12°C	257	1	36.54	*< 0.001*
	12°C vs 15°C	230	1	0.04	*n.s.*
Autumn	Ambient vs 15°C	240	1	12.96	*< 0.001*
	Ambient vs 12°C	226	1	4.35	*< 0.05*
	12°C vs 15°C	256	1	2.59	*n.s.*

(a)

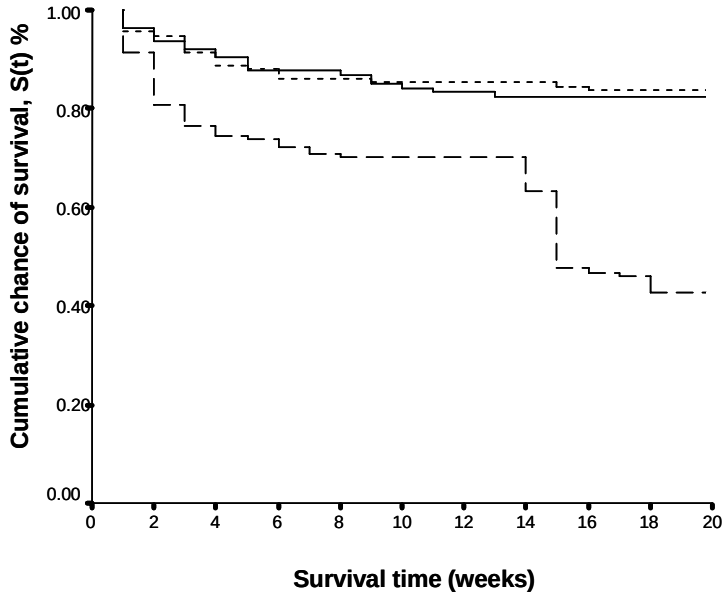

Figure 2.6: (a) Kaplan-Meier estimators for Deroceras reticulatum *hatching in spring and reared at ambient temperature (dashed line), 12°C (dotted line) or 15°C (solid line).*

(b)

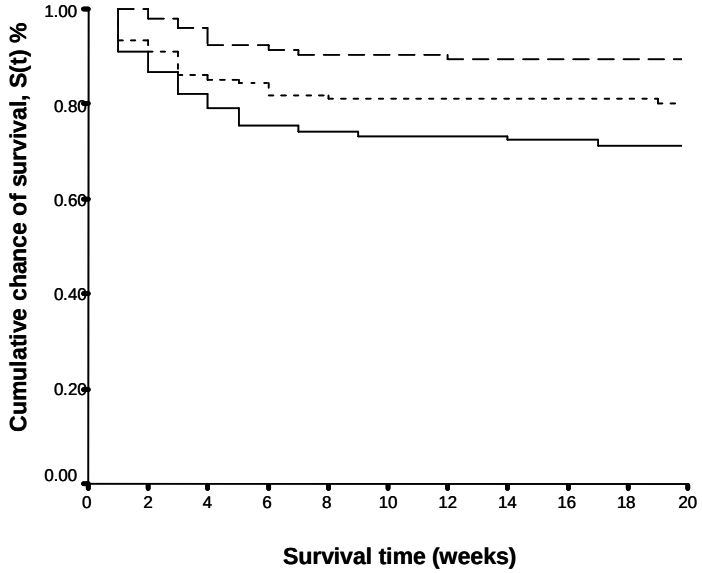

Figure 2.6 (cont../): (b) Kaplan-Meier estimators for Deroceras reticulatum *hatching in autumn and reared at ambient temperature (dashed line), 12°C (dotted line) or 15°C (solid line).*

Over the full 34 week observation period rearing temperature was found to have no effect on growth (ANOVA: $F_{2, 95} = 1.844$, *n.s.*). Contrasting the mean weights of these slugs (*Fig. 2.7*) with autumn hatching slugs alive for 20 weeks, as described previously (*Fig. 2.4*), indicates that the mean weights are lower at all rearing temperatures in the 34 week group. This is observed on a week by week basis, but becomes particularly marked with increasing time.

Fig. 2.7 shows that slugs reared at ambient conditions eventually 'caught-up' with and exceeded the growth rate of slugs reared at constant temperatures. This 'overtaking' by ambient reared slugs occurred at week 26 for slugs at 12°C and week 29 for those reared at 15°C.

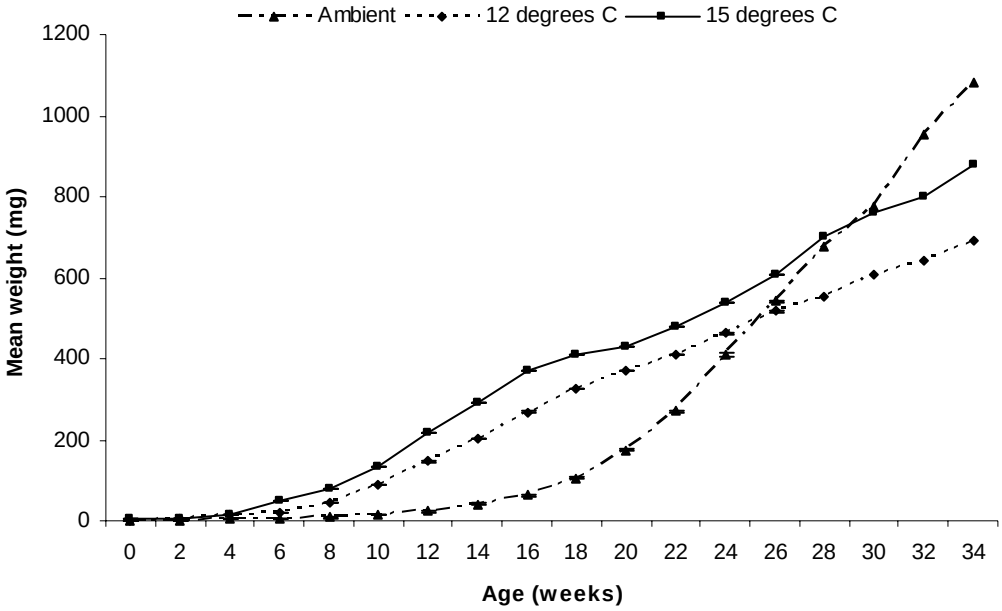

Figure 2.7: Mean weight (± S.E.) (mg) of Deroceras reticulatum *hatching in autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-34).*

Slugs for which monitoring ceased after laying batches of five or more eggs are classified as 'censored' in survival analyses because their ultimate fate within the monitoring period is unknown, i.e. it is not known whether they would have stayed alive or died between the time monitoring stopped and week 34. This is taken into account during the analysis and such individuals are indicated by a cross on Kaplan-Meier estimator plots.

The mean survival time was highest for slugs reared at ambient conditions, followed by those at 12°C and finally 15°C which survived, approximately 6 weeks less than those at ambient conditions (*Table 2.7*).

Table 2.7: Mean survival times (± S.E.) (weeks) for autumn hatching Deroceras reticulatum *at each rearing temperature during the 34 week monitoring period.*

Rearing Temperature (°C)		
Ambient	*12*	*15*
30.31 ± 0.89	27.88 ± 1.11	24.67 ± 1.19

Although the mean survival times indicate that ambient conditions are generally more favourable for autumn hatching slugs, the patterns in survival rates over the entire 34 week monitoring period show that this becomes less so with increasing time (*Fig. 2.8*). The differences seen between rearing temperatures in weeks 0-20 are maintained up to week 26. After this time the survival rate of ambient reared slugs begins to decline until, at week 30, it equals that of slugs reared at 12°C and at week 32 it falls below this group. Slugs reared at 15°C consistently exhibit the lowest survival throughout this monitoring period.

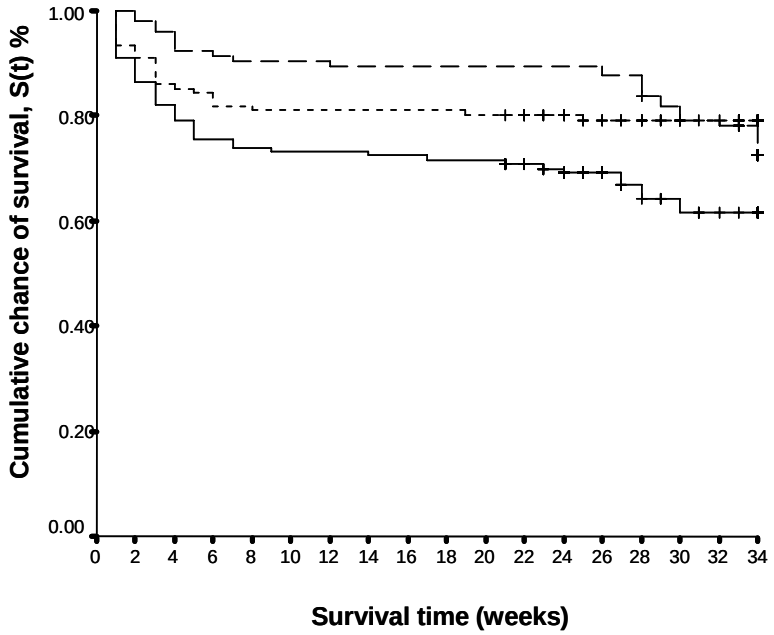

Figure 2.8: Kaplan-Meier estimators for Deroceras reticulatum *hatching in autumn and reared at ambient temperature (dashed line), 12°C (dotted line) or 15°C (solid line). Crosses indicate weeks when there were censored individuals (slugs that were removed from the experiment when they laid batches of 5 or more eggs).*

Overall, the differences in survival times were significant between slugs reared at 15°C and each of the other two temperatures, but there were no differences between those reared at ambient and 12°C (*Table 2.8).*

Table 2.8: Results of Kaplan-Meier survival analysis (with Breslow test) to compare differences in survival rates between Deroceras reticulatum *hatching in the autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-34).*

Laying season	*Pairwise comparison between rearing temperatures (°C)*	*N*	*df*	*Breslow statistic (G2)*	*P-value*
Autumn	Ambient vs 15°C	240	1	12.05	*< 0.001*
	Ambient vs 12°C	226	1	0.02	*n.s.*
	12°C vs 15°C	256	1	4.04	*< 0.05*

Discussion

This paper comprises the second part of a study of *D. reticulatum* development, continuing the work described in *Paper 1*. The experiments investigated the extent to which slugs hatching at different seasons of the year exhibit the same growth trajectories under identical rearing temperatures and whether this impacts on survival. It was also possible to contrast the growth and survival of slugs reared at different temperatures within a single season allowing a comparison with published studies.

That there is considerable variation in the size of slugs at hatching and during subsequent growth, even when reared under identical conditions, has been widely reported in the literature for a number of species (e.g. Hunter, 1978; Prior, 1983; Shibata & Rollo, 1988; South, 1992). Such variation is common in pulmonates generally (Peake, 1978) and is confirmed in the current study for *D. reticulatum*. Since individuals of the same age varied between approximately 5-100 fold in their weight and, by inference, slugs of a given weight vary considerably in their age, the results presented here support the conclusion of Prior (1983) for *Limax maximus* (L.) that 'one cannot use body weight to estimate the absolute or relative age of animals accurately'. It is clear that the terms 'juvenile' and 'adult' should be used to describe the developmental state rather than the chronological age of slugs.

Little is known about the mechanisms underlying this exceptional divergence in size, although it is unlikely to be adaptively neutral since it is so consistently maintained over time (Rollo & Shibata, 1991). For example, it allows that there will be some mature individuals in the population capable of mating whenever conditions are favourable, maximising reproductive opportunities. Shibata and Rollo (1988) put forward a number of hypotheses to explain the basis of this phenomenon in *Deroceras laeve* (Müller), a largely self-fertilising species. These included maternal diet and egg quality, 'nutritional imprinting' (i.e. influence of early nutritional experience), density effects and egg size. Of these, they found that only egg size had a significant effect on growth with slugs hatching from smaller eggs growing faster than those hatching from larger ones. This was, however, only the case for slugs fed a high quality diet post-hatching and since size variation was still observed in experiments where egg size was controlled and diet was of a standard quality, it would suggest that there are likely to be multiple factors that influence growth trajectories either singly or in concert. This is supported by the experiments presented in this paper; egg size was not measured, but since batches were allocated to treatments at random prior to incubation (*Paper 1*) any variations in egg size would be expected to be distributed evenly amongst treatments. Whilst differences in egg size may, therefore, explain growth variation within treatments this would not account for the significant differences observed between them.

Size differences between slugs of the same age generally became more pronounced with time, agreeing with the findings of South (1982). Shibata and Rollo (1988) suggest that growth during early development is exponential and small differences in rates as a result of different weights at hatching translate into a 'rapid divergence in body sizes'. Certainly the growth rate increased with time in all the experimental treatments

presented here and this explanation would seem feasible. Since slugs were fed the same diet and were reared in isolation, nutritional imprinting and density effects can also be ruled out as explanations of growth variation in *D. reticulatum*. Indeed, it has been shown that intraspecific competition in this non-aggressive species is minimal and has little effect on growth (Rollo, 1983b). Furthermore, size variation is comparable between isolated and communally reared individuals (South, 1982).

Hatching season influenced growth at all temperatures assessed. Although seasonal differences in size between slugs from week 0-4 were small in all treatments, there was a rapid divergence after this time. The ambient reared slugs acted as controls and confirmed previous work that showed under field temperatures *D. reticulatum* grows faster in spring than autumn (Carrick, 1938). At constant temperatures of 12°C and 15°C, however, the converse was observed; growth of slugs hatching in autumn was faster than that of those hatching in spring. The 20 week monitoring period encompassed the months of May–September for spring hatching slugs and November/December–March/April for the autumn hatching slugs, depending on the hatching date. Ambient autumn temperatures were considerably lower, albeit fluctuating, than the constant treatments assessed in this study during the same monitoring period whereas ambient spring temperatures were more similar (mean ambient temperature (± S.E.) for autumn hatching slugs = 5.6 ± 0.1°C; spring hatching slugs = 12.7 ± 0.1°C). It may be that the autumn hatching slugs exhibited a much greater growth response to the constant temperatures than those hatching in spring because they are more 'unseasonably high' for this group. If there is something inherently different about slugs hatching from autumn laid eggs that adapts them to withstand over-wintering then it might be expected that they would show a greater capacity to capitalise on consistently and markedly more favourable conditions than usually experienced through the winter months. In a species considered to be an r-strategist (South, 1982) the ability to respond to disturbed and changing environments is key. The finding that *D. reticulatum* is able to 'step up' its growth in response to prolonged and unexpectedly mild conditions for the time of year concords with this. Whilst, therefore, these results support the importance of temperature on growth, it seems that season is, independently, also influential.

The influence of photoperiod on growth of *D. reticulatum* is not clear. Work on reproduction in this species indicates that the weight of individuals mirrors closely the development of the reproductive tract (Runham, 1978). Sokolove and McCrone (1978), working on *L. maximus*, showed that photoperiod affected reproductive development and it could, therefore, be inferred that it also has an effect on growth. South (1989a), however, argued that since *D. reticulatum* is capable of breeding throughout the year whenever temperature conditions are favourable it is unlikely that day-length is influential. The results of the current study cannot resolve this question. The photoperiod was appropriate to the time of year for the ambient reared slugs whereas it was maintained at 16L:8D throughout for the slugs reared at constant temperatures and therefore cannot be separated from the effects of season in either case and, additionally, temperature in the former. It could be that the longer day regime acted as an environmental cue to the autumn laid eggs

reared at constant temperatures in tandem with the 'unseasonably good' temperatures conditions for growth as proposed above, but further experiments would be required to investigate this hypothesis.

Most published studies based on slugs hatching at a given point in time have shown a high positive association between growth and temperature up to an optimum, after which there is a decline in growth along with other physiological functions leading to rapid mortality (e.g. Abeloos, 1944; Arias & Crowell, 1963; Dmitrieva, 1969; Pinder, 1969; South, 1982). Judge (1972), however, found the opposite; growth was greater at lower temperatures. In the experiments presented here a positive association was confirmed for slugs hatching in autumn, but not for those hatching in spring where individuals reared at 15°C were observed to grow significantly more slowly than those at 12°C and ambient temperature, agreeing with Judge (1972). Studies in the literature rarely state the season in which slugs hatched. It could be that those indicating a positive association between growth and temperature were based on slugs hatching in autumn whereas those indicating a negative association used slugs hatching in spring. The results of the current experiments, therefore, may not be contradictory, but rather help to explain this discrepancy.

Seasonal modification of the temperature-growth relationship brings into question the optimal temperature for growth of *D. reticulatum*. Reports suggest that this is between approximately 17-19°C (Dainton, 1954a; Dmitrieva, 1969; South, 1982). The autumn results of the current experiments support this range in that the maximum temperature assessed approaches these values and was indeed that at which growth was fastest. It would be advantageous for slugs to grow quickly in mild conditions during autumn so that they mature more rapidly and increase the likelihood of oviposition before the onset of winter. This would allow time for some embryonic development and a concomitant increase in tolerance of eggs to freezing conditions before the coldest months of the year arrive (Arias & Crowell, 1963). In spring, however, depressing growth at warmer temperatures, e.g. transient mild snaps, would serve to restrict maturation so that peak egg laying occurs, as observed, in autumn and not during the hotter months of summer when desiccation is more of a risk. There is evidence that slugs are able to make seasonal adjustments in their physiology that better adapt them to survive at the prevailing temperatures. It has, for example, been shown that the mean supercooling point (SCP) of *D. reticulatum* changes between summer and winter (Cook, 2004). Furthermore, it has been suggested that *L. maximus* and *Philomycus carolinianus* (Bosc) are 'cold adapted' in spring and 'warm adapted' in autumn (Rising & Armitage, 1969). It seems feasible that this seasonal adjustment could also include a change in the optimal temperature for growth.

Seasonal differences in activity may also be relevant to the results of the current experiments. Wareing and Bailey (1985) showed that slug activity is affected by constant temperatures and that some seasonal adjustment occurs. They suggest that this is controlled partly by endogenous rhythm and partly by day-length. Under long-days the optimum temperature for locomotion was 17°C, whilst under short-days it was 13°C. The optimum temperature for feeding remained at 14°C regardless of day-length. In the experiments presented here the long-days were maintained for the 15°C and 12°C treatments in both hatching seasons.

The results of Wareing and Bailey (1985) would imply that, as 15°C is nearer the optima for both locomotion under long-days and feeding, slugs reared at this temperature are likely to grow more than at 12°C in both seasons as they are likely to have eaten a larger quantity of food and been more active. Since this was only observed for the autumn hatching slugs it suggests that endogenous factors may have a greater influence than day-length.

Egg laying was initially sporadic with isolated eggs being produced, rather than batches. This is in accordance with the observations of Carrick (1938) who states that 'a few abnormal eggs are laid before the normal egg-masses begin to be produced by young slugs which have just reached maturity'. When five or more eggs were laid they were collected for the next stage of the experiment (*Paper 3*). Although five is an arbitrary number to constitute a batch it was chosen because it was noted that once about five eggs were laid they tended to be in obvious groups, rather than spread at random individually around the Petri dish. It was, therefore, felt that this number reasonably represented a proper batch and would be likely have a greater chance of hatching than the earlier isolated eggs. After an egg batch was collected weight monitoring ceased as it was not practical to continue rearing all slugs until they died naturally and at this point they were considered to be mature. Rollo (1988) suggests that there is a trade-off between growth and reproduction such that once oviposition begins all reserves are switched to reproduction and growth stops, i.e. the two processes are mutually exclusive. Growth was therefore regarded as complete once the first batch of eggs was laid.

The results of prolonged monitoring of the slugs hatching in autumn need to be interpreted with caution. As noted the number of autumn hatching slugs alive from weeks 0-34 was considerably less than from weeks 0-20, particularly in the constant temperature treatments, due to the gradual removal of egg-layers from the experiment. These two groups are not, therefore, composed of the same individuals; those in the 34 week analysis exclude any that laid batches of five or more eggs. The results are, nevertheless, informative. At constant rearing temperatures the mean weight of slugs in the 34 week group was lower at all time intervals than the 20 week group suggesting that the considerable variation in growth that is seen within a treatment may be partitioned into 'slow growers' and 'fast growers'. Fast growers seem to be able to take advantage of the warmer than ambient, constant conditions, growing to large sizes and laying eggs rapidly. Slow growers, in contrast, gain weight at comparable rates to ambient reared slugs, regardless of the elevated constant temperatures (there were no significant differences in growth between rearing temperatures over the 34 week period). Despite within treatment variation in growth rate, the slugs reared at ambient temperature generally grew more slowly and began laying eggs later than slugs at constant temperatures and would, therefore, have consisted of a mixed group of fast and slow growers for longer. During weeks 20-34, however, their mean growth overtook that of slugs at 12°C and 15°C. These weeks correspond to May-August, i.e. the conditions experienced by spring hatching ambient reared slugs during weeks 0-20. The emerging pattern, with ambient slugs beginning to grow faster than those at constant temperatures reflects what was seen in the spring hatching group and could be explained by an increase in mean weight as faster growers reach

maturity. It may also be that fluctuating temperatures affect growth in a different way to constant temperatures. These results require further investigation

Egg size may be an important determinant of whether a slug is a fast or slow grower (Shibata & Rollo, 1988), but genetic differences may also play a role. Self-fertilisation is possible in species that normally cross-fertilise e.g. the genus *Philomycus* (McCracken & Selander, 1980), although it is not the norm. Furthermore, some species lay mixed batches of eggs, fertilised by both autosperm and allosperm, e.g. *Arion* (Duncan, 1975). Whilst it is reported in the literature that *D. reticulatum*, a normally cross-fertilising species, lays eggs when reared in isolation these are said to be infertile (South, 1982; Nicholas, 1984). In the current study, this was found not to be the case; some eggs laid by isolated slugs were fertile. It may be that *D. reticulatum* is also capable of both cross-fertilisation and self-fertilisation. If this were so then fast and slow growth may be determined by whether the egg is fertilised by autosperm or allosperm. These ideas are expanded upon in *Paper 3*; additionally, genetic studies to investigate this hypothesis would be of great value.

Whilst the mortality of *D. reticulatum* under different environmental conditions has been described in the literature in terms of absolute or relative numbers, there is little work that has formally assessed survival over time and, as for growth, none that compares this between seasons. In the experiments presented here it was found that season significantly affected the survival rate at ambient temperature and 15°C, but not at 12°C. Under ambient temperature slugs survived longer in autumn than in spring whereas at 15°C survival was greater in spring. The ambient results indicate that under field temperatures *D. reticulatum* is better able to survive in cool than warm conditions. It is known that slugs possess physiological mechanisms to cope with low temperatures, for example, they can enter a state of chill coma (Mellanby, 1961), i.e. they cool to below the temperature at which freezing would normally occur (supercooling point) without becoming immobilised, and can survive freezing temperatures for longer in winter than other times of the year (Cook, 2004). They are more vulnerable to warm temperatures, however, having to rely to a greater extent on behavioural adaptations to withstand extremes. Although the mean ambient temperature in spring (± S.E.), at 12.7 ± 0.1°C, is not at the upper limit of their tolerance, this group were subject to a mean daily range in temperature that was almost double that of the autumn hatching ambient reared slugs (± 4.9°C c.f. ± 2.8°C). Furthermore the maximum recorded temperature in spring was 24.1°C compared to 14.0°C in autumn; hence spring hatching slugs were subject to larger extremes of temperature and this may also impact negatively on their survival.

Comparisons of survival between rearing temperatures within a season showed that, although there was no difference between the two constant temperatures in spring or in autumn, in both seasons these differed significantly from ambient. In spring survival at ambient temperature was lower than at both constant temperatures whereas in autumn it was higher. It is not clear why there was no difference between the constant temperatures; perhaps survival is simply not very sensitive to small temperature changes. The

ambient results are, however, consistent with previous suggestions that slugs are better adapted to lower temperatures.

South (1982) included an assessment of mortality in his experiments of growth at different controlled temperatures. In general it was found that the percentage of *D. reticulatum* surviving with time was inversely related to temperature, however no formal assessment of whether the differences between rearing temperatures were statistically significant was presented. South's results cannot be directly contrasted with the current experiment as the time of year that studies were carried out was not stated and nor were the same rearing temperatures assessed. A small subset of individually reared slugs was, nevertheless, compared with a larger group of communally reared individuals and it was found that their survival was similar, indicating that the results of the current experiment are unlikely to be inflated due to the rearing procedure.

Over the longer 34 week period survival patterns of autumn hatching slugs from weeks 0-20 were largely maintained. The chances of survival continued to decline with time for slugs reared at 15°C and this group had the lowest survival rate throughout. The survival of ambient reared slugs, though remaining high for most of the 34 week observation period, began to decline towards the end and eventually fell below that of the slugs reared at 12°C. At the time when ambient survival began to decline it was late May/early June and, as observed with growth patterns, the survival rates begin to revert to the trends observed for spring reared slugs from weeks 0-20. Had the slugs been monitored for longer it may be speculated that the ambient survival would also have dropped below that of the slugs reared at 15°C.

This study was laboratory based and as such provides information on the influence of different rearing temperatures and laying seasons on the potential capacity for growth and ultimately survival of *D. reticulatum*. Under field conditions many other factors will modify this response, e.g. predation, parasitic infestation (Glen *et al.*, 2000), food availability (Rollo, 1988) and climatic factors (Young & Port, 1989). It is, therefore, necessary to be cautious in the extent to which the present findings can be extrapolated to such situations. It was seen that growth accelerates under unseasonably mild conditions and that survival is greater at lower temperatures. It may be, therefore, that population booms after mild autumns are caused not by an increase in survival, but by a subset of fast growing slugs that mature rapidly. If, over the much longer term, mean temperatures were to rise as a result of global warming the decrease in survival at warmer temperatures may cause the distribution of *D. reticulatum* to move northwards; small changes in minimum temperatures have been shown to affect the range of other invertebrates (Crozier, 2004).

If the survival results are contrasted with those for growth it is immediately obvious that, at least for slugs reared at ambient and 15°C, there is an inverse relationship between growth and survival; as slugs grow faster their chances of survival become progressively lower, agreeing with South (1982). At 12°C, however, no such relationship was apparent. At this temperature the growth rate increased significantly in autumn compared to spring with no change in survival rate yet at 15°C, a rise of just 3°C, the much greater increase

in growth of autumn hatching slugs was accompanied by a significant decrease in survival. If there is a 'trade-off' between growth and longevity, it would seem that this operates within certain confines. Perhaps 12°C represents a transition temperature, not warm enough to reduce survival in autumn, but not cool enough to enhance it in spring. At temperatures where this inverse relationship holds, the implication is that harsh conditions may not affect an entire generation equally; smaller, slower growing slugs have an increased chance of surviving to replace larger faster growing individuals providing a high degree of flexibility in response to a changing environment at the population level.

Hunter and Symonds (1971) suggested that there are overlapping ('leapfrogging') generations of *D. reticulatum* in temperate regions such as the United Kingdom. Under this scheme the slug population consists of two generations separated by an interval of about nine months. In generation A, slugs hatch in autumn, over-winter and lay eggs the following spring (equivalent to the autumn hatching slugs in the current study) whereas generation B hatch in late spring and then mature and lay eggs in late autumn (equivalent to the spring hatching slugs). This accounts for the two peaks in slugs numbers in spring and autumn whilst allowing that there cannot be two complete generations in a year due to a lifespan of nine or more months from egg to adult (Hunter, 1968b). South (1989a) points out that generations A and B are not necessarily distinct 'races' as some slugs may mature early/late and slip into the alternative generation. The results of the study presented here support South (1989a), suggesting that rather than being an exception, this intermixing of the two generations may be the norm. Perhaps the situation is more analogous to Aesop's 'hare and tortoise fable' (Gibbs, 2003); slow growing individuals may exist in the population simply growing steadily in small increments (the tortoises) while their fast growing contemporaries race ahead, but ultimately die early (the hares) and lose in the survivorship stakes. The 'tortoises' of one generation are to be found with the 'hares' of the following generation.

Any situation where the growth of a pest increases, but survival doesn't decrease poses a problem for control. For the constant rearing temperatures a difference of 3°C resulted in a significant change in growth; in spring growth was slower at 15°C than 12°C whereas in autumn this was reversed. Survival, however, remained unaltered at 12°C in both seasons. This could have appreciable short term effects on damage levels if a similar trend were confirmed for fluctuating temperatures. A cool spring where temperatures are nearer 12°C than 15°C could promote faster growth of *D. reticulatum* populations while a cool autumn would have the opposite effect with concomitant changes in crop damage. This would be particularly critical in winter crops sown in mild autumns, e.g. winter wheat. In the longer term, if the distribution of *D. reticulatum* were to move northwards due to a rise in mean temperature its status as a pest would be likely to increase in areas where it is not currently a significant problem.

The experiments supported the hypothesis that autumn hatching slugs exhibit different growth trajectories to spring hatching slugs when reared under identical conditions suggesting that the temperature-growth relationship is more complex than previously thought. Not only does it vary with rearing temperature, but

this is further modified by the hatching season. Studies of *D. reticulatum* growth, therefore, need to take into account the hatching season. A series of assessments may give misleading results unless they are all carried out at the same time of the year. Survival was inversely related to rearing temperature, but season modified this only at 15°C and ambient temperature.

In conclusion it was found that:

1. There was considerable variation in the growth of *D. reticulatum* reared under identical conditions.
2. Growth was influenced by hatching season at all rearing temperatures; at constant temperatures of 12°C and 15°C growth was faster in autumn than spring whereas at ambient temperature the reverse was observed.
3. Within a season the association between growth and temperature was low, but negative in spring; however, it was high and positive in autumn.
4. Survival was influenced by hatching season for slugs reared at ambient temperature and 15°C and was inversely related to growth rate, but had no effect at 12°C.
5. Within a season the chances of survival improved at lower rearing temperatures.

This series of experiments has suggested a number of avenues for future research. In particular, genetic studies would help to determine the influence of paternity on growth trajectories, i.e. fertilisation by allosperm or autosperm and would allow the detailed exploration of phenotypic plasticity. The ambient treatments acted as controls to confirm the growth and survival patterns of *D. reticulatum* under field temperatures. These temperatures were, of course, fluctuating whereas the other treatments assessed constant temperatures. South (1982) found that a fluctuating temperature of 10/18°C did not have the same effect on the lifespan of *D. reticulatum* as the corresponding mean temperature of 12.7°C. This may also be the case for growth and survival and repeating the experiments at a wider range of constant and fluctuating temperatures would be informative.

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PAPER 3 - *Objective 1.1*

Hatching, Growth and Survival of Self-Fertilised *Deroceras reticulatum*

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Abstract

Eggs laid by *Deroceras reticulatum* (Müller) reared in isolation were incubated at ambient temperature, constant 12°C or constant 15°C. Appreciable numbers developed only at 15°C. These eggs laid by self-fertilised slugs developed more slowly and the hatching rate was lower than the parental population. There was considerable variation in the growth of those that hatched, despite identical rearing conditions. Their growth rate was significantly faster and their cumulative chance of survival was lower than the parental population. Eggs laid by this self-fertilised generation failed to hatch. The possible adaptive advantages of low level self-fertilisation in this predominantly cross-fertilising species are discussed.

Introduction

In the literature, *Deroceras reticulatum* is regarded as an obligate outcrosser (e.g. Runham & Hunter, 1970; McCracken & Selander, 1980; Niklas and Hoffmann 1981 in South, 1992). Whilst some authors have recorded instances of this species laying eggs when reared in isolation these are said to have been infertile (South, 1982; Nicholas, 1984). Many other members of the genus *Deroceras*, however, can successfully self-fertilise. For example, this has been observed in *D. meridonale* (Reygrobellet) (Maury & Reygrobellet 1963 in South, 1982), and *D. agreste* (L.) produced five successive generations by self-fertilisation in the laboratory (Chen *et al.*, 1984). A study of genetic variation of different slug species in the eastern USA showed that *D. laeve* (Müller) reproduced by both cross- and self-fertilisation (McCracken & Selander, 1980), supporting the assertion of Duncan (1975) that different modes of fertilisation may even operate in the same species under different conditions.

Self-fertilisation may have adaptive benefits. Foltz *et al.* (1984), in a survey of genetic variation in terrestrial slugs, showed that self-fertilising species were considerably more successful at colonising new habitats than cross-fertilised species. They concluded that the type of fertilisation system was, therefore, related to colonisation ability, a conclusion that was supported by Anderson and McCracken (1980) working on the family Philomycidae. *D. reticulatum* is classified as both a protandric and simultaneous hermaphrodite because the male system develops first and in mature slugs both the male and female systems are functional at the same time (Runham & Hunter, 1970). The sequence of development has been described in detail at the histological level (Runham & Laryea, 1968). These authors found that there was considerable overlap

between the male and female-phases; stages of development 'graded into each other'. Depending on the extent of this overlap it is possible for mature gametes of both types to be present in the same place at the same time which provides a cellular basis for self-fertilisation.

Given, therefore, that most other members of the genus *Deroceras* are able to self-fertilise either as their main method of reproduction or in addition to cross-fertilisation, that there is a cellular basis for the process in *D. reticulatum* and that this is one of the most successful species at thriving in disturbed habitats such as agricultural environments, whilst it may predominantly use cross-fertilisation, it would be surprising if it were incapable of self-fertilisation. The data presented in this paper explore the potential of this species to self-fertilise and the development of viable offspring is monitored. It is an extension of the work described in *Paper 2* on the growth and survival of *D. reticulatum*.

Materials and Methods

The eggs used in these experiments were laid by the autumn hatching slugs described in *Paper 2* (i.e. generation one slugs). Generation one slugs were reared in isolation hence any eggs that hatched were self-fertilised and constitute generation two. Only batches of five or more eggs were used. Up to a maximum of 20 such batches were collected from generation one slugs per rearing temperature (ambient, 12°C or 15°C). There were no significant differences in batch size between treatments (Kruskal-Wallis: $N = 51$, $df = 2$, $H = 0.224$, *n.s.*). Each batch was placed on fine grade netting and rinsed with distilled water to remove any soiling before being transferred into a Petri dish lined with moist laboratory tissue. The number of eggs per batch was recorded. Hatched slugs were fed on a mixed diet of Chinese cabbage and carrot *ad libitum*. Cuttlefish bone was provided as a source of calcium.

The temperature treatments described in *Paper 1* were maintained in this third phase of the study i.e. ambient temperature, 12°C or 15°C. Methods of temperature control and photoperiod remained unchanged. Egg batches and subsequent hatchlings were incubated and reared at the same temperature at which they were laid by their generation one 'parent'.

Egg maintenance and hatching were largely as described in *Paper 1*. Egg batches were prevented from drying out by remoistening with distilled water as required and hatching was checked weekly. After it had commenced, monitoring continued until two full weeks had elapsed since the last slug hatched. On each checking occasion any offspring were removed from the Petri dishes and cultured as in *Paper 2*. The development time and number of slugs hatching per batch were recorded. Slugs were cultured and weighed as described in *Paper 2*. Slugs were monitored for a total of 20 weeks.

Continuous data were tested for normality and transformed if necessary. Percentages were arcsine transformed. In all cases this resulted in parametric data which were then analysed using an independent sample t-test or analysis of variance (ANOVA). ANOVA was followed by Tukey post-hoc tests as

appropriate. Regression was used to analyse variables between which a cause-effect relationship was postulated; for binomial data binary logistic regression was carried out, otherwise linear regression was used. Discrete counts were compared using chi-squared goodness of fit. Continuous weight data were analysed using a repeated measures analysis of variance (ANOVA). To account for the non-parametric nature of the data, as confirmed by Mauchley's test of sphericity and Box's M-test, the ANOVA was adjusted by applying the lower-bound epsilon correction. Tukey post-hoc tests were performed as appropriate. Survival data were analysed with the Kaplan-Meier procedure, using the Breslow test to compare between treatments. A hazard ratio was calculated.

Results

Although egg batches were collected for all three temperature treatments, appreciable numbers of generation two slugs hatched only in the 15°C treatment (*Table 3.1*). Furthermore, of these, the majority were from the same egg batch. Analyses are, therefore, interpreted with caution and treated as exploratory.

Table 3.1: Number of egg batches collected, mean number of eggs (± S.E.) and total number hatching at ambient, 12°C and 15°C incubation temperatures.

	Incubation Temperature		
	Ambient	*12°C*	*15°C*
Number of batches collected (>=5 eggs)	11	20	20
Mean number of eggs (± S.E.)	10.27 ± 1.97	11.90 ± 2.68	14.90 ± 3.96
Number of eggs hatching	0	1	18

Analyses of parental effects on egg laying use data from all three rearing temperatures. All other analyses are confined to slugs reared at 15°C due to insufficient numbers at the other temperatures. Since these slugs hatched in May-July 2003 they are analogous to the spring hatching generation one slugs reared at 15°C and inter-generational comparisons are based on these groups.

Parental weight at laying differed significantly depending on the rearing temperature (ANOVA: $F_{2, 48} = 5.725$, $P < 0.01$). Tukey post-hoc tests showed that parents reared at 15°C were heavier at laying than those at 12°C or ambient temperature, but there were no differences between these latter two treatments (*Table 1.2*). There were also significant differences in mean parental age at laying between all three rearing temperatures (ANOVA: $F_{2, 48} = 50.910$, $P < 0.001$). Parents reared at 15°C were youngest and those at ambient temperature were oldest (*Table 3.2*). There was no correlation between parental weight and batch size (linear regression: $N = 51$, $R^2 = 0.036$, *n.s.*).

Table 3.2: Mean (± S.E.) parental age and weight at laying batches of 5 or more eggs at each of three incubation temperatures for Deroceras reticulatum *reared in isolation.*

Incubation Temperature (°C)	*Parental weight at laying (mg)*	*Parental age at laying (wks)*
Ambient	1004.10 ± 65.63	31.82 ± 0.67
12	992.83 ± 56.35	24.60 ± 0.72
15	1217.44 ± 46.00	21.30 ± 0.39

At 15°C batch size was not a significant predictor of whether any of the eggs in a batch hatched for generation two eggs (binary logistic regression: N = 20, Z = 0.37, *n.s.*, percent concordant pairs = 37.3%). When compared with spring hatching generation one slugs it was seen that, for batches of five or more eggs, there was a significant difference between batch size; generation two batches were smaller (ANOVA: $F_{1, 38} = 10.74$, $P < 0.01$) (*Table 3.3*). In addition, fewer of these batches had at least one egg hatching when compared to generation one (Fisher's Exact Test: N = 40, P = 0.04).

There was a significant relationship between the batch size and the number of eggs hatching in generation two (Linear regression: N = 20, $R^2 = 0.39$, $P < 0.01$), however this was mainly due to an outlier. This outlier was a particularly large batch of eggs which produced a large proportion of the slugs that hatched. When this was removed, the relationship was not significant (Linear regression: N = 19, $R^2 = 0.04$, *n.s.*) (*Fig.3.1*). In both cases, the regression line was forced through the origin as the number of eggs hatching must equal zero when batch size is zero.

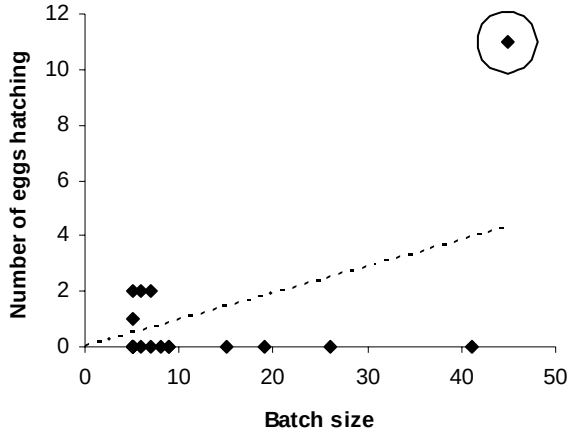

Figure 3.1: Scatter plot of the number of eggs hatching and batch size for generation two eggs reared at 15°C. The circled data point is an outlier. The dotted line shows the relationship between batch size and number of eggs hatching when the outlier was included. Regression equation: y = 0.096x.

At 15°C generation two slug eggs took longer to develop than spring hatching generation one eggs, i.e. the parental population (t-test: N = 25, df = 23, t = -6.503, *P < 0.001*). Similarly, there was a significant difference in hatching rate between these groups of slugs, with generation two slugs having a lower hatching rate (t-test: N = 25, df = 23, t = 6.830, *P < 0.001*). Mean values are summarised in *Table 3.3*.

Table 3.3: Mean (± S.E.) development time (weeks), hatching rate (%) and batch size for spring hatching generation one and generation two Deroceras reticulatum *reared at 15°C.*

Generation	*Development time (wks)*	*Hatching rate (%)*	*Batch Size (No. eggs)*
1	3.53 ± 0.09	74.5 ± 4.72	22.3 ± 2.1
2	5.00 ± 0.27	29.2 ± 3.48	11.9 ± 2.7

Although the sample size was considerably smaller, at a rearing temperature of 15°C generation two slugs were consistently heavier at all weeks from 0-20 compared to spring hatching generation one slugs. This became most marked from week 4 onwards (*Fig. 3.2*). This was a highly significant difference (ANOVA: $F_{1, 99}$ = 185.41, *P < 0.001*). The mean weight at week 20 (± S.E.) was 121.55 ± 11.56 mg (N = 94) and 794.22 ± 186.12 mg (N = 7) for generation one and two slugs respectively.

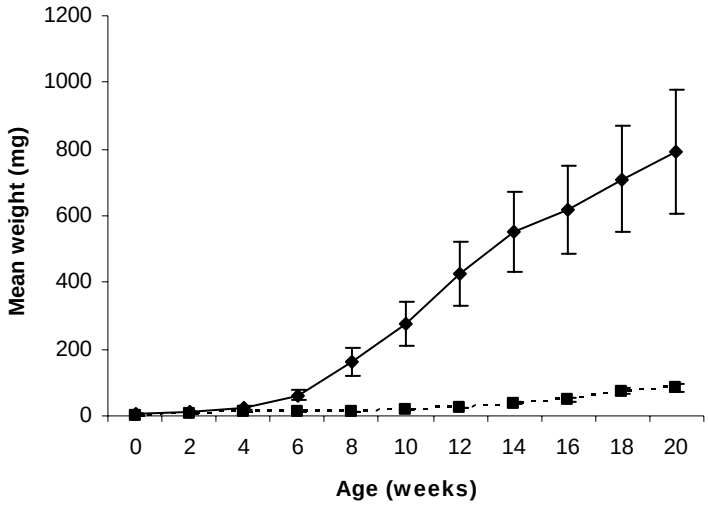

Figure 3.2: Mean weight (mg) of Deroceras reticulatum *from spring hatching generation one (dotted line) and self-fertilised generation two (solid line) reared at 15°C. Bars represent S.E.*

At a rearing temperature of 15°C there was a significant difference in the survival of self-fertilised generation two slugs and spring hatching generation one slugs at all times from week 0-20 (Kaplan-Meier (Breslow test): N = 101, df = 1, χ^2 = 18.22, *P<0.001*) (*Fig.3.3*). The self-fertilised slugs had a lower chance of survival from weeks 0-20 (hazard ratio = 6.56); the mean survival times (± S.E.) during this period are 17.36 ± 0.55 weeks and 11.56 ± 1.92 weeks for generation one and two slugs respectively.

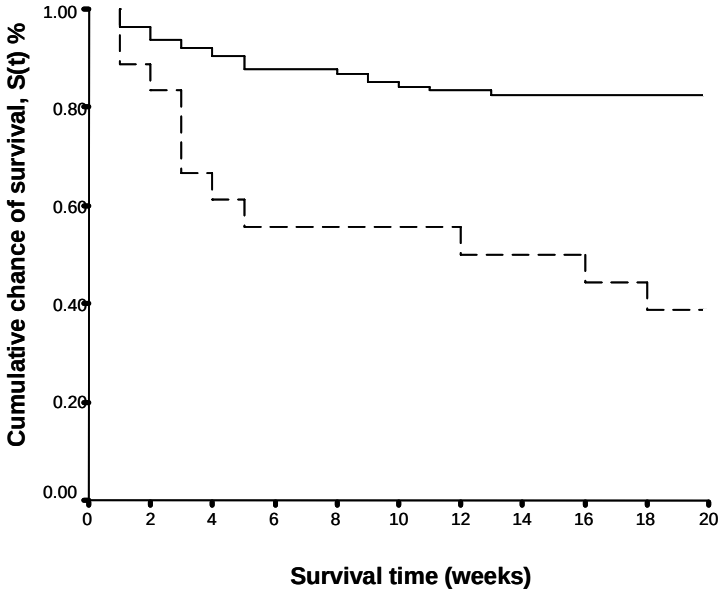

Figure 3.3: Kaplan-Meier estimators for Deroceras reticulatum *from spring hatching generation one slugs (solid line) and self-fertilised generation two slugs (dashed line) reared at 15°C.*

One of the self-fertilised generation two slugs laid three batches of eggs, i.e. a third generation. None of these eggs hatched.

Discussion

The current experiment contradicts published reports that *D. reticulatum* is an obligate outcrosser and that eggs laid by individuals reared in isolation are infertile (South, 1982; Nicholas, 1984); some such eggs were found to be viable and the offspring were, by definition, produced by a self-fertilised parent. Consequently, this is the first study that contrasts the relative development of self-fertilised individuals of this species (generation two) with those representing an 'average population' (generation one, *Paper 2*), hitherto assumed to be cross-fertilised. It was hypothesised in *Paper 2* that, whilst many of the individuals comprising generation one were probably cross-fertilised by allosperm, some may have been self-fertilised by autosperm. Formally testing this was beyond the scope of this work and therefore these slugs are considered an 'average population' to avoid ambiguity.

It is reiterated that the analyses presented in this paper are exploratory and further work is required to test the hypotheses they generate. In comparisons between generations one and two, the analyses concerning events prior to hatching were balanced as the same number of egg batches were collected for both generations. The number of generation two offspring that hatched, however, was small and hence inter-generational comparisons of growth and survival are highly unbalanced. This was unavoidable, but means that the results of these analyses must be treated with caution.

Parent slugs reared at 15°C were significantly heavier when they began laying batches of five or more eggs than those at 12°C or ambient temperature; there was no difference between these latter two groups. This finding is in broad agreement with South (1982) who found that the mean weight at egg laying tended to increase with temperature for *D. reticulatum*, although this was somewhat obscured at particularly high or low temperatures. It is not clear why there was no difference between parents reared at 12°C and ambient temperature. The mean ambient temperature was, unfortunately, not recorded due to malfunction of the Tinytalk® datalogger, but it could have been close to 12°C and thus the actual differences in treatments may have been small. South (1982) however, found that cycling and equivalent mean temperatures had different effects on the weight at egg laying implying that other factors must also have influenced the current results. One such factor may be photoperiod, which differed between constant and ambient treatments; there is evidence that photoperiod is involved in initiating reproductive maturation in *Limax maximus* (L.) with the transition to long-days promoting male-phase development (Sokolove & McCrone, 1978). It seems, therefore, that a combination of factors is likely to modify the onset of egg laying in *D. reticulatum* and additional work is needed before any further conclusions can be drawn.

There was a clear inverse relationship between rearing temperature and age at laying; the higher the temperature, the younger were the parent slugs when they began laying eggs, i.e. the shorter the lifecycle. Again, this supports the findings of South (1982). Since it was shown in *Paper2*, however, that rearing temperature was positively associated with growth (i.e. weight) for the parent slugs and that weight was a poor indicator of age, it would seem likely that weight may be confounding this apparent association between rearing temperature and age.

There was no correlation between parental weight and batch size. This may be partly because parent slugs had only recently begun to lay eggs and the process can be somewhat sporadic initially (*pers. obs.*). In retrospect it may have been more appropriate to collect batches nearer to the mean size reported for this species (twenty two) (Carrick, 1938). It was seen, however, that for the wild caught slugs that laid the eggs constituting generation one the correlation between parental weight and batch size was only significant in spring and even here the R^2 values were very low. Taken together, it would seem that parental weight has a very weak influence, if at all, on batch size and other factors are more important.

For generation two eggs incubated at 15°C batch size was not a significant predictor of whether any of the eggs in the batch hatched. This is not surprising given that the majority of those hatching were from the same batch (*Fig. 3.1*). Although batch size was 'artificially manipulated' to a certain extent by only collecting those with five or more eggs, *Fig. 3.1* suggests that it is unlikely that this would unduly bias the results; it can be seen that for batches smaller than 10 eggs, similar numbers had at least one egg hatching as had none hatch at all and very much larger batches also had no eggs hatching. A more likely explanation is that many of the eggs collected may not actually have been fertilised. It has been suggested that sperm

production may vary among individuals in the snail *Arianta arbustorum* (L.) (Locher & Baur, 1999) and this may also be the case for *D. reticulatum*, particularly for those that have just reached maturity.

It has been suggested that in *D. reticulatum* copulation usually precedes egg laying and that, as a consequence, egg laying is delayed in slugs reared in isolation (Runham & Laryea, 1968), perhaps due to a shift in reproductive physiology. A basic assumption of sex-allocation theory in simultaneous hermaphrodites is that there is a trade-off between male and female function because the individual has a fixed amount of resources to allocate to each gender (Charnov, 1982). Locher and Baur (2000) suggest that the optimal allocation to male versus female function in *A. arbustorum* may depend on density such that at higher densities there is a higher risk of sperm competition and therefore a higher mating frequency would lead to a larger allocation to the male reproductive function. The converse of this argument, which mirrors the situation in the current experiments, is that when there is a low density and therefore low mating frequency, there is a similarly low risk of sperm competition leading to a shift in resource allocation favouring the female reproductive function, i.e. egg laying. If this is the case in *D. reticulatum* then perhaps the eggs collected soon after oviposition commences are unfertilised by autosperm because reproductive physiology is still stabilising.

Generation two batches were significantly smaller than those of generation one. Whilst this may indicate that self-fertilisation is a 'back-up strategy' and not the most effective means of reproduction in this species, it could also be explained by initial sporadic laying and reflect a change in reproductive physiology as described above.

Of the generation two slugs that hatched, the development time was slower and the hatching rate was more than 50% lower than for generation one slugs. This supports the suggestion of Duncan (1975) that allosperm is usually more effective in fertilisation. He speculates that this is due to a biochemical barrier to self-fertilisation or to the prostatic secretions that allosperm receive following copulation.

To confirm the preliminary observations of the current study a larger sample of generation two eggs would be required along with a greater number of different parents. If this were to bear out these findings it would support the suggestion that self-fertilisation is a secondary reproductive strategy in *D. reticulatum* acting as a buffer against adverse conditions affecting the main cross-fertilised population. By developing more slowly, and consequently hatching later, the self-fertilised slugs may provide a background 'pool' to replace cross-fertilised slugs that hatch earlier and subsequently suffer mortality due to harsh conditions or predation, for example. The reduction in hatching rate indicates that it is not the predominant mode of fertilisation, but would, nevertheless, maintain a small pool if individuals to rebuild the population after a catastrophic decline in numbers.

Although many of the self-fertilised slugs were from the same batch, it is known that variation in growth rate is considerable even between slugs hatching from the same batch (Prior, 1983; Shibata & Rollo, 1988; South, 1992). Whilst, therefore, taking into account the low numbers of generation two slugs in this analysis, it may be that the range of weights observed are still representative of self-fertilised *D. reticulatum* generally.

That the growth of self-fertilised slugs was much faster than that of generation one slugs reared under the same conditions supports the hypothesis proposed in *Paper 2* that the division of *D. reticulatum* into slow and fast growers may be due to whether the eggs are fertilised with allosperm or autosperm. Moreover, these results go further and indicate that if this is the case, then the faster growing slugs would probably be fertilised with autosperm.

There were much wider standard errors in the growth data for self-fertilised generation two slugs compared to generation one which are due to the markedly smaller sample size, although these still do not overlap between generations. The similarity in mean weight from weeks 0-4, regardless of the rearing treatment as reported in *Paper 2*, is also apparent in these results. After this time, again, there is a rapid divergence in weight between the generations which increases with time.

Unfortunately, as for generation one, the egg size in generation two was not measured. Despite the slower development time of the self-fertilised generation two eggs, the significantly higher growth rate of the offspring would predict that the eggs were smaller than those of generation one, according to Shibata and Rollo (1988).

The cumulative chance of survival of self-fertilised *D. reticulatum* was much lower than for generation one slugs at all ages, the difference between the generations increasing with time. By week 20, self-fertilised slugs had a less than 50% chance of being alive compared to more than 80% for generation one slugs. The difference in survival between these generations was much greater than between any of the treatments compared within generation one (*Paper 2*). There was, however, the same apparent trade-off between growth and survival.

It was seen in *D. agreste*, which frequently self-fertilises, that self-fertilised slugs had a longer lifespan than those that were cross-fertilised (Chen *et al.*, 1984). The reverse was seen in the current experiment with *D. reticulatum* suggesting, again, that self-fertilisation is a 'back-up strategy' in this species. Whilst self-fertilised slugs are generally not as 'fit' as generation one slugs in terms of hatching rate, longevity etc. they may at least provide a 'stop gap' cohort. If these individuals were then to mate, resulting in cross-fertilised offspring, this may explain the sudden explosion in *D. reticulatum* populations reported in areas where numbers had previously been severely reduced by adverse conditions (Miles *et al.*, 1931; South, 1989b).

One of the second generation self-fertilised slugs laid a batch of eggs. This third generation batch failed to hatch and did not show any signs of development during the 10 week monitoring period. Although no conclusions can be drawn from the results of a single batch, it may be speculated that if self-fertilisation acts as an emergency 'back up strategy', it is not an effective long-term coping mechanism and might only have the potential to 'buffer' a population against adverse conditions for one generation.

In conclusion it was found that:

1. Generation one *D. reticulatum* laid eggs when reared in isolation (*Paper 2*). Some of these hatched giving rise to a self-fertilised second generation.
2. For the generation one parental population there was no clear relationship between mean weight and age at egg laying.
3. For the second generation eggs development time was longer and hatching rate was lower compared to the parental population. The egg batches were also smaller and batch size was a poor predictor of whether any of the eggs in a batch hatched.
4. The growth rate of self-fertilised *D. reticulatum* showed marked variation under identical conditions and was significantly faster than that of the parental population. It is suggested that growth rate in field populations may be connected to whether eggs are fertilised by autosperm or allosperm.
5. Survival of self-fertilised *D. reticulatum* was significantly lower at all ages than the parental population.
6. Fast growth and longevity seem to be mutually exclusive.
7. Eggs laid by the self-fertilised *D. reticulatum* did not hatch.

Acknowledgements

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PAPER 4 - *Objective 1.1*

The Relationship between Weight and Female-Phase Sexual Maturity in *Deroceras reticulatum*

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Abstract

Sexual maturity of *Deroceras reticulatum* (Müller) was classified using a five category system based on body, ovotestis and albumen gland weights. This categorical system was shown to be efficient in allocating individuals to a maturity class. There was a significant relationship between body weight and female-phase maturity for laboratory reared individuals and this was described with a probability equation. The relationship was validated against field-collected slugs and found to predict female-phase maturity with 86% accuracy. The probability of slugs being mature females was relatively low, even at high body weights. There may be a male-phase bias in populations of *Deroceras reticulatum* with less than 20% physiologically female individuals at any time during the year. This requires further testing in other locations. The implications of these findings for control are discussed.

Introduction

Growth rate in slugs has been shown to be a very 'plastic' characteristic varying considerably even between individuals from the same egg batch (e.g. Hunter, 1978; Shibata & Rollo, 1988; South, 1992). All size classes of slugs may, as a result, be found in a population at any one time (Haynes *et al.*, 1996) and it is of interest to know whether this is reflected in the maturity structure. There is considerable disagreement in the literature concerning the relationship between body weight and maturity, in large part due to differences in the way that maturity is defined and assessed. For example, Runham and Laryea (1968) defined maturation on a gland by gland basis, Smith (1966) divided maturity into male and female-phase, Hunter (1968b) considered slugs to be mature if the hermaphrodite duct contained sperm or eggs and Haynes *et al.* (1996) based their conclusions on combined gland and body weight analyses. Assessments of maturity have, consequently, been based on one of two approaches: histological classifications (Smith, 1966; Hunter, 1968b; Runham & Laryea, 1968; Parivar, 1978; Runham, 1978) or the use of gland indices, i.e. gland/body weight ratios (Sokolove & McCrone, 1978; Duval & Banville, 1989; Haynes *et al.*, 1996). The former are time consuming and difficult to quantify reliably whereas the latter, while remedying the disadvantages of

the former, result in numerical values that are not particularly intuitive to interpret and do not allow the separation of body weight from gland weights.

The experiments presented in this paper were designed to explore the relationship between body weight and female-phase maturity in *Deroceras reticulatum* and used multivariate techniques to develop a new system of maturity classification. This was based on laboratory reared slugs described in *Paper 2* and was validated against field collected slugs. By focusing on the female-phase the results relate to that section of the population capable of egg laying and may, therefore, be of applied use in the indirect estimation of population egg banks. Such estimates are particularly difficult to obtain by direct methods and would be of considerable benefit in studies of population dynamics.

Materials and Methods

The slugs used in these experiments were preserved specimens from the study of growth and survival described in *Paper 2*. They had been laid in autumn by field collected 'parents'. After hatching they were reared in isolation and fed *ad libitum* on Chinese cabbage and carrots. Their growth was monitored regularly; when monitoring ceased they were preserved in 70% ethanol. This was changed and replaced with fresh 70% ethanol two weeks after they were preserved. Their weight at preservation was recorded. Field collected specimens were used to validate the results from laboratory reared slugs. These were preserved in the same way as the laboratory specimens and came from Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659).

Forty two preserved laboratory reared slugs and 50 preserved field collected specimens were dissected to remove the ovotestis (also known as the hermaphrodite gland) and albumen gland. Both samples comprised a similar range of body weights over 200 mg; the glands in slugs weighing less than this were too small to be identified with certainty. Each slug was weighed before dissection using a Mettler MT 5 balance to an accuracy of 0.01 mg. Due to the evaporation of ethanol from the body surface on exposure to air the recorded weight fluctuated and therefore a standard 'settling period' of five seconds was allowed between placing the slug on the balance and taking the reading. The glands were dissected from each individual according to the procedure outlined in Bullough (1970), augmented with modifications from Reise and Hutchinson (2001) and Block (1967), the latter being a method for snail dissection. The final protocol was as follows:

1. Any mucus was wiped from the surface of the slug. The slug was then placed in a wax filled dissection tray.
2. The first incision was made by cutting the integument along the left foot fringe using a pair of small spring loaded scissors. This resulted in less damage to the distal genitalia than following the usual recommendation of a median cut (J Hutchinson, *pers. comm..)*.

3. The mantle was peeled back over to the right hand side and pinned out, taking care not to puncture any of the internal organs (*Fig. 4.1)*.

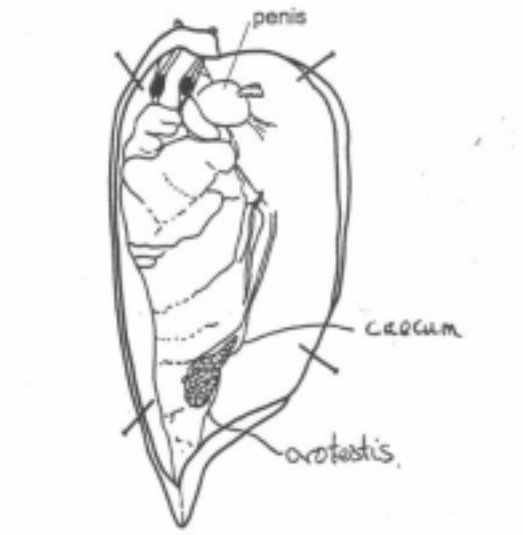


Figure 4.1: Pinning out the slug for dissection (after Reise & Hutchinson, 2001).

4. The dissection tray was filled with 70% ethanol to completely immerse the slug. [*The ovotestis is immediately visible lying to the posterior of the animal, dorsal to the visceral mass. It is a brown/purple coloured structure. The reproductive system runs very close to the digestive system and the two are entwined*].
5. The reproductive and digestive system were separated by carefully teasing apart, beginning at the ovotestis and working forwards.
   [The narrow hermaphrodite duct emerges from the ovotestis. It is unconvoluted and passes between the crop and the digestive gland, entering the large, creamy-coloured albumen gland].
6. The hermaphrodite duct was cut from between the ovotestis and the albumen gland. The ovotestis was placed into a glass tube containing 70% ethanol prior to weighing.
   [The convoluted common duct arises from the albumen gland and continues towards the anterior of the animal where it separates into two distinct male and female ducts].
7. The emerging common duct was cut from the albumen gland at the point of entry and the gland was placed into a second glass tube containing 70% ethanol prior to weighing.

The dissected ovotestis and albumen gland were weighed using a Mettler MT 5 balance to an accuracy of 0.01 mg. A small plastic dish (3.5 cm diameter) was used as a weighing receptacle and the balance was tared to zero before the gland was added. As for weighing the intact slug, a standard settling time of five seconds was allowed to account for fluctuation in the reading due to the evaporation of ethanol from the glands upon exposure to air.

The relationship between fresh and preserved weight was assessed using linear regression. For laboratory reared slugs principle components analysis (PCA) was used to explore the extent to which the measured body and gland weights accounted for the overall differences between individuals in the sample. The outcome of this was used to define a 'maturity score' which classified slugs into one of five groups (immature, early maturation, mid maturation, late maturation and mature). Discriminant analysis (DA) was carried out to assess the accuracy of the maturity scoring system derived from PCA and binary logistic regression was used to evaluate the relationship between maturity score and body weight. Data from field collected slugs were used to validate the binary logistic regression equation.

Results

Linear regression showed that there was a strong significant relationship between the fresh and preserved weight of laboratory reared slugs (Linear regression: N = 42, $R^2 = 0.993$, $P < 0.001$) (*Fig. 4.2*).

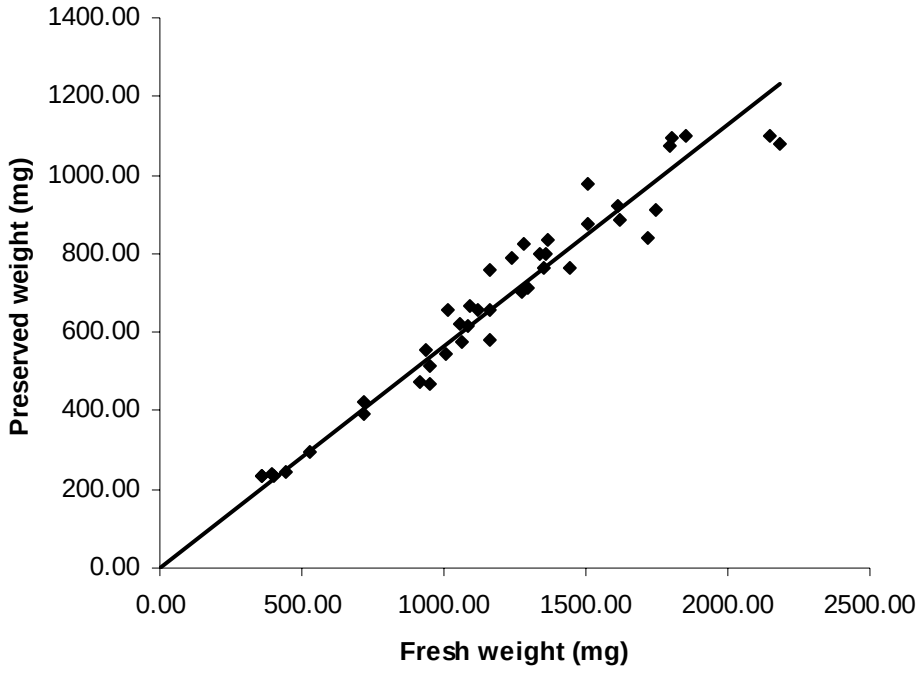


Figure 4.2: Relationship between fresh and preserved weight of laboratory reared Deroceras reticulatum. *Regression equation (line intercepts at origin): Preserved weight= 0.565 x fresh weight.*

A sample of 20 field collected slugs were also weighed before and after preservation and the relationship was almost identical (Linear regression: N = 20, $R^2 = 0.996$, $P < 0.001$; Regression equation: preserved weight = 0.567 x fresh weight). It was assumed that the change in intact weight due to preservation would also apply approximately to the gland weights. The regression equation of the laboratory reared slugs was, therefore, used to estimate the fresh weights of dissected glands (*Table 4.1*).

Table 4.1 shows the body and gland weights of the laboratory reared slugs. There were no significant differences in weight of selected slugs that were reared at different temperatures and therefore the data were pooled (ANOVA: $F_{2, 39} = 1.222$, *n.s.*). In one specimen (slug 9) there was no albumen gland. *Table 4.2* shows similar data for the field collected slugs. Principle components analysis showed that all three weight variables were required to adequately describe the differences between individuals in the sample (*Table 4.3*).

Table 4.3: Eigenvalues and component weightings for principle components analysis on body weight, ovotestis and albumen gland weights in Deroceras reticulatum.

	Principle Component		
Variable	*1*	*2*	*3*
Body weight	-0.577	0.576	0.579
Ovotests weight	-0.568	-0.793	0.221
Albumen gland weight	-0.586	0.201	-0.785
Eigenvalue	2.182	0.441	0.377
% total variance accounted for	72.7	14.7	12.6

Pearson correlation confirmed that body weight and both gland weights significantly contributed to principle component 1. In addition to body weight, component 2 was correlated significantly with ovotestis weight and component 3 was correlated significantly with albumen gland weight (*Table 4.4)*.

Table 4.4: Pearson correlation coefficients between weight variables and each principle component with associated P-values.

Principle Component	*Body Weight*	*Ovotestis Weight*	*Albumen Gland*
1	-0.856	-0.841	-0.878
	P < 0.001	*P < 0.001*	*P < 0.001*
2	0.406	-0.517	0.100
	P < 0.01	*P < 0.01*	*n.s.*
3	0.322	0.182	-0.469
	P < 0.05	*n.s.*	*P < 0.01*

Table 4.1: Body and gland weights of laboratory reared Deroceras reticulatum.

Slug ID	*Body Weight (mg)*		*Gland Weight (mg)*			
			Ovotestis		*Albumen Gland*	
	Fresh	*Preserved*	*Fresh* §	*Preserved*	*Fresh* §	*Preserved*
1	440.42	243.37	1.29	0.73	1.66	0.94
2	528.61	297.09	5.26	2.97	6.85	3.87
3	719.57	391.74	28.55	16.13	6.96	3.93
4	915.29	471.70	10.80	6.10	78.65	44.44
5	934.95	554.07	8.25	4.66	94.85	53.59
6	1015.20	654.49	9.96	5.63	168.42	95.16
7	1092.63	666.13	11.06	6.25	110.46	62.41
8	1117.35	655.28	17.75	10.03	125.20	70.74
9	1161.11	759.09	31.66	17.89	n/a†	n/a†
10	1271.20	700.37	11.40	6.44	120.64	68.16
11	1293.14	714.88	6.92	3.91	153.40	86.67
12	1444.05	764.13	13.47	7.61	126.60	71.53
13	1720.34	840.69	14.97	8.46	70.34	39.74
14	1745.30	911.59	12.51	7.07	105.70	59.72
15	358.96	233.16	0.44	0.25	0.27	0.15
16	403.22	235.11	1.27	0.72	4.53	2.56
17	1057.03	623.13	24.04	13.58	132.41	74.81
18	1159.26	657.73	15.49	8.75	140.25	79.24
19	1161.10	579.20	23.10	13.05	128.42	72.56
20	1282.78	825.69	21.13	11.94	168.16	95.01
21	1341.26	798.06	30.39	17.17	187.96	106.20
22	1351.40	762.34	27.49	15.53	197.26	111.45
23	1503.96	877.24	20.62	11.65	207.33	117.14
24	1611.62	921.67	32.39	18.30	191.04	107.94
25	1621.20	885.23	26.97	15.24	165.58	93.55
26	1803.63	1093.84	14.78	8.35	100.37	56.71
27	2147.36	1100.33	17.79	10.05	151.43	85.56
28	2181.35	1078.76	25.75	14.55	189.22	106.91
29	356.18	233.21	2.16	1.22	2.81	1.59
30	397.41	237.73	3.93	2.22	2.85	1.61
31	721.75	420.66	6.48	3.66	91.91	51.93
32	948.80	468.22	15.56	8.79	161.10	91.02
33	952.26	512.39	18.41	10.40	185.70	104.92
34	1008.49	546.25	22.99	12.99	60.21	34.02
35	1064.35	576.72	17.73	10.02	236.16	133.43
36	1082.12	615.85	13.03	7.36	183.84	103.87
37	1238.46	789.84	15.59	8.81	170.30	96.22
38	1360.47	797.38	17.91	10.12	44.57	25.18
39	1367.16	835.91	19.15	10.82	199.27	112.59
40	1509.59	977.60	17.93	10.13	170.50	96.33
41	1796.23	1073.58	12.71	7.18	156.73	88.55
42	1850.78	1098.72	16.00	9.04	129.26	73.03

§ These weights were predicted from the regression shown in Fig. 4.2.

† There was no albumen gland in this specimen.

Table 4.2: Body and gland weights of field collected Deroceras reticulatum *from Close House Field Station, Northumberland.*

Slug ID	*Body Weight (mg)*		*Gland Weight (mg)*			
			Ovotestis		*Albumen Gland*	
	Fresh	*Preserved*	*Fresh §*	*Preserved*	*Fresh §*	*Preserved*
1	363.29	213.84	18.74	10.30	n/a†	n/a†
2	410.04	238.91	11.72	6.44	26.66	14.65
3	410.19	238.99	16.54	9.09	21.45	11.79
4	411.18	239.52	16.32	8.97	n/a†	n/a†
5	416.53	242.39	10.24	5.63	24.09	13.24
6	442.00	256.05	19.47	10.70	23.76	13.06
7	443.03	256.60	7.22	3.97	41.90	23.03
8	457.07	264.13	29.06	15.97	15.43	8.48
9	468.01	270.00	9.10	5.00	26.47	14.55
10	470.60	271.39	28.04	15.41	17.61	9.68
11	484.05	278.60	8.64	4.75	30.33	16.67
12	493.91	283.89	9.17	5.04	19.27	10.59
13	497.03	285.56	18.18	9.99	13.79	7.58
14	501.82	288.13	37.97	20.87	15.99	8.79
15	511.16	293.14	13.99	7.69	23.62	12.98
16	540.19	308.71	17.01	9.35	18.81	10.34
17	558.50	318.53	23.82	13.09	21.02	11.55
18	567.10	323.14	18.65	10.25	40.63	22.33
19	568.11	323.68	15.10	8.30	47.73	26.23
20	575.23	327.50	11.75	6.46	36.15	19.87
21	590.07	335.46	10.90	5.99	38.03	20.90
22	596.04	338.66	15.81	8.69	50.64	27.83
23	607.92	345.03	8.66	4.76	49.60	27.26
24	627.46	355.51	13.57	7.46	26.46	14.54
25	645.25	365.05	16.28	8.95	29.86	16.41
26	651.16	368.22	7.68	4.22	34.57	19.00
27	651.73	368.53	8.11	4.46	45.41	24.96
28	692.18	390.22	12.95	7.12	31.48	17.30
29	708.59	399.02	14.26	7.84	36.19	19.89
30	763.28	428.35	21.80	11.98	23.60	12.97
31	812.24	465.95	16.05	8.82	52.98	29.12
32	815.68	459.43	25.86	14.21	68.85	37.84
33	871.11	477.41	22.62	12.43	84.59	46.49
34	873.10	477.34	13.23	7.27	80.75	44.38
35	875.26	446.57	16.27	8.94	83.70	46
36	896.29	551.80	17.94	9.86	64.23	35.30
37	925.54	526.85	27.55	15.14	85.43	46.95
38	928.28	485.20	30.99	17.03	58.10	31.93
39	957.46	591.57	12.72	6.99	55.95	30.75
40	961.26	571.60	14.63	8.04	167.92	92.29
41	961.85	535.2	19.21	10.56	111.46	61.26
42	981.96	567.59	34.15	18.77	80.68	44.34
43	1012.41	610.49	10.70	5.88	112.52	61.84
44	1016.80	530.47	22.05	12.12	94.43	51.90
45	1020.04	596.05	26.11	14.35	36.90	20.28
46	1025.36	656.28	14.94	8.21	55.70	30.61
47	1146.24	616.93	6.15	3.38	170.14	93.51
48	1156.72	689.04	17.69	9.72	108.70	59.74
49	1189.52	614.51	8.66	4.76	117.78	64.73
50	1264.75	705.54	23.67	13.01	76.64	42.12

§ These weights were predicted from the regression shown in Fig. 4.2).
† There was no albumen gland in this specimen.

Each of the principle components was plotted against the weight variables with which it was significantly correlated. The plots were inspected to determine where there were divisions between clusters of data points and individuals were given a maturity code for each plot according to the cluster they belonged to. If there were two clusters the codes were 1 for immature and 2 for mature; if there were three clusters the codes were 1 for immature, 2 for maturing and 3 for mature. An example is shown in *Fig. 4.3*. These codes were then combined to assign each individual a single overall value for that principle component based on the protandric sequence of reproductive development in this species. There is some overlap between developmental stages and hence some judgement had to be exercised, but *Table 4.5* summarises the general 'decision matrix' for the assignment of these codes. Finally, the three principle component scores for each individual were used to categorise it in one of five overall 'maturity groups' (*Table 4.6*). The first digit in the three figure codes shown in *Table 4.6* refers to the maturity score for the first principle component (as detailed in *Table 4.5*), the second digit is the score for the second principle component and finally the third digit is that assigned for the third principle component.

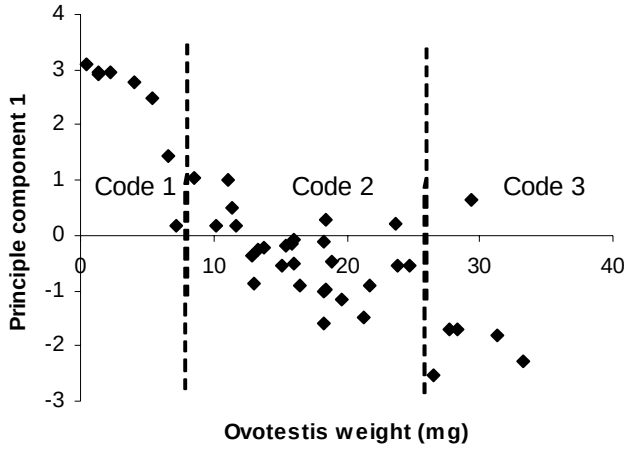


Figure 4.3: An example to show how individuals were assigned maturity codes. Weight variables were plotted against each principle component with which they were significantly associated. The plots were then divided into clusters by visual inspection and each individual was assigned a code according to the cluster it belonged to.

Discriminant analysis was used to assess how well the classification of weight variables into five maturity groups distinguished between individuals. This analysis generates a 'discriminant function' based on combinations of predictor variables (in this case weight) that best divide individuals into a requested number of groups (in this case five). This was compared with the five maturity groups derived from the principle components analysis and there was 95.1% agreement between the two (*Table 4.7*).

Table 4.5: Maturity scores for each principle component. Brackets indicate codes where judgement most commonly had to be exercised due overlapping between gland developmental stages.

Principle Component	*Maturity Code*	*Body Weight*			*Ovotestis Weight*			*Albumen Gland Weight*		
		Sml	*Med*	*Lge*	*Sml*	*Med*	*Lge*	*Sml*	*Med*	*Lge*
1	1	+			+			+		
	2		+			+			+	
	3			+			+			+
2	1	+			+					
	2		+		+	(+)				
	3			+			+			
3	1	+						+		
	2		+					(+)	+	
	3			+						+

Table 4.6: Final 'maturity group' categories.

Category	*Maturity*	*Separate PCA Codes*
1	Immature	111
2	Early maturation	222
3	Mid maturation	232/231
4	Late maturation	223
5	Mature	233/333

Table 4.7: Results of discriminant analysis to assess accuracy of maturity classification.

Maturity group assigned by PCA	*Group predicted from Discriminant Analysis*				
	1	*2*	*3*	*4*	*5*
1	6	0	0	0	0
2	0	12	0	1	0
3	0	0	4	0	0
4	0	1	0	12	0
5	0	0	0	0	5
Total	6	13	4	13	5
Number Correct	6	12	4	12	5
% Correct	100.0	92.3	100.0	92.3	100.0

The two individuals misclassified in the maturity groups assigned by principle components analysis were numbers 10 and 17 (*Table 4.1*). These had been put into groups 2 and 4 respectively (early and late maturation stages) and the misclassification is likely to have arisen due to overlap between the development of the ovotestis and albumen gland making them difficult to classify. The probabilities of them belonging to the alternative groups assigned by the discriminant analysis (4 and 2 respectively) were only marginally higher than those of the groups they had been classified in, but values were, in any case, reassigned.

Binary logistic regression showed that there was a significant relationship between body weight and maturity (Binary logistic regression: N = 41, Z = 1.06, $P < 0.05$; percent concordant pairs = 71.3%). The curve predicted from the regression describes the probability that a slug of a given body weight is female-phase mature (*Fig. 4.4*). As can be seen, this curve is smooth and flat, which reflects the gradual nature of maturation in *D. reticulatum*. Although age data were available for each slug, this was not included in the binary logistic regression as it cannot be used in classifying field collected specimens, whose ages are unknown.

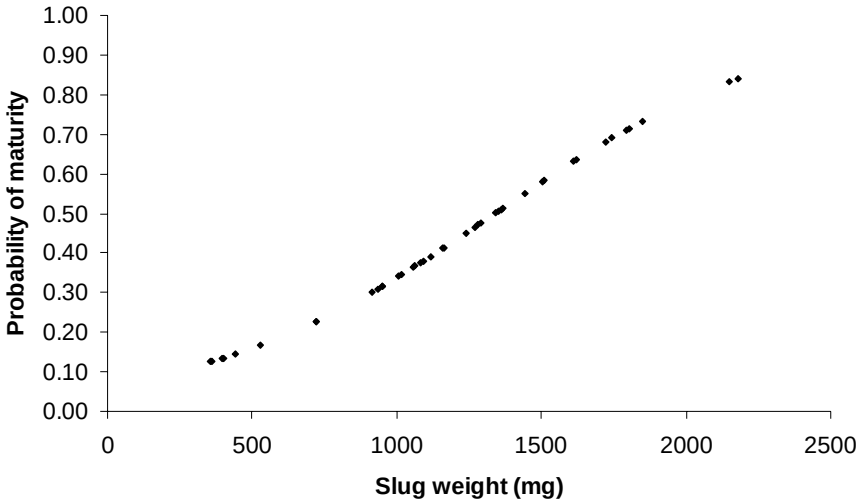


Figure 4.4: The relationship between weight and maturity for laboratory reared Deroceras reticulatum *as predicted by binary logistic regression. Binary logistic regression equation: Probability of maturity = (exp (-2.654 + 0.001981 x weight)) / (1 + exp (-2.654 + 0.001981 x weight)).*

The 50 field collected *D. reticulatum* were classified as female-phase mature or immature based on gland weights. The criteria upon which this decision was made are in accordance with the known protandric sequence of development of this species, namely that the ovotestis develops first and enlarges, followed by development and enlargement of the albumen gland and gradual reduction in size of the ovotestis as the slug enters the egg laying, female-phase (Runham & Laryea, 1968).

The weights of albumen glands were plotted against ovotestis weight to observe the spread in sizes. Data were divided into ‘large’ and ‘small’ groups for each gland; this was only an approximate division as there

were no very clear delineations between individuals and some judgement had to be exercised to decide whether a slug was mature. In general, all slugs with a small albumen gland were considered immature and those with a large albumen gland were assessed as mature. For individuals on the borderline between small and large, the weight of the ovotestis was taken into account; if this was small, the slug was classed as mature (*Fig. 4.5*).

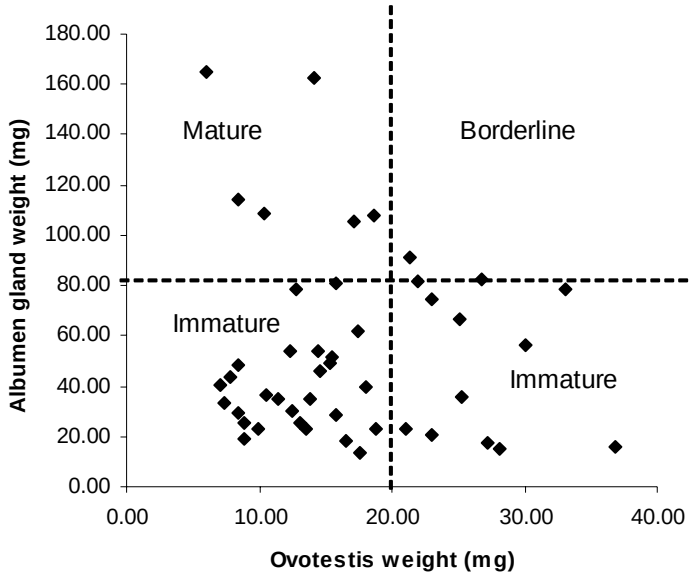


Figure 4.5: Approximate divisions of field collected Deroceras reticulatum *into mature and immature groups. A value judgement was exercised for borderline individuals (see text).*

The logistic regression equation based on the relationship derived in the laboratory (*Fig. 4.4*) was applied to this field collected sample to indicate the probability of a slug being mature. Since probability is a continuous variable, it needed to be dichotomised in order to compare agreement between the predicted and actual categorisation of field collected specimens which were assigned a binary classification (mature/immature). Various thresholds were applied to divide the laboratory reared slugs into mature and immature groups based on their probability of being mature, according to Fielding & Bell (1997). Calculations of the percentage of correct classifications, sensitivity (false negative errors) and specificity (false positive errors) were performed to select the optimum threshold that minimised false negative errors whilst giving the highest agreement between classifications and as low a false positive rate as possible (false negatives errors were regarded as more serious than false positive errors with respect to predicting the need for control in the field). Results are summarised in *Table 4.8* and indicate that a probability threshold of 0.25 optimised the performance of the model with 86% agreement and 100% sensitivity. The minimum weight corresponding to this threshold was approximately 700 mg, i.e. 700 mg was the minimum weight at which a slug is considered female-phase mature.

Table 4.8: Performance of binary logistic regression model.

Probability Threshold	*% Agreement with visual classification of field specimens*	*Sensitivity*	*Specificity*
0.15	40	1.00	0.81
0.20	74	1.00	0.35
0.25	86	1.00	0.19
0.30	86	0.77	0.11
0.35	82	0.31	0.00
0.40	82	0.31	0.00
0.45	76	0.08	0.00
0.50	74	0.00	0.00

Probability thresholds refer to the cut-off points tested to categorise slugs as mature and immature (binary classification) based on the predicted probability of maturity.

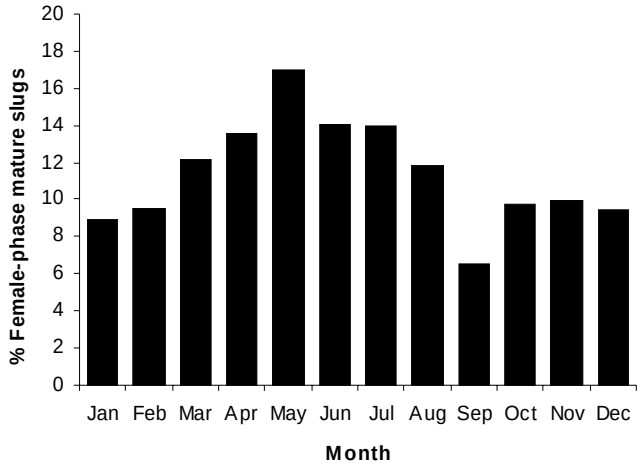
The binary logistic regression equation (*Fig. 4.4)* was applied to a database of weights from field populations of *D. reticulatum* collected from the same site between August 1997 & June 1999 (MAFF project CSA 3396). Data for each month were pooled for all years and the minimum slug weight for each 10% increase in the chances of being mature was calculated. The numbers of the population in each maturity bracket are shown in *Table 4.9*.

Table 4.9: Numbers of field collected Deroceras reticulatum *of the minimum weight required for sequential 10% increases in the chances of maturity as predicted by binary logistic regression equation derived from laboratory reared individuals (Fig. 4.4). (Based on data from Heddon Banks Farm, Northumberland 1997-1999,* MAFF project CSA 3396*).*

Month	*Total catch*	*Minimum weight (mg) for given percentage chance of maturity (in brackets)*									
		0- (<10%)	*230 (10%)*	*640 (20%)*	*920 (30%)*	*1140 (40%)*	*1340 (50%)*	*1550 (60%)*	*1770 (70%)*	*2040 (80%)*	*2450 (>90%)*
Jan	706	457	227	16	5	1					
Feb	566	383	158	2	5	12	3	2	1		
Mar	792	429	293	19	14	13	11	6	4	3	
Apr	200	89	87	8	8	3		3	1	1	
May	1011	375	452	61	23	20	28	22	22	5	3
Jun	3856	672	3002	119	36	14	4	3	2	1	3
Jul	2060	418	1447	180	13	2					
Aug	897	301	589	5			1				1
Sep	2487	2120	363		1	2					1
Oct	1253	666	582	2		3					
Nov	1501	871	553	52	19	5	1				
Dec	611	375	207	23	4	2					

By taking the mid-point of each maturity bracket the number of mature slugs of the required minimum weight expected to be found can be estimated. For example, in January 457 slugs in the 0-230 mg weight range have a 0-10% chance of being mature, hence approximately 5% of these are actually likely to be mature, i.e. 23 slugs. Summing the numbers of mature slugs estimated for each month suggests that less than 20% population are predicted to be female-phase mature at any time, i.e. the majority of the field population are immature, and the pattern of the percentage of mature slugs appears to be cyclical (*Fig. 4.6*).

Figure 4.6: Monthly percentages of female-phase mature Deroceras reticulatum *in the field (1997-1999 combined) as predicted by binary logistic regression equation derived from laboratory reared individuals (Fig. 4.3). (Based on data from Heddon Banks Farm, Northumberland 1997-1999,* MAFF project CSA 3396*).*



This trend was also consistent when years were looked at separately; data for part-years in 1997 and 1999 followed the corresponding pattern for the same period shown in *Fig. 4.6* and the complete data for 1998 mirrored the cyclical pattern for combined years extremely closely (*Fig. 4.7).*

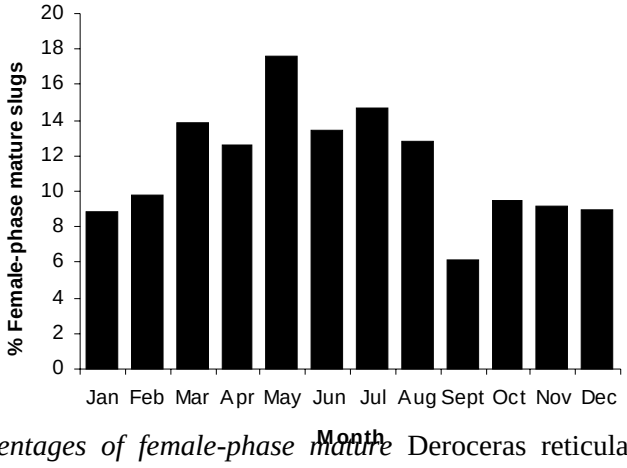


Figure 4.7: Monthly percentages of female-phase mature Deroceras reticulatum *in the field (1998) as predicted by binary logistic regression equation derived from laboratory reared individuals (Fig. 4.3). (Based on data from Heddon Banks Farm, Northumberland 1997-1999, MAFF project CSA 3396).*

Discussion

The few studies in the literature concerning the effect of temperature on the reproductive development of terrestrial slugs concern the species *Arion ater* (L.). They report that temperature can affect the speed of development of the reproductive tract, but not the sequence (Smith, 1966) and that constant very high (27°C) or very low (4°C) temperatures affect the development of gametes (Lusis, 1966), but temperatures between these extremes do not markedly disrupt the process. Although there is nothing published that has investigated this in *D. reticulatum*, it is known to be less sensitive to temperature effects than many other temperate slugs, including *Arion* species (Mellanby, 1961). In the current experiment there were, in addition, no differences in the mean weights between individuals sampled from each rearing temperature and it seems unlikely that pooling the slugs would have unduly confounded the results.

There was a very consistent relationship between the fresh and preserved weight of laboratory reared slugs across the whole range examined; the relationship was almost identical for those collected from the field. Slugs lost just under half their body weight due to preservation and this reflects the movement of body fluids from the animals to the more concentrated ethanol preserving medium. It was assumed that the relationship shown for body weights also applied to gland weights. The glands had different surface area/volume ratios and water content compared to the intact body and, therefore, this assumption may be criticised. However, these differences in surface area/volume ratio and water content also apply to different body sizes, and since the relationship held across the full range of intact weights, it is considered to be a reasonable approximation in the absence of any other data.

The field specimens were collected during routine trapping work and individual weights were, unfortunately, not recorded for all slugs. Of the sample whose weights were available, the relationship between fresh and preserved weight was remarkably similar to that for laboratory reared slugs implying that they lost body fluids at a similar rate. The equation for laboratory reared slugs was used to estimate the fresh gland weights of all slugs; since the equations for this group and the sample of field collected slugs were virtually identical it would make little difference which was used, but for consistency, the equation derived from the laboratory reared slugs was applied as it was based on a larger sample.

Many authors have highlighted the difficulty of dissecting glands from very small slugs (e.g. Lusis, 1961; Runham & Laryea, 1968; Duval & Banville, 1989). In the current experiments it was found that for slugs below a preserved weight of ~200 mg (fresh weight ~350 mg) the glands, particularly the albumen gland, could not be identified with certainty. It is reported that very early on in maturation, whilst body size increases, glands remain completely undifferentiated and the albumen gland and common duct are impossible to separate (Smith, 1966). Furthermore, once differentiation begins, early changes in glands occur at a cellular level without an accompanying increase in gland weight (Runham & Laryea, 1968). These factors, in addition to the ‘shrinking’ effect of ethanol on the weights of already small glands,

probably explain the problems encountered with small slugs in this study and it can be confidently inferred that such individuals were immature.

This study may be criticised on the grounds that some analyses have been based on the results of other analyses rather than empirical data and hence any uncertainties in the outcome of one are propagated in those that follow. This is a valid consideration, but is inevitable in exploratory work that generates hypotheses. The limitations of the study presented here are, therefore, acknowledged, but it is emphasised that it should be regarded as investigative. The possibilities it raises pose interesting questions, but these require further examination in order to take this work further.

The present study used principle components analysis to separate the effects of body, ovotestis and albumen gland weights arriving at a more flexible classification system than histological or gland indices approaches used previously with the particular advantage that overlap between male and female-phases could be interpreted in relation to the overall body size. A disadvantage is that the decisions regarding classification are subjective and may, therefore, be less reproducible than a strict numerical system, requiring knowledge of *D. reticulatum* biology. It does, however, result in a higher proportion of correct classifications as assessed by discriminant analysis than the gland indices approach where this has been explicitly reported (Duval & Banville, 1989; Haynes *et al.*, 1996).

The five group system used to discriminate between different maturity stages was found to be optimum, which agrees with Haynes *et al.* (1996). A classification system with fewer groups did not distinguish sufficiently between individuals to divide them into classes that were significantly different from one another. The basis of this five group system (*Table 4.4*) reflects broadly the development of the two glands as revealed by histological analysis (Runham & Laryea, 1968) and, therefore, has an empirical foundation. In this study the authors showed that there is an initial very slow and small increase in weight of both organs, the albumen gland lagging the ovotestis, followed by a rapid increase in the ovotestis size due to spermatogenesis and male-phase maturation. This is followed by an increase in the weight of the albumen gland due to accumulation of secretory products in the female-phase. The size of the albumen gland continues to increase to a maximum whilst that of the ovotestis decreases. This is also comparable with the stages described for *A. ater* (Smith, 1966).

In the current study, maturity was defined anatomically as having a large albumen gland following an increase in ovotestis size (maturity class 5), i.e. being physiologically female. There was a significant relationship between body weight and this measure of maturity for laboratory reared *D. reticulatum,* agreeing with the anatomical assessment of Haynes *et al.* (1996). The work of Lusis (1961) and Parivar (1978) on gland volumes in *A. ater* also supports this finding, showing a strong relationship between albumen gland volume and body weight in laboratory reared specimens. The study of Smith (1966) found no relation

between body weight and maturity, but assessed this at a cellular level, resolving maturity into a much larger number of stages some of which involved changes only discernable by histological techniques.

If egg laying had commenced in individuals when they were preserved it had done so only recently. In most individuals classified as mature, therefore, the albumen gland was reaching its peak size. It has been shown that the processes of growth and reproduction are antagonistic; adult *D. reticulatum* generally lose weight during reproduction even when food is plentiful and death ensues very rapidly after egg laying is completed (Rollo, 1988). It may be speculated that the relationship between body weight and maturation would have been modified had egg laying been a little more advanced in the mature slugs.

Sokolove and McCrone (1978), working with *Limax maximus* (L.), showed that male-phase maturation in this species was initiated by a short to long day-length transition in photoperiod whilst female-phase maturation seemed to be independent of the light regime. Although this has not been specifically tested in *D. reticulatum* it is suggested that it is less important in this species which is capable of breeding at any time of the year if temperature and humidity are favourable (South, 1989a). Other environmental factors such as quality of diet (Rollo & Shibata, 1991) and humidity (Lusis, 1966) have been shown to influence reproductive development, but it seems probable that these are mediated not directly, but rather through effects on feeding and growth and should not, therefore, alter the applicability of the relationship derived in the current study to different populations; for the derivation of the relationship the diet of the laboratory reared slugs was uniform and relative humidity remained high due to the culture conditions so there should be no confounding effect between samples.

There was 86% agreement between those field collected slugs judged to be mature through visual inspection of gland weights and those predicted to be mature by the binary logistic regression equation based on body weight alone. This ostensibly suggests that the binary logistic regression equation is a very reliable means of estimating female-phase maturity. The probability threshold at which its performance was optimised was, however, low at just 25%, i.e. the minimum weight at which *D. reticulatum* were considered to be female-phase mature by visual inspection (~700 mg) corresponded to just 25% chance as predicted by the binary logistic regression equation and, therefore, only the largest slugs were considered female-phase mature. It may be that there are differences in the weight at maturity between field and laboratory reared slugs. This could be tested by performing binary logistic regression on field collected slug data. Conventional wisdom would suggest that *D. reticulatum* of approximately 700 mg would be highly capable of laying eggs. Perhaps, therefore, field slugs mature at lower weights and the chances of them laying eggs at 700 mg are, in reality, much greater than 25%. On the other hand, independent studies that have explicitly investigated female-phase slugs in the population support the suggestion that numbers in this group may be relatively low. Lusis (1961) reports a consistent rarity of female-phase individuals in random samples of a wild population of *A. ater* and suggests that this is due to the female-phase being much shorter than the male-phase. Furthermore it has been shown in *D. reticulatum* that once egg laying commences the parent is

'sacrificed to augment reproduction' (Rollo, 1988) and hence the mature female-phase is short-lived and at any single point in time the chances of a slugs being female-phase mature is probably fairly low.

Application of the binary logistic regression equation to routinely collected weight data from field-caught specimens (MAFF project CSA 3396) indicated that at any time of the year less than 20% of the field population were mature by the definition used in the current study, i.e. in the female-phase. This requires further testing, but is supported by Haynes *et al.* (1996) who found that at any month of the year wild populations of *D. reticulatum* from a nearby site were rarely comprised of more than about 20-30% in maturity class 5 (large albumen glands and therefore in the female-phase). Application of the equation to routine data also suggested that changes in the percentage of mature slugs were cyclical, with approximately equal proportions of mature individuals in December as in the previous January. Moreover, the population of mature slugs is highest around summer and into autumn and then falls rapidly, coinciding with the known decline in adult numbers following egg laying (South, 1992). The pattern was consistent between years, indicating that there may be some underlying natural pattern increasing confidence in the result. Further study is required to explore this finding further, for example, at different sites. It seems, however, that such a scenario would be feasible on biological grounds. For example, the cumulative numbers of eggs laid by individuals are high (Carrick, 1938) suggesting large numbers of mature females are not necessarily required to maintain the egg bank of a population. Furthermore, there may be selective disadvantages to being large such as the need for more food to maintain metabolic processes and greater vulnerability to environmental stress if adequate refuge sites cannot be found. This result, therefore, raises the possibility that populations of *D. reticulatum* may naturally be male biased, but since there is always a small chance that low weight slugs may be physiologically mature 'females', a small pool of egg-producing individuals would be maintained in the population at all times.

Directly estimating the 'egg bank' in slug populations is notoriously problematic due to the small size of eggs and the difficulties in extracting them from soil samples without damage. The relationship between body size and maturity demonstrated in this study may help to overcome this problem indirectly; the size structure of a population can be ascertained through sampling and the probability that slugs in different size classes will be mature may then be estimated from the binary logistic regression equation. This would indicate the proportion of slugs likely to be female-phase mature and hence capable of contributing to the egg bank of that population. Relative comparisons between years could help to predict an increased risk of population expansion which would help to refine our understanding of population dynamics in this species, aiding decisions regarding the timing of control measures.

If the growth rate of slugs in the field could be predicted and the minimum weight at which slugs become female-phase mature were to be confirmed by further investigations, it may be possible to 'back calculate' in order to estimate the size of slugs in spring that consequently reach egg laying condition by autumn. If there

are large numbers of such slugs then control measures could be applied in a more targeted way before they lay eggs, drastically reducing the potential population in the following year.

In conclusion, it was found that:

1. A five-category system of maturity classification for *D. reticulatum* based on body, ovotestis and albumen gland weight was found to be very efficient in distinguishing between individuals.
2. There was a significant relationship between body weight and female-phase maturity for laboratory reared *D. reticulatum* and this could be described by a probability equation (binary logistic regression model).
3. The relationship between weight and maturity was validated against field data and was shown to predict female-phase maturity with 86% accuracy. The minimum weight at which *D. reticulatum* is considered to be female-phase mature by visual inspection of gland weights, however, corresponds to a probability of just 25% as predicted by the binary logistic regression equation.
4. There may be a male-phase bias in natural populations of *D. reticulatum* in Northumberland with fewer than 20% of physiologically female individuals throughout the year. This requires further testing in other locations.

As discussed above the exploratory work in this paper needs testing further. In particular, in order to evaluate concerns regarding whether laboratory reared and field collected *D. reticulatum* differ in their weight at maturity the equation could be validated with data from more laboratory reared specimens or, conversely, the binary logistic regression equation could be derived from field collected specimens. The results could then be compared with the current experiment to gauge its general applicability. An interesting extension to this work would be to divide up the population of slugs studied into male-phase mature and female-phase mature to see whether it is possible to derive a relationship between body weight and maturity, specific to each stage in reproductive development.

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PAPER 5 – *Objective 1.1*

The Abundance and Population Size Structure of *Deroceras reticulatum* and other Pest Slug Species in Arable Fields

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Abstract

Slug populations were assessed by soil sampling and refuge trapping in arable fields from spring until autumn each year from 2002 to 2004. Defined area trapping was also done in 2002. Soil sampling provided an estimate of population density in the upper 10 cm of soil, whilst the other methods provided estimates based on slug activity. Our findings highlight the potential for rapid increase in numbers and biomass of slugs, especially *D. reticulatum*, as a result of its ability to breed whenever conditions are suitable, high fecundity and the rapid growth rate of juveniles. They also highlight differences in breeding success between species. Refuge traps baited with chicken layers' mash were put out in the same fields on the same occasions when soil samples were taken provided that conditions were suitable (moist soil and minimum and maximum temperature in the range 5-25^{o}C). The great majority of slugs recorded in refuge traps were large (>100mg) even at times when smaller slugs were common in the soil. Thus, refuge traps did not provide proportionate and useful information on the densities of neonates and juveniles. The total numbers of *D. reticulatum* in traps were related to the density of larger individuals (>100mg) in soil when conditions were suitable for trapping. The relationship differed for traps in standing cereal crops compared to traps in stubble and allowance needed to be made for sunny conditions on the morning of examination. Defined area traps (DATs) recorded considerably more neonatal and juvenile *D. reticulatum* than refuge traps, but numbers of these in DATs were still significantly lower than in soil samples. Numbers of slugs >100mg did not differ significantly between DATs and soil samples.

Introduction

Slug numbers in arable fields and the risk of crop damage are normally estimated by refuge trapping (Paper 14, this report). The numbers of slugs recorded in refuge traps are dependent on suitable weather conditions for slug surface activity (principally a moist surface soil and mild temperatures, Young *et al.* (1991, a,b). Glen & Wiltshire (1986) reported that trap catches of two slug species (*Deroceras reticulatum, Arion silvaticus*) were correlated with the density of slugs weighing > 100mg in soil samples, but bore no

relationship to numbers of smaller slugs of these species. The reasons why smaller slugs are under-recorded in traps are unknown (Paper 15, this report). However, this does represent a potential problem for damage risk assessment, since we have shown (Paper 6, this report) that small slugs are capable of killing wheat seeds and moreover, smaller individuals of *D. reticulatum* and *Milax gagates* kill more seeds in proportion to their weight than larger slugs. Defined area trapping (DAT) is reported to provide a good estimate of the numbers of smaller slugs (Ferguson *et al.*, 1989; Ferguson & Hanks, 1990, Clements & Murray, 1991) and because trapping is done in a defined area, the numbers in these traps can be related to slug density in soil.

The aim of this study was to investigate the dynamics of slug populations in arable fields over a three year period and to compare soil sampling with refuge trapping and DAT to evaluate these different methods for assessing slug activity and density for the purpose of assessing the risk of potential damage to winter wheat and oilseed rape.

Materials and methods

Each year from 2002 to 2004, we assessed slug populations in arable fields by soil sampling and refuge trapping. Defined area trapping was also used in 2002.

Soil sampling

The densities of slug populations in the upper 10 cm of soil were estimated by soil sampling and flooding, using the method described by Glen *et al.* (1989). Each soil sample covered an area of 0.0625m^2 of soil (0.25m x 0.25 m), to a depth of 0.10 m. On each date, one soil sample was dug from each plot in an arable field. In some fields molluscicide treatments were applied to some plots as part of the experiments described in Paper 11, this report. In these cases, we used only data for slug populations and trap catches from plots that were untreated at the time of sampling. The numbers of plots and numbers of samples from each site used for analysis on each date are shown in Table 5.1. The samples were placed in opaque plastic containers with opaque lids, sealed with draft-excluder strip to prevent slugs escaping, and the samples were gradually flooded. The samples were examined daily and all slugs were collected, identified and weighed fully hydrated.

Small samples of soil were taken for estimation of soil moisture, at the same time as the soil samples were dug for slug population estimations. Soil moisture samples were taken using a trowel, from two depths (0-2 cm and 2-8 cm) from 5-10 plots on each date (Table 5.1).

In each of the three years, 2002-04, we sampled slug populations from spring/ summer to early autumn in one field going from winter wheat into oilseed rape. Because of crop rotation, this sampling was done in a different field each year. We also sampled in two fields going from oilseed rape into winter wheat in 2004.

Refuge trapping

Whenever the weather was suitable for trapping (the soil surface was visibly moist and temperature was in the range 5 – 25°C (Young *et al.* (1991)), traps were put out in the field, either on the day before soil sampling or on the day of sampling. The traps, bait and procedures were as described in Paper 14, this report: upturned flowerpot saucers, terracotta coloured, 25 cm diameter, with 20 ml of chicken layers' mash placed in a small heap on the soil in the middle of the area to be covered by the trap. One trap was placed in each plot, 1-2 m from the soil sampling point (no closer to avoid soil disturbed by the process of taking the samples), in the afternoon or early evening. Traps were examined the following morning, and soil surface moisture and weather conditions were recorded at the time of trap examination. A sample of up to 20 slugs of each species was collected from the traps for weighing in the same way as slugs from the soil samples.

Defined area trapping

The defined area traps (DATs) were those used by Clements and Murray (1991) – collars of galvanised sheet steel 30 cm high, each enclosing an area of 0.1m^2. The lower edge of each collar was driven about 9 cm into the soil and a large Chinese cabbage leaf was placed on top of the enclosed soil surface, which was then covered with wet paper towel roll. A lid of weatherproof plywood was placed on top (held in place by a stone) to keep out predators and maintain a dark moist environment within. Slugs that emerged from the enclosed soil and rested on the soil surface, leaf or moist paper were collected, but slugs were unable to enter the trap from outside. One three occasions (July, August and September) in 2002, one DAT was put in place in each of ten plots on Field 75, Long Ashton Research Station, North Somerset on the same date as one soil samples was taken and one refuge trap was placed in each plot.

Results

Soil sampling

Slugs extracted from soil were classified into three weight classes: 1-10mg (neonates), 11-100mg (juveniles) and >100mg (large slugs). The reasons for using these weight categories are outlined in the Discussion.

Deroceras reticulatum was present in each field over the three years but in different numbers each year (Fig. 5.1). In 2002 (Field 75, Long Ashton Research Station, North Somerset), there were 30 – 60/m^2 of neonatal *D. reticulatum* (1-10mg) in soil samples taken at monthly intervals during the period from 20 May to 12 August, with peak numbers recorded in June. Juveniles (10-100mg) were also present in good numbers during this period, with a peak of about 90/m^2 in July 2002. The large numbers of slugs in both size classes covering the range 1-100mg corresponded with a period of generally wet weather from May to early August 2002 (Fig. 5.2), which was presumably suitable for egg-laying as well as egg, neonatal and juvenile survival. As expected, there were fewer *D. reticulatum* (10-21/m^2) in the largest size category (>100mg), during this period. Numbers in all size classes declined to low levels in September and October, following non-inversion tillage and drilling of oilseed rape in late August 2002. This cultivation and drilling was done during a generally dry period of weather from mid August to late October (Fig. 5.2). In 2003 (Glebe Field, Higher

Clapton Farm, Somerset), densities of *D. reticulatum* >100mg were similar to those recorded in Field 75 in 2002, but recently hatched and juvenile *D. reticulatum* were virtually absent. Thus, breeding success and survival of juveniles were poor in the dry conditions of 2003 (Fig. 5.1). In 2004 (Eight Acres, Higher Clapton Farm), all size categories of *D. reticulatum* were present in small numbers up to early August, but a large peak of recently hatched and juvenile slugs was recorded on 21 August, presumably as a result of successful breeding in the wet weather from early August. Numbers then declined following cultivation and drilling of oilseed rape.

Two other species were recorded frequently in Glebe Field in 2003, as shown in Fig. 5.3: *Arion distinctus* Mabille and *Milax gagates* (Draparnaud). In contrast to *D. reticulatum* in that year, there were good numbers of the two smallest weight classes of *A. distinctus* (1-10mg up to 65/m^2; 1-100mg up to 80/m^2) in samples taken from June to the end of July. This species breeds in spring/early summer and it is likely that its breeding was successful because of a period of wet weather in late April and May (Fig. 5.3). However, numbers of *A. distinctus* collapsed in the dry weather that started at the beginning of August and continued to late October (Fig. 5.2). The effects of non-inversion tillage and drilling of oilseed rape in dry conditions in late August would also have contributed to this decline. *Milax gagates* was present in smaller numbers (Fig. 5.3), with the most abundant size class being 11-100mg, which peaked at almost 20/m^2 at the end of July.

Slug populations in the soil were sampled in two fields going from oilseed rape to winter wheat in 2004. In Glebe Field, Higher Clapton Farm, Somerset (Fig. 5.4), which had been sampled in 2003 (Figs. 5.1, 5.3), the densities of *D. reticulatum*, the commonest species, were initially low (7/m^2) in soil samples taken in oilseed rape in April 2004, showing no increase from September 2003 (Fig. 5.1), presumably as a consequence of poor survival of this species in the dry autumn of 2003. When sampling was next done, on 2 August, just after harvest of the oilseed rape crop, the population of *D. reticulatum* had grown to 62/m^2, with a population size structure indicating recent successful breeding and good survival of juveniles. The next soil samples were taken on 20 August, after the oilseed rape stubble had been cultivated by discing. Despite this disturbance, the population of *D. reticulatum* had grown to 108/m^2, with evidence of particularly good survival to the second weight category (11-100mg), but, as yet, no increase in the heaviest class (>100mg). By mid September 2004, the population of *D. reticulatum* had shown further growth to 252/m^2, with the increase being particularly great in the 11-100mg class, which had reached more than 120/m^2. Moreover, the heaviest weight class (>100mg) had reached a density of more than 60/m^2 – the highest density of this largest size class of *D. reticulatum* recorded in the three years of study. This increase in density of *D. reticulatum* in August and September appeared to be the result of a period of wet weather in August 2004 (Fig. 5.2). Following ploughing and drilling of winter wheat, numbers of *D. reticulatum* were considerably lower by mid October. *Arion distinctus* was present in only small numbers in Glebe Field in 2004, in contrast to its high densities in June to early August 2003. *Milax gagates* was also present in small numbers in 2004 (Fig. 5.4), as in 2003.

We also studied slug populations in Green Triangle, Lawn Farm, Wiltshire, going from oilseed rape to winter wheat in 2004. This field was direct-drilled with winter wheat following a very light scratch cultivation, which left the soil surface mostly undisturbed. In contrast to Glebe Field, *D. reticulatum* was not the commonest slug species present (Fig. 5.5). Numbers of *D. reticulatum* in Green Triangle did not increase from August to September, as they did in Glebe Field, even though Green Triangle was undisturbed whereas Glebe Field was cultivated. Only a few *D. reticulatum* were recorded on 1 November, showing poor reproduction and survival of this species, despite the favourable weather and lack of cultivation. Numbers of *A. distinctus* increased slightly from August to September (with the increase being accounted for by the middle weight class), but numbers of this species were remarkably constant throughout the period of study, indicating good survival of this species which had bred earlier in the year. The commonest species in Green Triangle on all three sampling dates in 2004 was *Milax gagates*. Overall numbers of this species increased from August to September then remained constant. The smallest size class was absent, except for a few in mid August. The population showed a gradual progression from the middle to the largest size class from September to 1 November, when the density of the largest size class peaked at a high level (120/ m^2).

Soil moisture contents (Table 5.1) were measured at two depths (0-2 cm and 2-10 cm) in the 0-10 cm layer sampled for slugs. Soil moisture content was normally in the 20-30% range in both layers, with some notable exceptions. The surface 0-2 cm layer was considerably drier than this on three occasions (3 September 2002, 30 August 2003 and 2 August 2004, but on all these occasions the moisture content of the lower layer was greater than 20% and this probably provided sufficient moisture for slugs to live in that layer.

A comparison of slug densities in soil just before cultivation with the densities just after cultivation and drilling of winter cereals or oilseed rape (Table 5.2) provides some tentative information on the effects of cultivations. Densities after cultivation by non-inversion tillage before drilling oilseed rape (four sites) or ploughing before drilling winter wheat (one site in 2004) were reduced by 85-90%. In contrast, slug populations increased by 21% after non-inversion tillage (discing) of rape stubble in 2004. Following subsequent ploughing, this population was reduced by 85%. Slug densities after scratch cultivation and direct-drilling winter wheat at Green Triangle in 2004 were lowered by only 16%.

Defined area trapping

Sufficient *D. reticulatum* were extracted from defined area traps put in place on 10 July and 12 August 2002 and from soil samples taken on those dates for a comparison of the numbers recorded by these two methods. Only a few slugs were recorded by both methods in September, so results are not presented for this date. In order to allow for the difference in area sampled by each method (0.1 m^2 for DATs, 0.0625 m^2 for soil samples), numbers per soil sample were multiplied by 1.6 to make the samples comparable. The comparative results for the different methods of sampling did not differ between July and August (Fig. 5.6), with no significant interactions between sampling method and date of sampling. Significantly fewer *D .reticulatum* in the two smallest weight classes were collected from defined area traps compared to soil samples (mean

values for 1-10mg slugs were 1.85 and 3.60/0.1m^2, respectively (LSD 1.22, $P < 0.01$); for 11-100mg slugs 4.75 and 8.64/0.1m^2, respectively (LSD 3.08, $P < 0.05$)). The numbers in the largest size class were not significantly different between defined area traps and soil samples (2.45 and 1.36/0.1m^2, respectively (LSD 1.42, $P > 0.05$). On both dates (July and August), there had been substantial rain in the days just before sampling. However, dry weather followed both sampling dates (Fig. 5.7) and this may have deterred smaller slugs (up to 100 mg) from coming to the soil surface inside the defined area traps.

Refuge trapping

The vast majority of *Deroceras reticulatum* recorded in refuge traps were >100 mg (Table 5.3), even on several dates when the densities of smaller slugs (1-100mg) in the upper 10 cm of soil were considerably greater than the densities of slug >100mg (Table 5.3). Given this characteristic of trap catches, it was decided to compare the numbers of *D. reticulatum* in traps with the numbers of this species >100 mg in soil. This relationship showed considerable variability (Table 5.3). However, knowing that slug surface activity is affected by soil surface moisture and that the numbers remaining in traps are likely to be related to weather and crop conditions, it was decided to separate the data based on soil moisture, cropping and weather (Fig. 5.8). As expected, when the soil was recorded as drying at the time of examination, low numbers were recorded in traps in relation to the numbers >100mg in soil. When the soil surface was moist, there appeared to be a clear distinction in the relationship for standing cereal crops compared to stubble (Fig. 5.8).

In the fields where substantial numbers of *A. distinctus* were recorded in soil (Table 5.4), rather few were recorded in traps. As for *D. reticulatum*, the majority of slugs in traps were >100mg. Given the annual life cycle of this species, which lays eggs in late spring, there were large numbers of smaller slugs (1-100mg) throughout the spring and summer, but few were recorded in traps. However, even in autumn 2004 when there were substantial numbers >100mg in Glebe Field, relatively few *A. distinctus* were recorded in the traps. In spring 2003, no *A. distinctus* were recorded in traps in Smithy's Field, even although large numbers >100mg were recorded in soil. However, on this occasion there were low catches of all slug species in traps, probably because the trap bait had been eaten overnight by mice. The results for trapping *M. gagates* were similar to those for *A. distinctus* (Table 5.5).

Discussion

The three weight classes used in this paper for analysis of slug populations (1-10mg, 11-100mg and >100mg were the same as those used by Choi *et al.* (2004) and Choi *et al.* (2006). These weight categories were based on (1) the weight range of *D. reticulatum* (from about 1 mg as neonatal individuals to large adults of about 1g) and (2) the logarithmic growth rate of *D. reticulatum* (South (1982)). Thus, each of the three weight categories covers an equal logarithmic range and together they cover the approximate normal weight range of this species. For the purposes of this paper, slugs in the range 1-10mg are considered to be recently hatched and their presence in the field therefore provides evidence of recent successful breeding; the numbers of 10-100mg individuals provide evidence of juvenile survival, whilst the largest size category,

>100mg, which includes adults, gives an approximate index of the reproductive potential of the population. For comparison, the same weight ranges were used for other slug species.

The differences in numbers of neonatal and juvenile *D. reticulatum* within and between the three years of study (Fig. 5.1) can be clearly related to weather conditions, with good numbers of neonates appearing after periods of wet weather and good survival to juveniles and to large slugs >100mg under these conditions. In contrast, the predominantly dry weather from March onwards in 2003 (Fig. 5.2) resulted in low numbers of neonates and juveniles and, although adults survived in reasonable numbers until late July, they were also reduced to low numbers in the dry conditions that lasted from August to late October 2003. Fortunately, it was possible to monitor populations of slugs in the same field (Glebe Field) in 2004 (Fig. 5.4) as in 2003. Numbers of *D. reticulatum* remained low in early April 2004 and showed a strong increase to moderate densities during the four-month period to August. Densities then increased further by late August and continued to increase so that by mid September there were large numbers of all size classes, including record densities (more than $60/m^2$) of large individuals, despite cultivation of the stubble by discing. This increase from low to high numbers over a period of six months clearly demonstrates the considerable potential for population increase in *D. reticulatum*. In particular, the population increase in August and September 2004 shows the importance of wet weather in late summer and early autumn in contrast to the dry weather of 2003. The mean air temperature in August 2004 was 17.5^{o}C, which is about the optimum for *D. reticulatum*. Mean air temperature in September 2004 (15.5 ^{o}C) was also close to the optimum. Given that August is also, on average, one of the wettest months of the year in the UK, this shows clearly why this species is such a troublesome pest of autumn-drilled crops. It also clearly illustrates that we are not able to make accurate forecasts of slug infestation levels in autumn because of our inability to forecast the weather more than a few days ahead.@@

Although a rapid method of estimating slug populations in soil has been developed and tested for oilseed rape crops in Germany and England (Glen *et al.*, in press), most risk assessments for winter wheat and oilseed rape rely on refuge trapping because of the convenience of this method. However, it has the disadvantage that it provides little direct information on the population densities of smaller slugs (1-100mg), which can be present in large numbers. In addition to the fact that slugs of 1-100mg weight are under-recorded in refuge traps, species other than *D. reticulatum* (*A. distinctus* and *M. gagates*) were recorded in low numbers in relation to the densities of large (>100mg) individuals of both species in soil. Hunter (1968) also noted that two species (*Arion hortensis* (agg.) and *Tandonia budapestensis* (Hazey)) were under-recorded in traps in comparison to *D. reticulatum*. Fortunately, the species that are under-recorded tend to occur in lower numbers than *D. reticulatum* in arable fields. There were, however, notable exceptions in our study (*A. distinctus* in Glebe field in summer 2003 and *A. distinctus* and *M. gagates* in Green Triangle in autumn 2004). In both cases this did not lead to unexpected damage, because of the collapse of the slug population in 2003 and because slug trap catches were above the threshold of four/trap in Green Triangle in 2004. Mice eating the trap bait in a crop of oilseed rape in April 2004 were probably responsible for lower

than expected catches of slugs at that time. This problem was not encountered later in the season when trapping is normally done to assess the risk of slug damage

Defined area trapping is sometimes recommended as a possible alternative because it is less labour-intensive than the standard method of soil sampling and flooding as used in this study. Also, because slugs are trapped from a defined area, it is possible to relate slug numbers trapped to the density of slugs in soil. Previous studies have shown that defined area trapping reveals similar densities of *D. reticulatum* compared to soil sampling, including smaller slugs ((Ferguson *et al.*, 1989; Ferguson & Hanks, 1990, Clements & Murray, 1991). However, in our investigations in July and August 2002, defined area trapping was less successful in recording smaller slugs (1-100mg) compared to soil sampling, although numbers were similar for larger slugs. We attribute the lower numbers of smaller slugs to the period of hot dry weather that followed the insertion of DATs into the soil on both dates and we believe that this would have deterred smaller slugs from emerging on the soil surface. This is clearly a disadvantage of the use of defined area traps for assessing he risk of slug damage to autumn sown crops, particularly oilseed rape, which is drilled in August or early September and, thus, slug population assessment needs to be made in late July or August.

We can only draw tentative conclusions on the effects of cultivations from the results of our studies because our fields did not include replicated uncultivated plots for comparison. However, the changes in slug population density after cultivations are interesting. Non-inversion tillage and drilling of oilseed rape resulted in substantial decreases in slug populations each year, whereas non-inversion tillage of oilseed rape stubble in August 2004 was followed by an increase in slug density. Ploughing subsequently caused a substantial reduction in slug densities at this site, in contrast to the other site which was direct-drilled. These findings are broadly in line with the findings reported in the literature, as reviewed by Glen & Symondson (2004).

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Table 5.1: Soil moisture content, as a percentage of wet weight, in soil samples taken from 0-2 cm and 2-10 cm at the same time as soil samples for estimating slug populations density.

Field	Date	No. samples for slug popn.	No. samples for soil moisture	% moisture	
				0-2 cm	*2-10 cm*
Field 75	20 May 02	25	10	26.1	25.8
	19 June 02	25	10	25.2	24.8
	10 July 02	25	10	27.1	26.5
	12 Aug 02	25	10	24.8	25.6
	3 Sept 02	10	10	11.8	21.8
Cowleys	9 Aug 02	10	5	33.2	30.8
Glebe	24 Jun 03	25	5	22.5	22.5
	18 Jul 03	25	5	23.6	22.3
	31 July 03	25	5	24.5	24.8
	1 Aug 03	25	5	17.2	22.2
	30 Aug 03	25	5	10.0	20.1
Eight Acres	15 July 04	10	9	23.7	23.6
	3 Aug 04	10	9	23.8	21.2
	21 Aug 04	10	9	28.4	27.6
	20 Sept 04	10	9	22.5	26.2
	7 Oct 04	10	9	24.3	27.1
Glebe	5 Apr 04	25	5	28.2	27.9
	2 Aug 04	25	5	11.8	21.7
	20 Aug 04	15	5	30.6	27.7
	16 Sep 04	15	5	23.5	20.5
	19 Oct 04	5	5	24.9	27.7
Green Triangle	16 Aug 04	25	5	22.1	19.5
	17 Sep 04	15	5	24.2	19.7
	2 Nov 04	5	5	29.6	22.1

Table 5.2: Reduction in slug population density in soil samples taken after cultivation compared to slug population density in samples just before cultivation.

Year	*Field*	*Method of cultivation*	*% Decrease in slug population density after cultivation*
2002	Field 75	Non-inversion	89.8
2002	Cowleys	Non-inversion	87.7
2003	Glebe	Non-inversion	87.0
2004	Eight Acres	Non-inversion	86.9
2004	Glebe (August)	Non-inversion	(20.8 increase)
2004	Glebe (September)	Ploughing	84.9
2004	Green Triangle	Scratch & direct drill	15.5

Table 5.3: Population densities of Deroceras reticulatum *in arable soil in relation to numbers per trap baited with layers' mash. At sites where slug pellets were applied, data are shown for untreated plots only.*

Field	Date	Slugs/m^2 in soil			Slugs in traps		Conditions at time of examination		
		No. Ò100mg	*No. >100mg*	*Total no.*	*No. per trap*	*% Ó100mg*	*Crop*	*Soil surface moisture*	*Weather*
Field 75	20 May 02	48.6	9.6	58.2	14.3	-	C	Moist	Cl & S
	19 June 02	85.1	20.5	105.6	7.6	100	C	Drying	B
	10 July 02	134.4	9.6	144.0	12.4	100	C	Moist	S & Sh
	12 Aug 02	96.0	13.4	109.4	0.5	100	CS	Drying+	Cl W
	3 Sept 02	11.0	3.0	14.0	0	-	OSR	Dry	B cool
Cowleys	9 Aug 02	44.0	18.0	62.0	7.5	100	C-S	Moist	Cl cool
Glebe	24 Jun 03	0	20.7	20.7	5.1	100	C	Moist	B W
	18 Jul 03	0	13.8	13.8	12.6	100	C	Moist	Cl
	31 July 03	6.9	23.0	29.9	30.8	100	C	Wet	Cl
	1 Aug 03	6.9	23.0	29.9	17.6	-	C	Moist	B
	30 Aug 03	9.2	0	9.2	0	-	OSR	Moist	B
Eight Acres	15 July 04	18.4	11.5	29.9	3.7	100	C	Moist	Cl Hum
	3 Aug 04	4.6	4.6	9.2	33.6	100	C	Moist	Mi
	21 Aug 04	80.5	6.9	87.4	4.9	100	C	Wet	B
	20 Sept 04	4.6	0	4.6	2.1	89	OSR	Moist	Cl
	7 Oct 04	4.6	4.6	9.2	0.8	75	OSR	Moist	B
Glebe	5 Apr 04	7.0	0	7.0	0.1*	-	OSR	Moist	Co B
	2 Aug 04	51.9	9.6	61.5	4.8	100	O-S	Moist	Misty
	20 Aug 04	96.1	11.7	107.8	5.2	45	O-SD	Wet	C B
	16 Sep 04	186.7	65.1	251.8	15.1	90	O-SD	Moist	Cl D
	19 Oct 04	32.0	6.4	38.4	13.4	90	WW	Moist	Cl D
Green Triangle	16 Aug 04	33.1	8.5	41.6	2.1	100	O-S	Moist	Cl D
	17 Sep 04	26.7	19.2	45.9	4.7	95	O-S	Moist	Cl D
	2 Nov 04	12.8	0	12.8	3.6	-	WW	Wet	Cl D

Crops: C = maturing cereal crop; C-S = cereal stubble; OSR = oilseed rape; O-S = oilseed rape stubble; O-SD = oilseed rape stubble, disced; WW = winter wheat

Weather: B = bright, Cl = cloud, Co = cold, D = drizzle, Hum = humid, Mi = Misty, S = sunshine, Sh = showers, W = warm

*Trap bait eaten by mice

Table 5.4: Population densities of Arion distinctus *in arable soil in relation to numbers per trap baited with layers' mash. At sites where slug pellets were applied, data are shown for untreated plots only.*

Field	Date	Slugs/m^2 in soil			Slugs in traps		Conditions at time of examination		
		No. Ò100mg	*No. >100mg*	*Total no.*	*No. per trap*	*% Ó100mg*	*Crop*	*Soil surface moisture*	*Weather*
Glebe	24 Jun 03	43.7	0	43.7	0.5	100	C	Moist	B W
	18 Jul 03	32.2	0	32.2	0.2	67	C	Moist	Cl
	31 Jul 03	147.2	0	147.2	1.6	27	C	Wet	Cl
	1 Aug 03	147.2	0	147.2	0.3	-	C	Moist	B
	30 Aug 03	9.2	0	9.2	0	-	OSR	Moist	B
Glebe	5 Apr 04	3.2	14.1	17.3	0*	-	OSR	Moist	Co B
	2 Aug 04	17.9	1.3	19.2	0.2	100	O-S	Moist	Misty
	20 Aug 04	3.2	0	3.2	0	-	O-SD	Wet	C B
	16 Sep 04	9.6	4.3	13.9	0.5	100	O-SD	Moist	Cl D
	19 Oct 04	0	0	0	0.8	100	WW	Moist	Cl D
Green Triangle	16 Aug 04	34.2	6.4	40.5	0.2	50	O-S	Moist	Cl D
	17 Sep 04	23.5	48.0	71.5	1.0	90	O-S	Moist	Cl D
	2 Nov 04	19.2	38.4	57.6	2.4	-	WW	Wet	Cl D

Crops: C = maturing cereal crop; C-S = cereal stubble; OSR = oilseed rape; O-S = oilseed rape stubble; O-SD = oilseed rape stubble, disced; WW = winter wheat

Weather: B = bright, Cl = cloud, Co = cold, D = drizzle, Hum = humid, Mi = Misty, S = sunshine, Sh = showers, W = warm

*Trap bait eaten by mice

Table 5.5: Population densities of Milax gagates *in arable soil in relation to numbers per trap baited with layers' mash. At sites where slug pellets were applied, data are shown for untreated plots only.*

Field	Date	Slugs/m^2 in soil			Slugs in traps		Conditions at time of examination		
		No. Ò100mg	*No. >100mg*	*Total no.*	*No. per trap*	*% Ó100mg*	*Crop*	*Soil surface moisture*	*Weather*
Glebe	24 Jun 03	9.2	9.2	18.4	1.5	80	C	Moist	B W
	18 Jul 03	0	4.6	4.6	0.1	100	C	Moist	Cl
	31 Jul 03	18.4	0	18.4	1.9	94	C	Wet	Cl
	1 Aug 03	18.4	0	18.4	0.4	-	C	Moist	B
	30 Aug 03	4.6	0	4.6	0	-	OSR	Moist	B
Glebe	5 Apr 04	3.2	3.8	7.0	0*	-	OSR	Moist	Co B
	2 Aug 04	11.5	3.2	14.7	0.4	67	O-S	Moist	Misty
	20 Aug 04	2.1	2.1	4.2	0.1	100	O-SD	Wet	C B
	16 Sep 04	1.1	9.6	10.7	5.3	100	O-SD	Moist	Cl D
	19 Oct 04	0	3.2	3.2	0.4	100	WW	Moist	Cl D
Green Triangle	16 Aug 04	56.6	51.2	107.8	0.6	50	O-S	Moist	Cl D
	17 Sep 04	45.9	98.1	144.0	4.7	90	O-S	Moist	Cl D
	2 Nov 04	22.4	128.0	150.4	1.4	-	WW	Wet	Cl D

Crops: C = maturing cereal crop; C-S = cereal stubble; OSR = oilseed rape; O-S = oilseed rape stubble; O-SD = oilseed rape stubble, disced; WW = winter wheat

Weather: B = bright, Cl = cloud, Co = cold, D = drizzle, Hum = humid, Mi = Misty, S = sunshine, Sh = showers, W = warm

*Trap bait eaten by mice

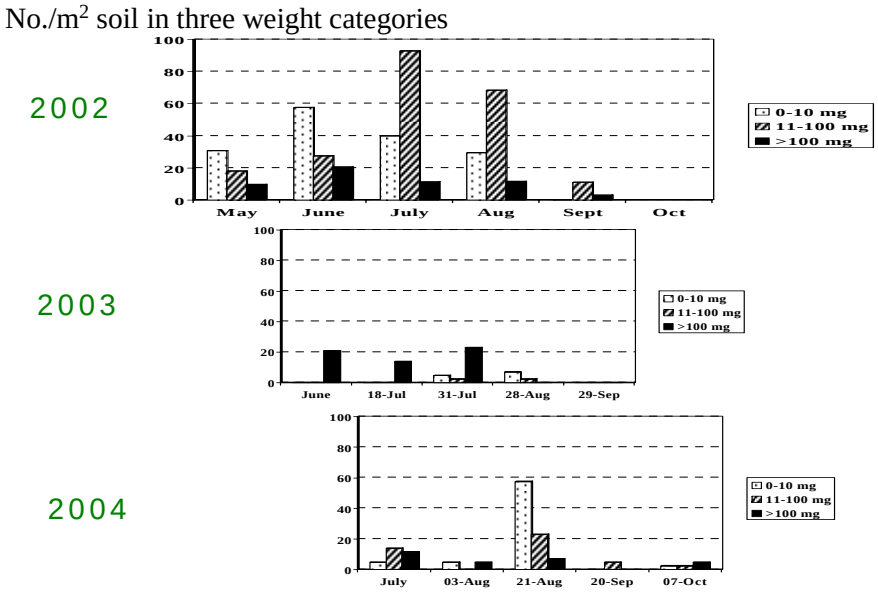

Figure 5.1.Densities of Deroceras reticulatum *in three weight classes estimated from soil samples taken from the upper 10 cm of soil in fields going from cereals to oilseed rape. 2002 – Field 75 Long Ashton Research Station; 2003 – Glebe Field, Higher Clapton Farm; Eight Acres, Higher Clapton Farm.*

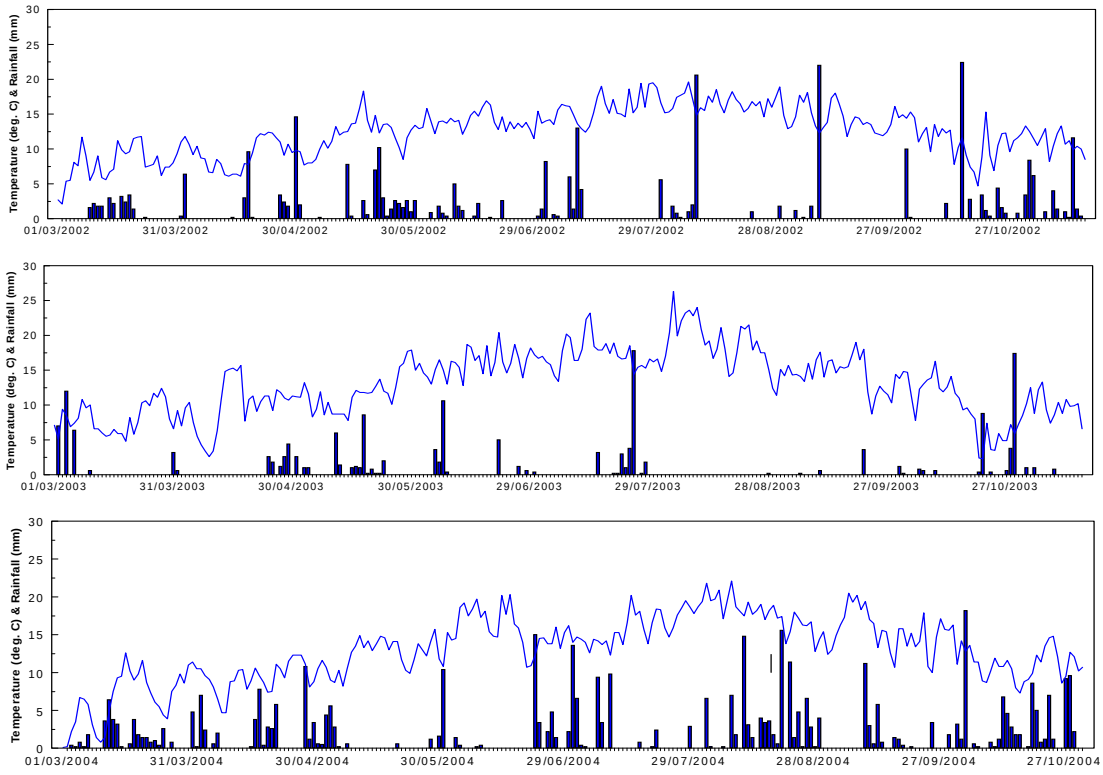

Figure 5.2.Daily mean air temperatures (lines) and rainfall (bars) recorded at the meteorological station at Yeovilton, Somerset from March to October, 2002-2004.

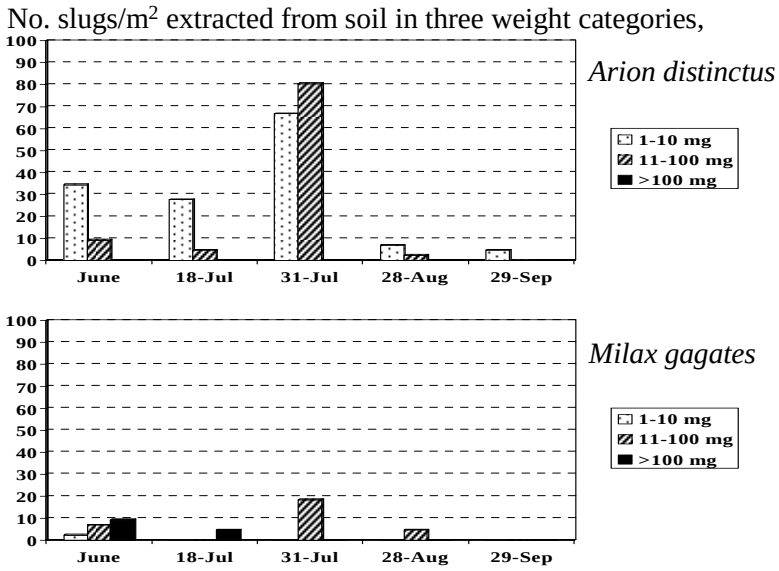

Figure 5.3. Densities of Arion distinctus *and* Milax gagates *in three weight classes estimated from soil samples taken from the upper 10 cm of soil in Glebe Field, Higher Clapton Farm, going from winter wheat to oilseed rape, 2003.*

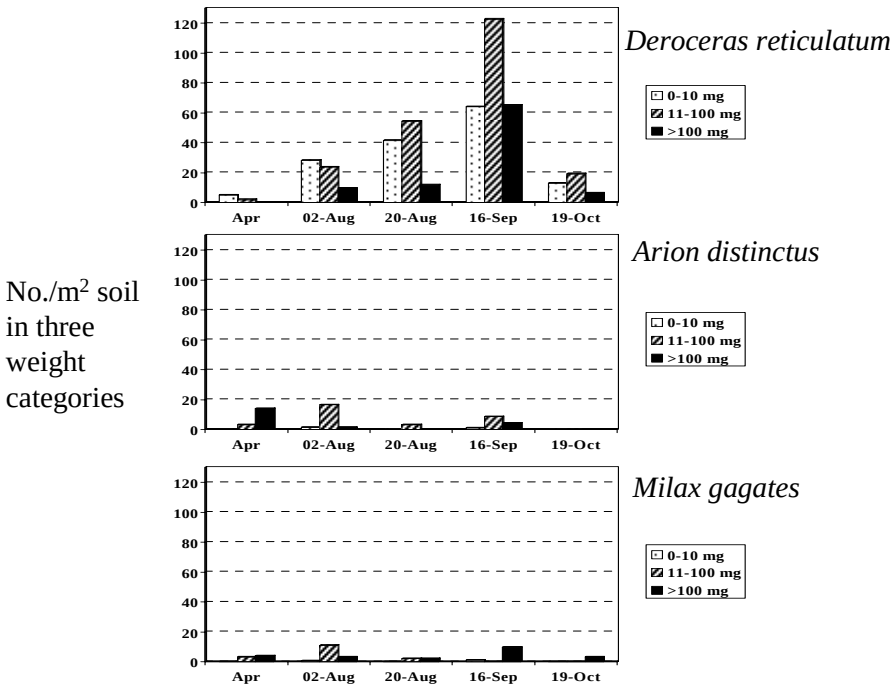

Figure 5.4. Densities of Deroceras reticulatum, Arion distinctus *and* Milax gagates *in three weight classes estimated from soil samples taken from the upper 10 cm of soil in Glebe Field, Higher Clapton Farm, going from oilseed rape to winter wheat, 2004.*

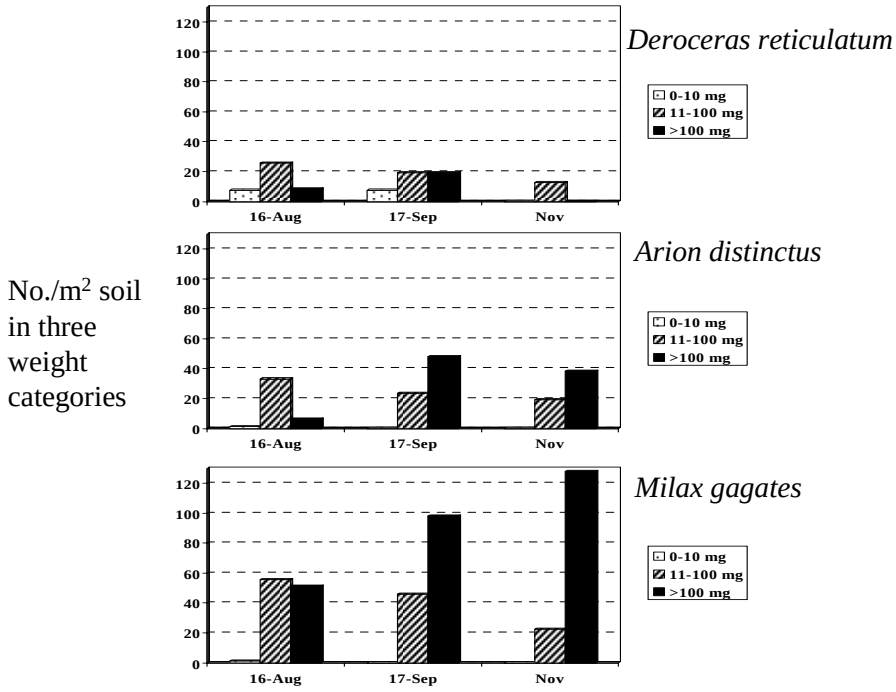

Figure 5.5. Densities of Deroceras reticulatum, Arion distinctus *and* Milax gagates *in three weight classes estimated from soil samples taken from the upper 10 cm of soil in Green Triangle, Lawn Farm, Wiltshire, going from oilseed rape to winter wheat, 2004.*

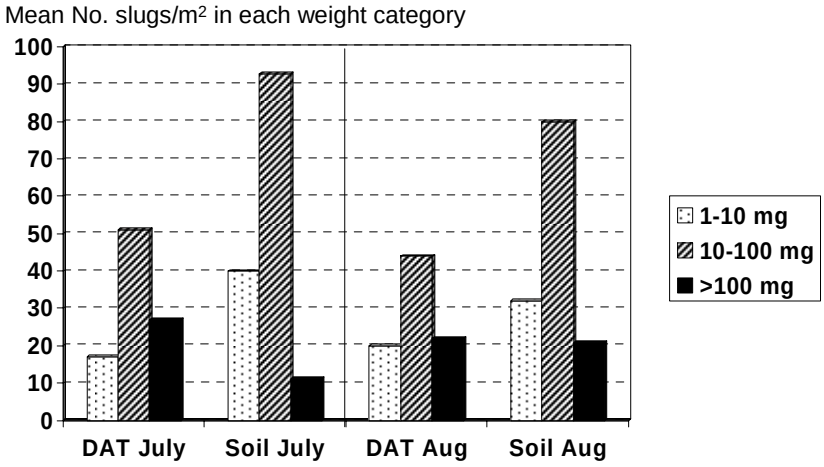

Figure 5.6. Densities of Deroceras reticulatum *in three weight classes estimated from defined area traps (DATs) compared with soil samples taken from the upper 10 cm of soil in Field 75, Long Ashton Research Station, North Somerset, in a crop of winter wheat in July 2002 and in wheat stubble in August 2002.*

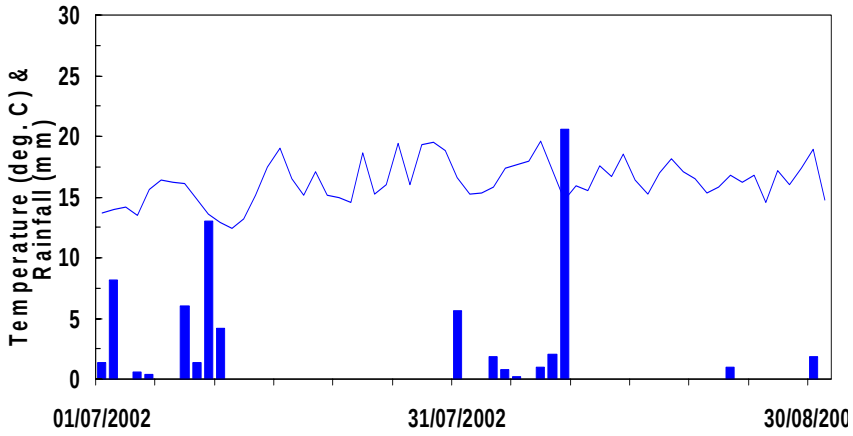

Figure 5.7.Daily mean air temperatures (lines) and rainfall (bars) recorded at the meteorological station at Long Ashton Research Station in July and August 2002.

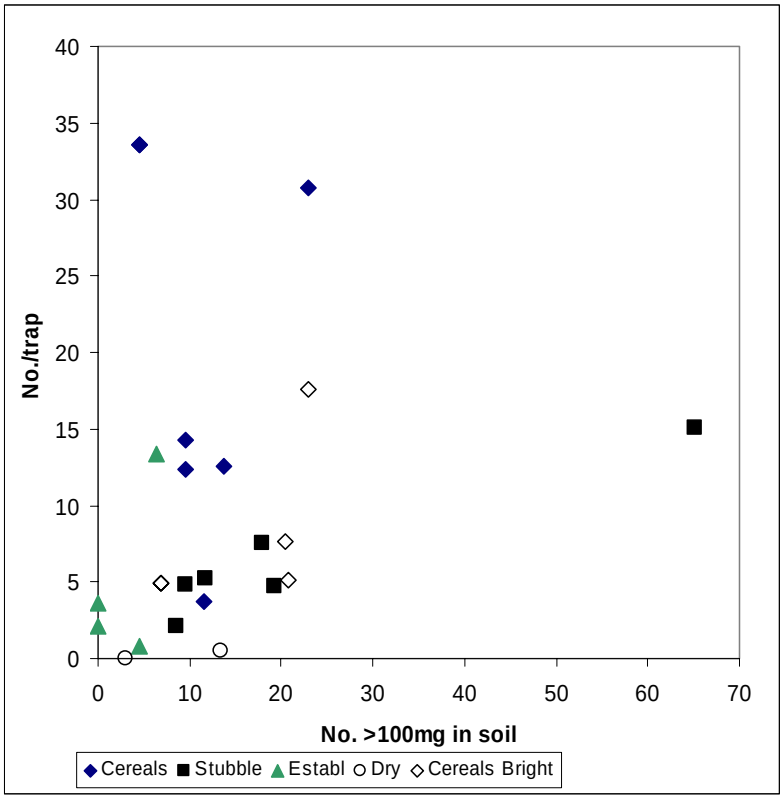

Fig. 5.8. Numbers of Deroceras reticulatum *recorded in refuge traps compared with the numbers of larger slugs of this species (>100mg) recorded in soil samples taken from the upper 10cm of soil at the same time as trapping, in standing cereals (Cereals), stubble of cereals or oilseed rape (Stubble) or in cereals and oilseed rape at establishment (Establ). In all those cases the weather conditions were suitable for trapping, with the soil surface remaining moist and the weather being cloudy on the morning following trap examination. Instances where the soil was recorded as drying (Dry) or there was bright sun (Bright) at the time of examination are shown separately.*

PAPER 6 – *Objective 1.1*

Slug Damage to Winter Wheat Seeds in Relation to Slug Body Weight

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Abstract

The capacity of individuals of three common pest slug species, *Deroceras reticulatum, Arion distinctus* and *Milax gagates*, to kill winter wheat was investigated in the laboratory by measuring the numbers of wheat seeds killed in the first week after sowing in relation to slug body weight. Individual slugs killed up to about 50 seeds during this period. For *D. reticulatum* and *M. gagates*, the number of seeds killed increased with slug body weight but at a declining rate towards an asymptote. Thus, weight for weight, smaller individuals of these species killed more wheat seeds than older individuals. One reason for this was that although juveniles ate less of each seed they always took the embryo resulting in seed death. For *A. distinctus*, the number of wheat seeds killed also increased with slug body but, weight for weight, this slug killed fewer seeds than the other species, partly because it took more from each seed than did the other species.

Introduction

Despite the importance of slugs as pests in winter wheat there is little information on the effect of slug size on their potential to cause damage. Glen *et al.* (1989) showed an empirical relationship between the square root of the biomass of slugs in soil and the percentage of wheat seeds and seedlings killed. This would indicate that the wheat seed kill increases with the biomass of slugs in the soil but that the rate of increase slows as biomass increases. However, the basis of this relationship is not understood. The decline in the rate of increase of damage with biomass could be due simply to the fact that, as damage increases, more and more of the seeds and seedlings that are vulnerable to slug attack have already been killed, so that there are fewer seeds left for slugs to feed on. However, it is also possible that this could be based on an underlying relationship between the numbers of wheat seeds killed and slug body weight. Given that small, immature individuals often make up the bulk of slug populations (e.g. Glen & Wiltshire, 1986 and Paper 5, this report), it is important to know whether small slugs are capable of killing wheat seeds and to understand the capacity of slugs to kill wheat seeds in relation to their body weight.

Materials and methods

Slugs of three species (*Deroceras reticulatum, Arion distinctus* and *Milax budapestensis*) in a range of sizes were collected from soil samples, as described in Paper 5 (this report), that were taken from oilseed rape stubble in autumn 2004. One week before the slugs were provided with wheat seeds, the slugs were placed in plastic boxes, with the bases lined with moist paper towel and with a large quantity of oilseed rape trash collected from the field, to simulate the conditions experienced by slugs in oilseed rape stubble before a wheat crop is drilled. The slugs were kept in these boxes, in the dark, at 11.5^{o}C for one week. Individual slugs were then taken from the boxes, weighed and placed in plastic containers (one slug per container) lined with moist paper towel in the base and containing eighty wheat seeds that had been soaked overnight in water. This number of wheat seeds was based on a preliminary study of the numbers of wheat seeds consumed by slugs, so that all slugs were provided with more seeds than they could consume. The slugs were left with the wheat seeds at 11.5^{o}C for one week then the slugs were removed and weighed individually. In each container where the slug was alive at the end of the week, the wheat seeds were examined carefully for signs of slug feeding and the feeding damage by at the end of the week was classified as follows: -

1. Embryo alone eaten
2. Embryo plus up to 25% of endosperm
3. Embryo plus 26-50% of endosperm
4. Embryo plus 51-75% of endosperm
5. Embryo plus 76-100% of endosperm.

Results

The number of seeds killed by slugs in the first week after seeds had imbibed water was positively related to the initial slug body weight (Fig. 6.1). For both *D. reticulatum* (ranging in weight from 15 mg to 590 mg) and *M. gagates* (ranging in weight from 42 mg to 700 mg), the number of seeds killed increased with body weight but at a decreasing rate towards an asymptote of about 40 – 50 seeds, in a way that was well described by a logarithmic relationship. *Arion distinctus* ranged in weight from 46 mg to 340 mg and individuals of this species killed fewer seeds than the other two species of equivalent body weight (Fig. 6.1). There was a linear relationship between the body weight of *A. distinctus* and the number of wheat seeds killed.

Negative correlations between the percentage of seeds with only the embryo eaten and the initial body weight of *D. reticulatum* and *M. gagates* (Fig. 6.2) showed that the smaller individuals of both species tended to consume only the embryo of the seeds whilst the larger individuals tended to consume both the embryo and at least part of the endosperm. A much smaller percentage of *A. distinctus* consumed only the embryo, compared to the other two slug species (Fig. 6.2) indicating that this species tended to consume a greater percentage of the tissue of each seed that it killed.

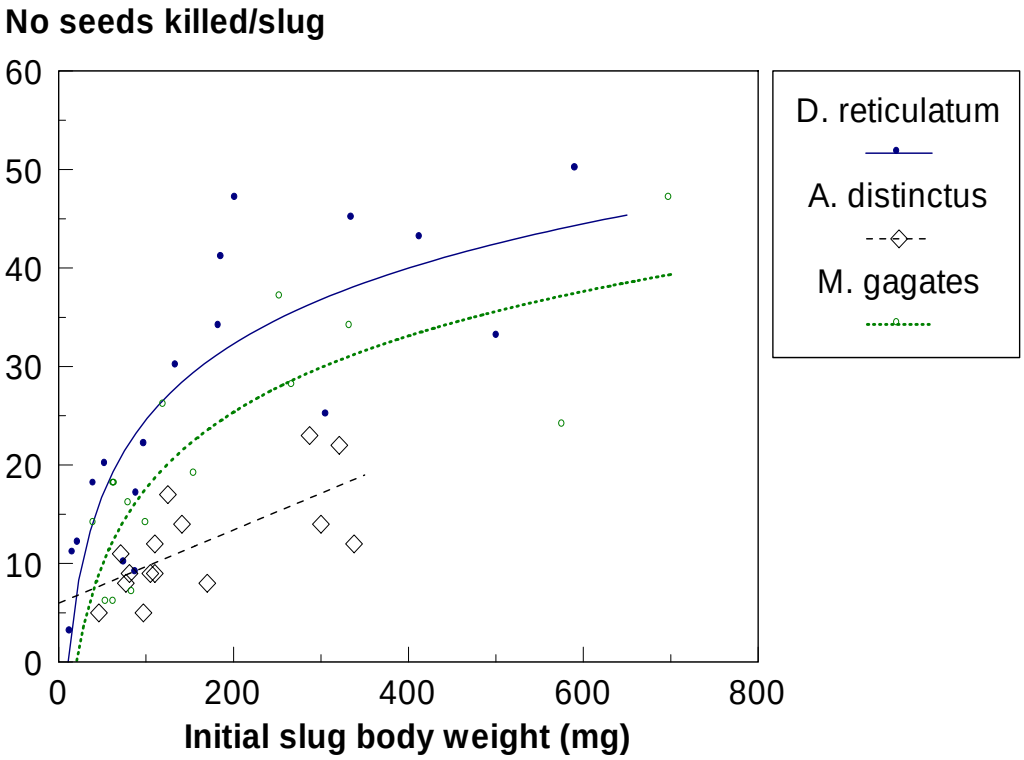

*Figure 6.1: The numbers of wheat seeds killed by slugs during the first week after the seeds had imbibed water, at 11.5°C, in relation to the initial body weight of three species of slugs (*Deroceras reticulatum, Arion distinctus *and* Milax budapestensis*).*

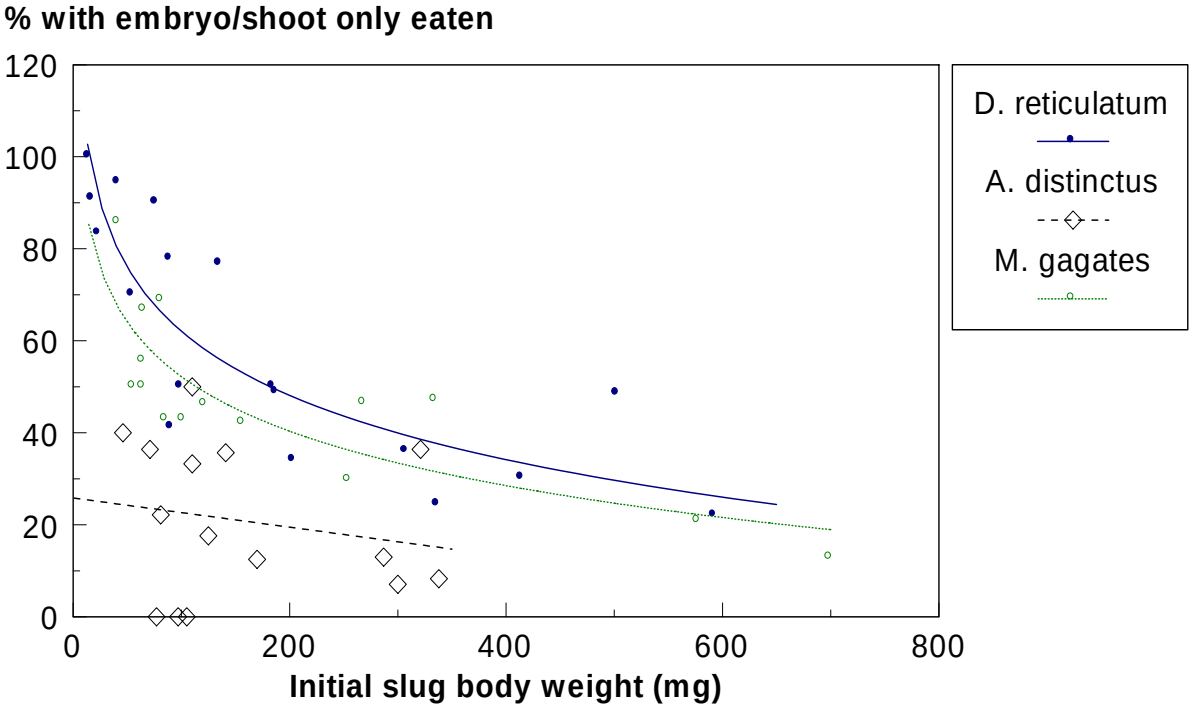

*Figure 6.2: Wheat seeds with only the embryo or shoot eaten as a percentage of all wheat seeds killed by slugs during the first week after the seeds had imbibed water, at 11.5°C, in relation to the initial body weight of three species of slugs (*Deroceras reticulatum, Arion distinctus *and* Milax budapestensis*).*

All three species of slugs increased in weight over the period of one week feeding on wheat seeds (Fig. 6.3). Some smaller *D. reticulatum* and *M. gagates* more than doubled their body weight, but larger individuals showed a smaller percentage increase in body weight, so that there was a negative regression between initial body weight and the percentage increase in body weight. *Arion distinctus* showed smaller percentage increases in body weight than the other two species (Fig. 6.3), with a stable relationship between initial body weight and percentage increase in weight.

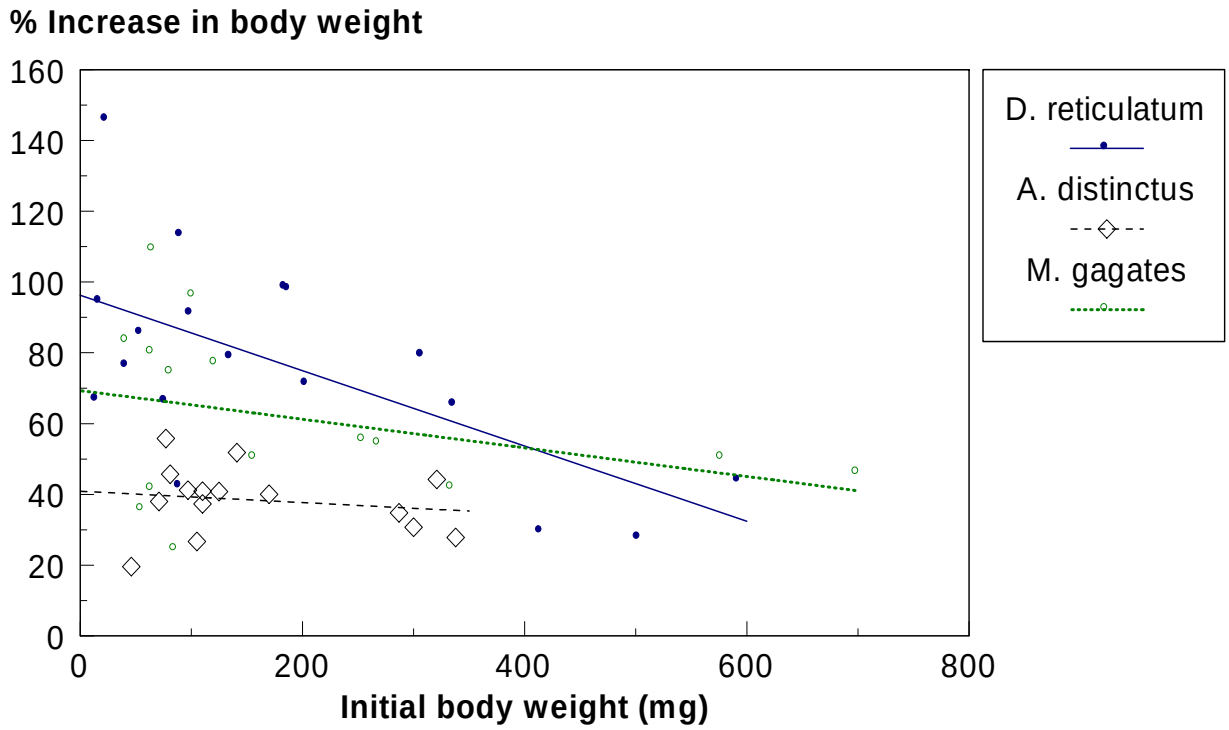

*Figure 6.3: The percentage increase in body weight over a period of one week at 11.5°C feeding on wheat seeds, in relation to the initial body weight of three species of slugs (*Deroceras reticulatum, Arion distinctus *and* Milax budapestensis*).*

Discussion

The field study of Glen *et al.* (1989) has indicated that slug biomass is more important than numbers in determining the severity of damage to wheat seeds and seedlings. However, although there have been studies of the comparative consumption of wheat seeds by different slug species (Duthoit, 1964; Hunter 1968c) these investigations have not included the influence of slug body weight. Thus, the study reported here is the first into the number of wheat seeds killed by slugs in relation to individual body weight.

Duthoit (1964) showed that all species of slugs that she found in cereal fields were capable of damaging cereal seeds and seedlings in the laboratory. All species tended to eat both the embryo and endosperm of wheat seeds. When given the choice of seeds or seedlings, *Deroceras reticulatum*, *Arion ater* L. and *Arion fasciatus* agg. all caused equal damage to both, whereas *Arion hortensis* agg. and *Tandonia budapestensis*

were more likely to damage seeds than seedlings. On balance, Duthoit (1964) concluded that *Deroceras reticulatum* and *Arion ater* were potentially the most damaging species present in cereal crops, mainly because of their greater appetite compared to other species, but also because of their feeding preferences. Given that *D. reticulatum* is much more prevalent than *A. ater* in arable fields with cereal-dominated crop rotations (Glen & Wiltshire, 1988), it is not surprising that the former species is generally considered to be the most important pest species.

Our study of wheat consumption by individual slugs showed that, in proportion to their weight, smaller individuals of *D. reticulatum* and *M. gagates* are capable of killing more wheat seeds than larger individuals. It must be remembered that the experiment was done under artificial laboratory conditions where the seeds were readily available and the slugs did not have to travel any distance to find more seeds. There could be differences between smaller and larger slugs that would differentially affect their consumption of wheat seeds in the field. For example, compared to smaller slugs, larger slugs are likely to be able to move over greater distances to find seeds in the field. However, this would be counteracted by the ability of smaller slugs to move through smaller spaces in the soil to gain access to the seeds. Thus, the results of our study provide a useful comparison of the influence of slug body weight.

Arion distinctus of a given weight killed fewer wheat seeds than the other two species of equivalent weight. The results show that there are likely to be two reasons for this. First, *A. distinctus* consumed more from each seed and, second, *A. distinctus* showed a slower growth rate over the experimental period than the other two species. Similarly, larger individuals of both *D. reticulatum* and *M. gagates* consumed more from each seed and showed a slower growth rate than smaller individuals. Thus, smaller slugs tend to feed only on the embryo before moving on to kill the next seed, whereas larger slugs tend to stay to feed on the endosperm as well as the embryo. However, the greater consumption from individual seeds by larger individuals of *D. reticulatum* and *M. gagates* and by all *A. distinctus* is irrelevant to crop damage because once the embryo is eaten, the seed is killed.

Although all slugs were provided with an excess of wheat seeds, it seems likely that the larger individuals of *D. reticulatum* and *M. gagates*, which killed more seeds would behave been more and more likely, as the week progressed, to encounter seeds that they had already fed from. This effect could have contributed to their tendency to feed on a larger percentage of the tissue of each seed than smaller slugs. However, it is likely that this would be representative of field conditions, where there is also a limited number of wheat seeds available for slug feeding.

The consumption of up to about fifty wheat seeds per slug during the first week after the seeds had imbibed water emphasizes the importance of cultural measures to prevent slugs from feeding on the seeds and, where this is not possible because seeds have been drilled into a cloddy seedbed, the importance of applying slug pellets as soon as possible after drilling to kill slugs before they can feed on seeds (see Paper 11, this report).

Acknowledgements

The work described here is part of a Defra-sponsored project in the Sustainable Arable LINK Programme. The industry partners are Bayer CropScience Ltd, CropTech, De Sangosse UK, Godfrey Farms Ltd, the Home-Grown Cereals Authority and Lonza Ltd. The academic partners are ADAS UK Ltd, Rothamsted Research and the University of Newcastle. DEFRA is the government sponsor. We thank all the partners in this project for their inputs.

PAPER 7 – *Objective 1.2*

Abundance and Vertical Distribution of Slugs in Soil Following Cultivation

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Abstract

Cultivation, especially ploughing followed by seedbed preparation, disrupts normal surface activity patterns of slug populations. We tested whether this disruption could lead to changes in the vertical distribution of slugs in the soil profile over time, which might have an effect on the efficacy of pellet applications after cultivation. In a replicated experiment in winter wheat, slug numbers were found to be considerably reduced by ploughing and their distribution changed, through burial, with up to 40% of the survivors being in the lower (10 – 20 cm) soil layer. Slugs moved back to the soil upper (0-10 cm) layer over a period of four weeks or more after ploughing. Thus, slugs buried by ploughing might return to the soil surface after molluscicide pellets applied at drilling have ceased to be effective.

Introduction

Cultivation, especially ploughing followed by seedbed preparation, disrupts normal surface activity patterns of slug populations. One consequence of this is that the numbers of slugs recorded in baited refuge traps on wheat seedbeds after drilling do not accurately reflect the risk of winter wheat seeds being killed by slugs (Glen *et al.*, 1993). We wished to investigate whether slugs work their way to the surface over an extended period of weeks after ploughing.

Materials and methods

A field experiment was established in autumn 2001 in Field 73 at Long Ashton Research Station, North Somerset. Winter wheat was drilled on 3 October, following oilseed rape. The experiment had two treatments: (1) ploughing on 3 October before drilling; and (2) zero-tillage with direct drilling. The treatments were laid out in a randomised block design, with 10 replicates of each treatment. The plot size was 12 m x 12 m, with 12 m wide discards between plot rows to permit machinery to turn. Two soil samples were taken from each plot on 3 October, immediately after drilling, and then at intervals up to 27 days after ploughing. This time period was chosen because previous unpublished work had indicated that most of the change in vertical distribution of slugs occurred during this period after ploughing. On each sampling date,

one soil sample was first dug at random from the upper soil level (0-10 cm) in the central region in each plot (ref?). This was done by inserting a metal frame 25 cm x 25 cm square x 10 cm deep into the soil and removing the sample by undercutting with a spade. Any soil debris that had fallen into the hole was carefully removed, and then a second soil sample was taken at 10-20 cm depth, from the same hole, by inserting a metal frame 25 cm x 25 cm square x 20 cm deep. The soil outside one side of the frame was then excavated so that the sample could be undercut with the spade and removed. Soil samples were placed immediately in plastic tubs with lids and transferred to a soil flooding unit where the samples were steadily flooded over nine days and all slugs coming to the soil surface were removed, identified and weighed individually.

Results

Numbers (Table 7.1) and total weights (not shown) of slugs per sample were square-root transformed for analysis of variance. ANOVA showed highly significant ($P < 0.001$) effects of cultivation, soil depth and date of sampling, with interactions ($P < 0.001$) between all three factors. In the plots without tillage, more than 99% of the slug population was found in the upper 0-10 cm layer of soil, where back-transformed mean numbers and weight of slugs were significantly greater than on ploughed plots. Initially, on 3 October, numbers in the upper layer were reduced by 96% by ploughing and on subsequent dates by 79%, 62% and 86%, respectively, compared to numbers on unploughed plots on the same dates.

Table 7.1: Mean square root numbers of slugs per soil sample at two depths after ploughing or without any cultivation, winter wheat, October 2001. (Back-transformed means in brackets).

Cultivation treatment	*Sampling depth*	*Days after ploughing*			
		0	*9*	*15*	*27*
Ploughed	0 –10cm	0.95 (0.9)	0.95 (0.9)	1.97 (3.9)	1.17 (1.4)
	10 – 20cm	0.79 (0.6)	0.52 (0.3)	0.82 (0.7)	0.54 (0.3)
No tillage	0 – 10cm	4.96 (24.6)	2.08 (4.3)	3.24 (10.5)	3.23 (10.4)
	10 – 20cm	0.20 (0.04)	0.00 (0.00)	0.20 (0.04)	0.10 (0.01)
LSD ($P = 0.05$)	0.76 0.75 for same method of cultivation				

The rapid reduction in slug numbers suggests that many slugs were probably killed by the mechanical action of ploughing. Immediately after ploughing and nine days later, there was no significant difference between numbers of slugs in the upper and lower soil layer in ploughed plots. By 15 days after ploughing, there were significantly more slugs in the upper compared with the lower layer. A similar trend was observed 27 days after ploughing (significant for weight but not numbers of slugs), indicating that surviving slugs had moved back to the surface layers from about 15 days onward.

Discussion

Despite the substantial effect of ploughing in reducing slug numbers, in the experiment described here, other experiments show that substantial numbers of slugs can survive ploughing and cause severe damage to crops such as winter wheat at establishment (Glen & Symondson, 2003). Farmers' experience also clearly bears this out. In this context, it is important that our findings show that surviving slugs are likely to move back to the soil surface layers over a period from 10 days to four weeks or more after ploughing. Thus, if sufficient slugs survive ploughing to be a risk to the following crop, there is the potential for a substantial proportion of them to live at a depth where they do not come into contact with slug pellets applied at drilling. These slugs could then return to the surface and cause damage to emerging wheat crops after slug pellets applied at about the time of drilling have ceased to be effective.

This in turn suggests that there could be benefits from broadcasting slug pellets on the stubble of the previous crop, before cultivation, at a time when the weather is suitable for slug activity, when most of the slug population will be close to the soil surface. We tested the efficacy of pellet applications to stubble in a series of field experiments from 2002 to 2004 (Paper 11, this report). Pellets applied to stubble were effective and not significantly different from pellets applied at drilling in the dry weather of late summer and early autumn 2002 and 2003. However, in the wet weather of late summer and autumn 2004, pellets applied to stubble were significantly less effective in protecting winter wheat from slug damage because slugs were able to recover and the treatment effects had diminished by the time that wheat was at risk (see Paper 11, this report for further details). Thus, we conclude that it is best to apply slug pellets around the time of drilling, just before damage is expected.

Acknowledgements

The work described here is part of a Defra-sponsored project in the Sustainable Arable LINK Programme. The industry partners are Bayer CropScience Ltd, CropTech, De Sangosse UK, Godfrey Farms Ltd, the Home-Grown Cereals Authority and Lonza Ltd. The academic partners are ADAS UK Ltd, Rothamsted Research and the University of Newcastle. DEFRA is the government sponsor. We thank all the partners in this project for their inputs.

PAPER 8 - *Objective 1.3 & 1.4*

Behaviour of *Deroceras reticulatum* in Response to Broadcast, Drilled and Soil Contaminated Molluscicide Pellets

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Abstract

The foraging, feeding and post-feeding behaviour of *Deroceras reticulatum* (Müller) in response to broadcast and drilled molluscicide pellets was investigated. The effect of pellet condition was evaluated by comparing 'clean' and soil contaminated pellets. After the onset of activity slugs began feeding on broadcast pellets much sooner than drilled pellets and were more likely to feed on the first pellet encountered. Methiocarb poisoned more slugs than metaldehyde when pellets were broadcast, but there was no difference between active ingredients when drilled. No recovery from poisoning was observed in the twenty four hours following pellet exposure for either method of application. Soil contamination did not reduce the efficacy of the pellets and had no differential effect on poisoning.

Introduction

Control of slugs in arable crops currently relies largely on the use of molluscicide pellets (Iglesias *et al.*, 2002). These are applied either to the soil surface (broadcasting) or beneath the soil surface with the seed (drilling) (Glen & Wilson, 1995). Despite an increase of almost fourfold in pellet use during the last decade (Garthwaite & Thomas, 2003) there has not been a concomitant reduction in levels of damage. For example, slug damage in wheat alone is estimated at £4 million per annum (Shirley *et al.*, 2001) compared to £2.69 million in 1985 (Port & Port, 1986). Much research in recent years has been directed towards understanding factors that influence the foraging and feeding behaviour of slugs. This includes the role of climatic factors, such as temperature and humidity, in determining activity levels (South, 1989b; Young & Port, 1989), the processes involved in meal initiation and termination (Wedgwood & Bailey, 1988; Bailey, 1989) and the means by which slugs locate food items (Howling, 1991). There has, however, been no work that specifically investigates the foraging and feeding behaviour of slugs in relation to the method of pellet application (i.e. broadcasting versus drilling). Furthermore, although it has been suggested that heavy rainfall may render molluscicides ineffective due to pellets becoming covered in mud splash (Hass *et al.*, 1999; Simms *et al.*, 2002) this has never been formally tested; these aspects are investigated in the experiments presented here. Subsequent mortality and recovery were also monitored.

Time-lapse, infra-red video techniques were employed to create permanent records of slug behaviour. This established methodology has a number of strengths. It allows whole nights of activity to be observed remotely and does not disturb the slugs themselves. The approach has been used successfully in several previous studies (e.g. Howling & Port, 1989; Bailey & Wedgwood, 1991; Hommay *et al.*, 1998; Grimm & Schaumberger, 2002). The species used in these experiments was *Deroceras reticulatum*, the most serious slug pest of arable crops in the United Kingdom (Schley & Bees, 2003).

Materials and Methods

D. reticulatum used in the experiments were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). They were maintained at 12°C in ventilated plastic containers (18 x 12 x 7 cm) filled with moist laboratory tissue for up to five days prior to use. This ensured that any slugs in the sample that were unhealthy following collection could be excluded from the experiments. During this time they were provided with Chinese cabbage and carrot *ad libitum*.

The experiments were carried out under controlled conditions at 12 ± 2°C in plastic arenas measuring 57 x 36 x 16 cm, the rims of which were painted with a Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) barrier to prevent slugs escaping. The arenas were filled to a depth of 8-10 cm with loamy soil dug from an agricultural plot at Close House Field Station and any stones, large lumps of organic matter and soil organisms were removed prior to use. The soil surface was raked to a fine tilth. Fresh soil was used for each replicate and it was watered such that the surface appeared damp at the start of each recording. Samples of soil from each replicate were dried in an oven at 80°C and the mean moisture content was found to be 28.6 ± 0.5%.

Slug activity was recorded using a Panasonic AG-6040 time lapse VHS video recorder. This was set to record 80 times more slowly than normal speed so that a total of 240 hours of activity could be stored on a single cassette. Recordings were played back at normal speed. The camera used was a Sanyo VCB3572 IRP ½" high resolution 570TVL infra-red sensitive camera, fitted with a Computar 8-48 mm lens and infra red bandpass filter. The arenas were illuminated at night using a Computar Uniflood LED infra red lamp (serial number CL057787). Light of this wavelength does not appear to disrupt slug activity yet permits recording to take place during darkness (Howling, 1990). Daytime lighting was provided by two 400 Watt halogen lamps suspended 1.55 m above the arena. These were controlled by a timer to match the prevailing sunrise and sunset times, which were updated weekly. Two types of commercial grade molluscicide pellets were used in the experiments; 3% a.i. methiocarb (Bayer, UK) and 5% a.i. metaldehyde (De Sangosse, UK). These were applied at the manufacturers recommended rate.

Methods

For each pellet type two application methods were assessed; broadcasting and drilling. The pellets were not pre-treated in any way prior to application. They were placed equidistant from each other in the arena either on the soil surface (broadcast) or 1 cm below the soil surface in drill lines 15 cm apart, lightly covered in soil (drilled). The configuration of the pellets in the arena is shown in *Fig. 8.1 (a)* and *(b)*.

Pellets can become splashed with soil as a result of heavy rainfall which may influence the behavioural response of slugs towards them. This was investigated by comparing clean and soil contaminated pellets of each type. The effect of soil contamination was achieved by rolling the pellets in dishes filled with wet soil. The pellets were applied to the arena as for broadcast pellets.

Five slugs were used per arena which is equivalent to a field density of 25 slugs per m^2. It has been suggested that when slugs are disturbed they exhibit an immediate, atypical feeding response and therefore it is advisable to allow them one day to acclimatise to their new environment before conducting experiments (Whelan, 1982). Accordingly the slugs were placed in the arena 24 hours prior to the commencement of recording. During this time they were starved to ensure that they were motivated to forage and feed. Following the acclimatisation period pellets were applied to the arena, recording began and continued overnight until the following morning, giving a total of 16 hours recording time per replicate. The six pellet application regimes were recorded in blocks. Each block was replicated six times and different slugs were used for each recording.

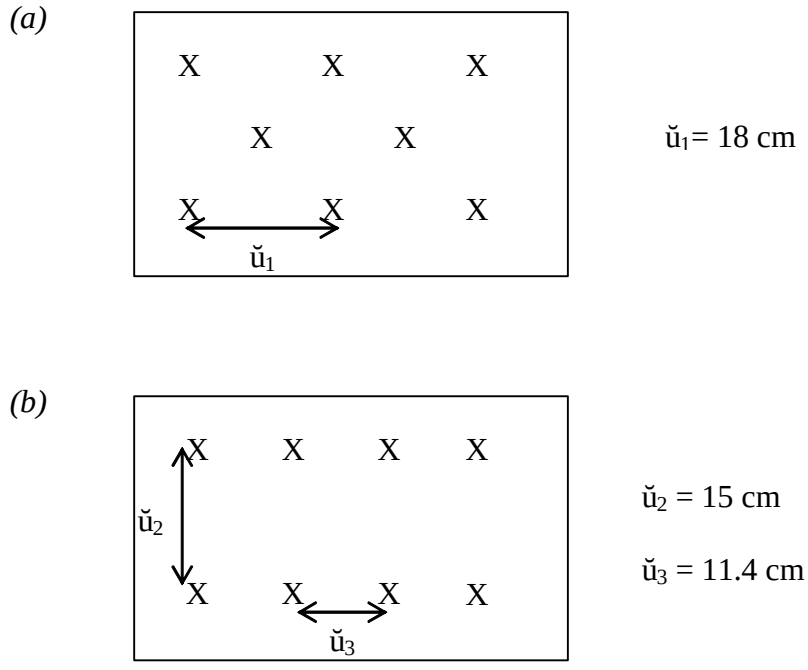


Figure 8.1: Configuration of pellets in arenas (a) broadcast and soil splashed pellets (b) drilled pellets. X indicates the position of a pellet.

After each night of recording slugs were removed from the arena and weighed. The appearance of each slug was recorded as poisoned or unpoisoned. Poisoned slugs showed a characteristic appearance depending upon the active ingredient; methiocarb poisoning tends to cause bloating of the body and may induce eversion of the buccal mass and head tentacles whereas metaldehyde poisoning is typically manifested by a shrivelled body and retracted buccal mass and tentacles (Frain & Newell, 1983). Individuals were then placed into separate Petri dishes lined with moist laboratory tissue at 20°C and supplied with Chinese cabbage *ad libitum*. After 24 hours the state of each slug was again recorded as poisoned or unpoisoned. Slugs were weighed prior to acclimatisation, at the end of recording and 24 hours later using a Mettler MT5 balance.

Video recordings were analysed using a computer programme called Inchworm™. This semi-automated system allowed pellets to be defined as zones within the arena. The path of the slug was then followed manually using the computer cursor. The software digitised the track output and resolved it into time and distance data for zoned and non-zoned areas. To discern feeding from non-feeding contact with a pellet, a threshold contact duration criterion of 0.5 minutes was used. If the slug was in contact with the pellet for more than this time it was assumed to be feeding; if it was in contact with the pellet for less than this time, it was categorised as having ignored the pellet. Feeds on the same pellet separated by less than three minutes were treated as a single meal. If consecutive feeds were on different pellets they were counted as separate meals regardless of the intervening time. The number of pellets ignored by slugs between the onset of activity and the first pellet feed was counted along with the number of pellets eaten after the first feed. Continuous data on times and distances travelled were non-parametric. They were analysed using the Mann-Whitey U-test. Discrete counts of events were assessed using the Chi-squared test with Yates' correction.

Results

For both methiocarb and metaldehyde the method of pellet application significantly affected the time elapsed between the onset of slug activity and the first feed. It took considerably more time for feeding to begin when pellets were drilled compared to broadcast (Mann-Whitney U-test: methiocarb: N = 48, W = 436.0, $P < 0.001$; metaldehyde: N = 56, W = 604.0, $P < 0.001$). In the case of methiocarb, this difference was almost 10-fold whereas for metaldehyde it took six times as long (*Table 8.1*).

Pellet condition did not significantly affect the time taken to start feeding on a pellet after the onset of activity for methiocarb, with no difference between soil contaminated and 'clean' broadcast pellets (Mann-Whitney U-test: N = 55, W = 773.5, *n.s.*). This difference was, however, significant for metaldehyde (Mann-Whitney U-test: N = 57, W = 946.0, $P < 0.001$), with it taking almost half the time for a slug to start feeding on a soil contaminated compared to a 'clean' pellet (*Table 8.1*).

Table 8.1: Median times (mins) elapsed between onset of activity and first feed (with interquartile range) for broadcast, drilled and soil contaminated methiocarb and metaldehyde pellets.

Active Ingredient	*Pellet Treatment*	*N*	*Median Time (mins)*	*Interquartile Range*	
				Q1	*Q3*
Methiocarb	Broadcast	27	27.20	10.40	43.85
	Drilled	21	268.90	143.30	496.70
	Soil contaminated	28	21.30	7.68	61.58
Metaldehyde	Broadcast	28	30.25	9.70	83.30
	Drilled	28	182.40	40.08	424.28
	Soil contaminated	29	16.50	4.20	26.50

There were highly significant differences in the distance travelled before the first feed for the different methods of pellet application. For both pellet types slugs travelled much further before feeding on a drilled compared to a broadcast pellet (Mann-Whitney U-test: methiocarb: N = 48, W = 413.0, $P < 0.01$; metaldehyde: N = 56, W = 574.0, $P < 0.01$). In the case of methiocarb they travelled six times as far when pellets were drilled and for metaldehyde they travelled almost five times further (*Table 8.2*). This difference is illustrated in *Fig. 8.2* which shows typical slug tracks for broadcast and drilled pellets.

Pellet condition had no effect on the distance travelled before the first feed for methiocarb (Mann-Whitney U-test: N = 55, W = 768.0, *n.s.*). Although, in the case of metaldehyde, the median distance slugs travelled before the first feed was greater for 'clean' rather than soil contaminated pellets this difference was not statistically significant (Mann-Whitney U-test: N = 57, W = 921.5, *n.s.*) (*Table 8.2*).

Table 8.2: Median distances (cm) travelled by Deroceras reticulatum *between the onset of activity and first feed (with interquartile range) for broadcast, drilled and soil contaminated methiocarb and metaldehyde pellets.*

Active Ingredient	*Pellet Treatment*	*N*	*Median Distance (cm)*	*Interquartile Range*	
				Q1	*Q3*
Methiocarb	Broadcast	27	33.70	20.85	62.05
	Drilled	21	211.40	98.60	415.10
	Soil contaminated	28	33.05	13.03	57.23
Metaldehyde	Broadcast	28	49.95	17.93	139.03
	Drilled	28	238.05	85.13	412.23
	Soil contaminated	29	18.40	9.10	52.80

(a) *(b)*

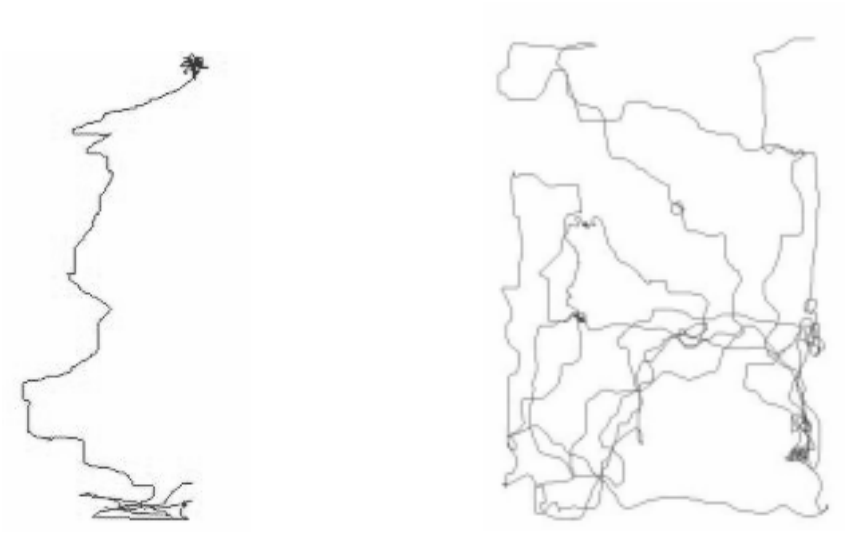


Figure 8.2: Typical slug tracks for (a) broadcast and (b) drilled pellets.

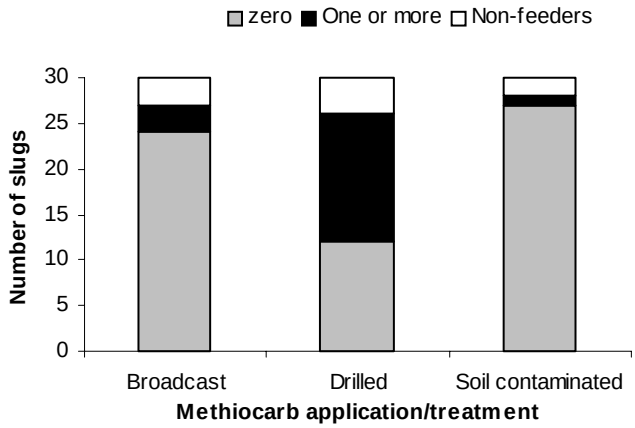
The results for the umber of pellets ignored before the first feed are displayed in *Fig. 8.3.* The number of pellets ignored before the first feed was categorised as 'zero' or 'one or more'. For both methiocarb and metaldehyde there was a significant difference between these categories for broadcast and drilled pellets. In the broadcast applications most slugs fed on the first pellet they encountered. This is in contrast to the drilled applications where considerable numbers of slugs were observed to ignore one or more pellets before the first feed of the night (Chi-Square: methiocarb: N = 53, df = 1, G^2_c = 9.228, *P < 0.01*; metaldehyde: N = 56, df = 1, G^2_c = 8.590, *P < 0.01*).

The condition of the pellet did not affect the number of pellets ignored before the first feed; there were no significant differences in the number of 'clean' and soil contaminated pellets ignored for either active ingredient (Chi-Square: methiocarb: N = 55, df = 1, G^2_c = 0.315, *n.s.*; metaldehyde: N = 57, df = 1, G^2_c = 0.228, *n.s.*).

The method of pellet application had no significant effect on the number of slugs feeding on subsequent pellets after the first feed for methiocarb (Chi-Square: N = 48, df = 1, G^2_c = 0.066, *n.s.*). For metaldehyde, however, this difference was significant with more slugs eating two or more pellets when drilled compared to broadcast (Chi-Square: N = 56, df = 1, G^2_c = 16.556, *P < 0.001*).

The condition of the pellet did not influence the number of slugs contacting and eating subsequent pellets after the first feed with no significant differences between soil contaminated and 'clean' pellets for either active ingredient (Chi-Square: methiocarb: N = 55, df = 1, G^2_c = 0.444, *n.s.*; metaldehyde: N = 57, df = 1, G^2_c = 0.809, *n.s.*). These results are shown in *Fig. 8.4.*

(a)



(b)

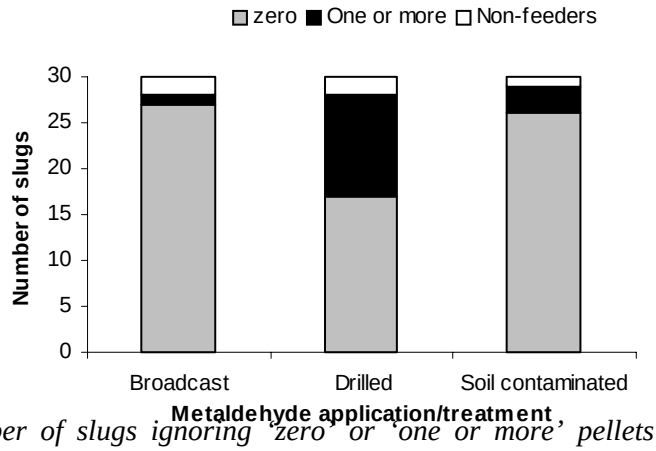
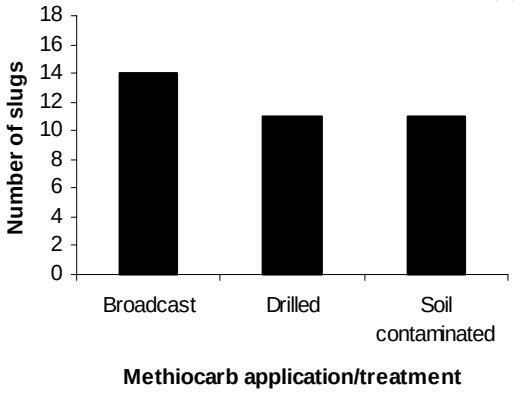


Figure 8.3: The number of slugs ignoring 'zero' or 'one or more' pellets before the first feed for (a) methiocarb pellets and (b) metaldehyde pellets.

(a)



(b)

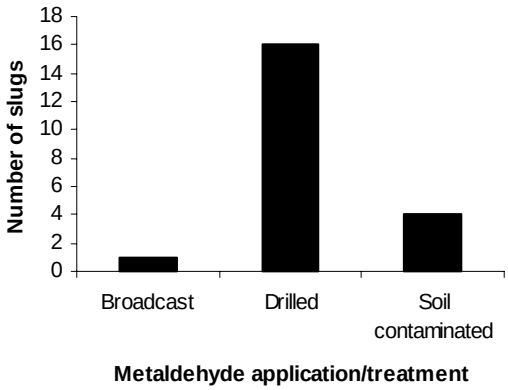


Figure 8.4: The number of slugs consuming subsequent pellets after the first feed for (a) methiocarb pellets and (b) metaldehyde pellets.

The effect of the application method and pellet condition on mortality and recovery was assessed by observing numbers of poisoned and unpoisoned slugs at the end of the night's recording (T_0) and 24 hours later (T_{24}). Homogeneity chi-square tests indicated that, for both metaldehyde and methiocarb, there were no

significant differences between replicates for either pellet application method or condition at T_0 or T_{24}. Results were therefore pooled over arenas. *Table 8.3* summarises the numbers of slugs in each of these categories for both active ingredients. It was not possible to recover every slug from the arena as some became obscured by a soil covering. Additionally, some reassessments at T_{24} were missed for logistical reasons; hence the numbers in *Table 8.3* do not represent a strictly a 'before and after' analysis.

Table 8.3: The numbers of poisoned and unpoisoned Deroceras reticulatum *at T_0 & T_{24} with metaldehyde and methiocarb pellets applied in different ways.*

Method of Application/ Pellet Condition	*Methiocarb*				*Metaldehyde*			
	T_0		*T_{24}*		*T_0*		*T_{24}*	
	UP	*P*	*UP*	*P*	*UP*	*P*	*UP*	*P*
Broadcast	2	23	1	19	12	13	10	15
Drilled	11	18	9	10	12	7	7	7
Soil contaminated	2	28	1	19	9	11	4	16
Total	15	69	11	48	33	31	21	38

UP = unpoisoned; P = Poisoned

The numbers of poisoned and unpoisoned slugs were compared for application method and pellet condition, using a Contingency Chi-square test, applying the tests for each active ingredient at T_0 and T_{24}. The results are shown in *Table 8.4*. At both T_0 and T_{24} the only significant difference in relation to the method of pellet application was between drilled and broadcast methiocarb with fewer slugs poisoned when pellets were drilled. There were no such differences observed for metaldehyde. Pellet condition did not affect the numbers of poisoned and unpoisoned slugs for either metaldehyde or methiocarb with no significant differences between soil contaminated and 'clean' pellets at T_0 or T_{24}.

Table 8.4: Contingency Chi-square values comparing, between methods of application and pellet condition, the numbers of Deroceras reticulatum *poisoned by metaldehyde or methiocarb pellets at T_0 and T_{24}.*

	Chi-Square Value	
Comparison	*Metaldehyde*	*Methiocarb*
T_0		
Broadcast vs. Drilled	0.484	*5.049
Broadcast vs. Soil contaminated	0.010	0.111
T_{24}		
Broadcast vs. Drilled	0.072	**7.094
Broadcast vs. Soil contaminated	1.243	0.526

*Significance level: *P < 0.05; **P < 0.01*

The difference in mortality caused by metaldehyde and methiocarb was directly compared at T_0 and T_{24} for each of the two methods of application and for pellet condition. At T_0 methiocarb poisoned significantly more slugs than metaldehyde when broadcast yet there were no differences when the pellets were drilled. This pattern was also observed for soil contaminated as opposed to 'clean' pellets where methiocarb poisoned significantly more slugs at T_0 than metaldehyde. By T_{24} the disparity between numbers of slugs poisoned by the two active ingredients had declined with only broadcast methiocarb pellets poisoning significantly more slugs than metaldehyde pellets applied in the same way.

When results were combined for all methods of application/pellet condition there were highly significant differences between active ingredients at T_0 with methiocarb poisoning more slugs than metaldehyde. At T_{24} these differences had completely disappeared and both active ingredients were seen to be equally effective. The results are summarised in *Table 8.5*.

Table 8.5: Contingency Chi-squared values comparing, between metaldehyde and methiocarb, the numbers of Deroceras reticulatum *poisoned when pellets are applied in different ways or are soil contaminated at T_0 and T_{24}.*

Application Method/ Pellet Condition	*T_0*				G^2	*T_{24}*				G^2
	MA		*MC*			*MA*		*MC*		
	UP	*P*	*UP*	*P*		*UP*	*P*	*UP*	*P*	
Broadcast	12	13	2	23	**8.036	10	15	1	19	*5.066
Drilled	12	7	11	18	2.011	7	7	9	10	0.042
Soil contaminated	9	11	2	28	**8.163	4	16	1	19	0.914
Total	33	31	15	69	***17.315	21	38	11	48	3.474

*UP = unpoisoned; P = Poisoned. Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001*

Crawford-Sidebotham (1970) expressed the differences in mortality between active ingredients as an 'Index of Efficiency', *I*. This is defined as $I=R_D/R_M$ where:

$$R_D = \frac{\text{Number of slugs poisoned by methiocarb}}{\text{Total number of slugs in methiocarb experiment}}$$

$$R_M = \frac{\text{Number of slugs poisoned by metaldehyde}}{\text{Total number of slugs in metaldehyde experiment}}$$

The index of efficiency serves as a useful guide to illustrate the magnitude of the differences observed between active ingredients. Of the results presented here, the indices of efficiency for those comparisons where differences between methiocarb and metaldehyde were found to be significant are given in *Table 8.6.*

It can be seen that, for example, at T_0 1.35 times as many slugs were poisoned by broadcast methiocarb as compared to metaldehyde. By T_{24} this had declined although methiocarb was still causing the higher mortality of the two active ingredients. For soil contaminated pellets methiocarb was observed to poison almost twice as many slugs as metaldehyde at T_0.

Table 8.6: Values for the index of efficiency of methiocarb as compared with metaldehyde for methods of application/pellet condition where there were significant differences in mortality between active ingredients.

Application Method/ Pellet Treatment	*Time*	*Index*
Broadcast	T_0	1.35
Broadcast	T_{24}	1.27
Soil Contaminated	T_0	1.94

Recovery was defined as an improvement in the appearance of the slug from poisoned to unpoisoned during the 24 hour observation period. There was no significant recovery for slugs consuming either active ingredient, regardless of the method of application or condition of the pellets. The results are summarised in *Table 8.7*.

Table 8.7: Contingency Chi-squared values comparing, for different methods of pellet application/condition, the number of Deroceras reticulatum *poisoned at T_0 and $T_{24\ for}$ methiocarb and metaldehyde.*

Application Method/	*Metaldehyde*					*Methiocarb*				
	T_0		*T_{24}*			*T_0*		*T_{24}*		
Pellet Condition	*UP*	*P*	*UP*	*P*	G^2	*UP*	*P*	*UP*	*P*	G^2
Broadcast	12	13	10	15	0.082	2	23	1	19	0.042
Drilled	12	7	7	7	0.160	11	18	9	10	0.120
Soil contaminated	9	11	4	16	1.822	2	28	1	19	0.133

There were no clear trends in the weight change of poisoned and unpoisoned slugs between T_0 and T_{24} regardless of the active ingredient or method of application/pellet condition.

Discussion

An understanding of how control measures influence the behaviour of pest species provides a valuable insight into their ecology and can lead to better pest management strategies resulting in more precise targeting of pesticides and a reduction in collateral damage.

The aim of the experiments presented here was to investigate how different methods of pellet application and pellet condition affect the foraging and feeding behaviour of *D. reticulatum.* Specifically foraging was assessed in terms of the time and distance travelled between the onset of activity and the first pellet feed. Analysis was confined to the first feed only because it was assumed that at least some active ingredient would be ingested during this feed and its toxic effects may influence subsequent behaviour. Since it was not possible to quantify these effects the interpretation of further foraging activity would have been compromised. Feeding behaviour was resolved into the number of pellets ignored before the first feed and the number of slugs that then went on to consume at least one further pellet. This gave a measure of how pellet attractiveness and sequential acceptability, as defined by Howling (1990), are affected by the method of application and condition. Slug activity was recorded overnight and consequent poisoning and recovery were monitored during the following twenty four hour period.

Control of slugs in arable crops is most crucial at the time of seed germination and emergence when the most serious damage is caused (Duthoit, 1964). This follows a period of relative food scarcity in the arable environment when fields have lain bare after ploughing and, therefore, no alternative food source was provided in these experiments. The addition of germinating seedlings to the arena would, in any case, have severely reduced the clarity of the video recordings.

It has been shown that food deprivation increases the locomotor activity of *D. reticulatum* (Airey, 1987) and it is common in studies investigating feeding activity to starve slugs for a short period prior to experiments (e.g. Briggs & Henderson, 1987; Bailey *et al.*, 1989). In the current work this practice was adopted to increase the motivation of slugs to forage and feed and to ensure that all individuals were at a similar level of food deprivation thereby reducing within-group variation.

Since *D. reticulatum* is largely surface dwelling (South, 1965; Hunter, 1968c) and feeds on leafy vegetation in preference to roots (Runham & Hunter, 1970) it might be expected that pellets broadcast on the soil surface would be encountered more readily by this species than drilled pellets and, indeed, this is supported by these results. After the onset of activity *D. reticulatum* travelled a shorter distance and began feeding on broadcast pellets much sooner than drilled pellets regardless of the active ingredient. This contrasts, however, with a study by Hogan (1985). Although Hogan's experiments are not directly comparable, as they assessed drilled wheat seeds versus those presented in a central seed pile, she found *D. reticulatum* fed on drilled seeds sooner. This observation may be explained by considering the means by which slugs encounter food items. Evidence regarding this has been contradictory in the past with some studies suggesting olfaction plays a key role (Gelperin, 1974; Pickett & Stephenson, 1980) whilst other conclude that it is an entirely random process (Duval, 1970). Most recent work indicates that slugs are attracted to food only when they are in close proximity to it, i.e. within 3-4 cm, and not over larger distances (Bailey *et al.*, 1989; Howling, 1991). It would seem, therefore, a likely explanation of Hogan's result is that the slugs simply did not follow a path that took them near enough to the central seed pile to detect its presence. In the current

experiments the broadcast pellets were distributed evenly throughout the arena and within the optimum density for control (Hunter & Symonds, 1970) thereby representing a more realistic field situation.

An inherent limitation of any video based assessment of individual slug activity is that animals must be contained within an arena of fixed size. This is relevant in studies of foraging in that it restricts movement along the margins and may, therefore, cause atypical behavioural patterns. These so called edge effects have been investigated in studies using arenas of various sizes and it was found that slug movement was not significantly affected by the area of the arena (Bailey, 1989; Howling, 1990). It is not, therefore, thought that edge effects would have had a major impact on the foraging behaviour observed in the experiments described here.

When pellets of either active ingredient were broadcast most slugs fed on the first one they encountered whereas when they were drilled considerable numbers of slugs ignored one or more pellets before commencing feeding. As discussed above this is likely to be because broadcast pellets are more immediately accessible to *D. reticulatum* than drilled pellets. Since soil contamination was clearly shown not to affect the ability of slugs to detect pellets it would seem unlikely that the loose covering of soil over shallow drilled pellets would itself markedly reduce their attractiveness to slugs. This is supported by a study which compared the efficacy of broadcast and drilled methiocarb pellets in protecting wheat seedlings from damage (Glen *et al.*, 1992). It was seen that, although slugs fed on and were killed by pellets drilled at the same depth as those used in the current study, they were not as effective as broadcast applications in reducing damage suggesting that slugs feeding activity was arrested sooner by broadcast pellets. Physical inaccessibility is probably the more important factor in explaining both the increase in damage and greater number of pellets ignored when drilling is used to apply molluscicides.

It has been suggested that drill lines are used as 'motorways' by slugs with foraging beginning at one end of the furrow and continuing along to the other as the looser soil in the furrow is easier to travel through (Allen 1981 in Martin & Kelly, 1986). The study presented here did not find any evidence in support of this. Slug tracks were seen to meander randomly across the arena both before and after feeding on pellets, as shown in the example of a typical track in *Fig. 8.2 (b).* The soil surface was, however, a uniform fine tilth and pellets were deposited in hollows rather than furrows before being loosely covered with soil. Had furrows been used, or if the tilth had been coarser slugs may have been able to detect drill lines more easily.

Analysis of the number of slugs eating at least one further pellet after the first feed gave mixed results depending both on the active ingredient and the application method. For methiocarb there was no difference between drilled and broadcast pellets whereas for metaldehyde more slugs went on to consume subsequent pellets when they were drilled compared to broadcast. A combination of two factors may explain these results; difference in the amount of pellet consumed and in the action of the active ingredient. When pellets are drilled, after the soil covering has been removed by a foraging slug, it is only the top of the pellet that is

exposed the rest remaining in a soil depression. This contrasts with the broadcast situation where the entire pellet is accessible. It is likely, therefore, that a smaller quantity of drilled pellet is eaten. Feeding activity is also inextricably linked to the means by which the active ingredients in molluscicides exert their toxic effects and this may account for the differences between methiocarb and metaldehyde. Both work by disrupting the control of the feeding apparatus of slugs (Mills *et al.*, 1989) and there is evidence that the effect of metaldehyde is cumulative (Kemp & Newell, 1985). It is possible that in eating a smaller quantity of pellet when drilled the amount of active ingredient ingested on the first feed with metaldehyde was not sufficient to interfere with further feeding. In the case of methiocarb toxic effects are not thought to be cumulative and so, despite less drilled pellet being consumed, enough active ingredient may nevertheless have been ingested for there to be no difference between methods of application. Further support for these results comes from a study that shows young *D. reticulatum* have a tendency to develop an aversion to methiocarb, refusing subsequent pellets after the initial feed (Kemp & Newell, 1985). No such effect was observed for metaldehyde which also helps to explain why slugs were seen to consume further drilled pellets of this active ingredient and not methiocarb.

A contact duration threshold of 0.5 minutes was used to distinguish whether slugs were feeding as distinct from simply resting next to pellets. Other studies vary as to how feeding has been determined. For example, Grimm and Schaumberger (2002) considered any time spent at the food constituted feeding whereas Howling (1990) used movement of the pellet as his criterion. It was observed in the recordings made in the experiments presented here that, if contact was made with a pellet, slugs tended either to move off relatively quickly, subsequently behaving normally, or they stayed in contact for a prolonged period of time, ultimately exhibiting signs of poisoning. It seemed, therefore, that a useful distinction could be made here rather than assuming any contact equated to feeding. Since it took some time for slugs to locate drilled pellets, however, involving removal of the loose covering of soil and inevitably movement of the pellet in the process Howling's criterion would have been inappropriate. Frequency plots of the numbers of slugs in contact with pellets for different time intervals showed that there was no clear boundary where transient contact changed to prolonged contact for either drilled or broadcast pellets i.e. there was no bimodal distribution. The majority of slugs that went on to exhibit signs of poisoning were in contact with the pellet for 0.5 minutes or longer and, therefore, this value was chosen as a compromise. Had a lower threshold been applied there would have been a greater chance of misclassifying feeds on drilled pellets yet a higher threshold could have underestimated feeds on broadcast pellets.

It is known that metaldehyde and methiocarb are, themselves, repellent to slugs (Wright & Williams, 1980) and this is why they are incorporated with attractants amongst other substances in bait formulations (Bailey, 2002). The amount of an active ingredient consumed is therefore influenced by its repellency and efficacy is a trade-off between there being enough active ingredient to poison the slug and not so much that it is deterred from consuming a toxic dose. Evidence suggests that metaldehyde is more repellent to slugs than methiocarb (Wedgwood & Bailey, 1988; Bailey *et al.*, 1989) and this is supported by these experiments.

When broadcast, metaldehyde poisoned fewer slugs than methiocarb which would be explained by a higher repellency of the former, resulting in fewer slugs consuming a lethal dose before terminating the meal. The experiments were carried out at 12 ± 2°C. This temperature was chosen to represent conditions typical of a mild spring or cool autumn in the UK. There is strong evidence to suggest, however, that temperature affects the toxicity of metaldehyde (e.g. Cragg & Vincent, 1952; Webley, 1965; Wright & Williams, 1980). Had the experiments been carried out at a higher temperature the mortality due to metaldehyde might have been enhanced. That there was no difference between active ingredients when drilled suggests that although slugs fed on more metaldehyde pellets than methiocarb when applied in this way, overall lethal amounts of each were ingested during the night of activity further underlining the differences in repellency and time course of action.

When comparing the mortality of slugs between methods of application for a given active ingredient it was found that broadcast methiocarb poisoned more slugs than when this molluscicide was drilled. The smaller amount of pellet consumed when drilled (as explained above) may account for this difference. Furthermore, methiocarb pellets were slightly smaller than metaldehyde pellets which could explain why this effect was observed for the former and not the latter along with the cumulative action of metaldehyde.

There was no recovery noted for slugs poisoned by either active ingredient over twenty four hours regardless of application method despite provision of moist humid conditions said to be conducive to recovery (Bourne *et al.*, 1988). This may be because the observation period was relatively short, although other studies where slugs were monitored for forty eight hours after feeding on molluscicide also showed no significant recovery for *D. reticulatum* (Crawford-Sidebotham, 1970; Airey, 1986). All these studies were, however, laboratory based. Results in the field would be expected to show more variation due, for example, to fluctuations in temperature and other factors (Glen & Orsman, 1986) or the availability of shelter under clods of earth (Bailey, 2002). It is, however, difficult to assess mortality of individuals in field conditions due to problems of containing the experimental population, scavenging of moribund and dead individuals by other animals and the rapid decay of poisoned slugs. Although the results on mortality and recovery presented here are informative, this assessment was a secondary aim of these experiments. Ideally more slugs would have been used and the study could be extended to investigate the effects of temperature and different recovery periods more fully.

It has been suggested that soil contamination of broadcast pellets may reduce their efficacy due to slugs being unable to detect them (Hass *et al.*, 1999; Simms *et al.*, 2002). In formally testing this it was found not to be the case. There was no reduction in the performance of soil contaminated pellets relative to 'clean' pellets of either active ingredient for any of the parameters assessed. Indeed, in one case soil contaminated pellets were shown to be more effective; slugs began feeding on soil contaminated metaldehyde pellets in half the time it took them to start feeding on clean pellets. An explanation for this could be that the increase in moisture content of the pellet imparted by the soil covering made it more palatable to the slug. It is not

clear, however, why this was observed only with metaldehyde and not methiocarb. Bait pellets contain, in addition to the active ingredient and other substances to reduce deterioration, a bulking material which is often a cereal base (Bailey, 2002). Perhaps the increased moisture had a differential effect on this base constituent in metaldehyde and methiocarb increasing the attractiveness of the former more than the latter. In summary these results indicate that soil contamination of molluscicide pellets is, of itself, unlikely to impede the feeding, foraging or mortality of *D. reticulatum*.

In conclusion, it was found that:

1. *D. reticulatum* travel shorter distances and feed on broadcast pellets sooner than drilled pellets.
2. *D. reticulatum* are more likely to feed on the first pellet encountered when they are drilled compared to broadcast.
3. Methiocarb poisoned more *D. reticulatum* than metaldehyde when pellets were broadcast, but there was no difference between active ingredients when drilled.
4. No recovery from poisoning was observed during the 24 hour period following pellet exposure for either active ingredient.
5. Soil contamination did not reduce the efficacy of pellets and had no differential effect on poisoning compared to 'clean' pellets.

With respect to the control of *D. reticulatum* the results of these experiments imply that broadcasting is a more effective method of molluscicide pellet application than drilling. Since slugs locate broadcast pellets sooner there may be less opportunity for them to cause damage, providing they consume a toxic dose. Furthermore, because soil contamination of broadcast pellets does not diminish their efficacy, re-application following heavy rainfall is unnecessary.

These experiments have shown how certain aspects of the feeding and foraging of *D. reticulatum* are influenced by the method of pellet application and pellet condition under a defined set of conditions. Activity is affected by temperature (e.g. Crawford-Sidebotham, 1972; Young & Port, 1989) and it would be interesting to see whether changing the temperature at which the experiments were carried out would alter the results. It has been suggested that in the presence of alternative food the susceptibility of *D. reticulatum* to molluscicides is reduced (Airey, 1986). Although it would be impractical to introduce seedlings to the arena due to problems with the clarity of recordings mentioned previously it would be feasible to introduce seeds, e.g. wheat. This would permit an assessment of whether any observed differences in susceptibility to poisoning could be due to changes in the foraging or feeding behaviour.

Acknowledgements

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PAPER 9 - *Objective 1.3 & 1.4*

Characterisation of the Surface Activity of *Deroceras reticulatum* on Coarse and Fine Seedbeds in the Presence and Absence of Metaldehyde Pellets

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Abstract

Temporal and spatial activity of *Deroceras reticulatum* (Müller) on coarse and fine seedbeds was assessed in the presence and absence of metaldehyde pellets. Seedbed conditions did not affect the time activity started. The total distance travelled per night was also comparable between seedbeds and was significantly reduced by metaldehyde pellets. Slugs that fed generally did so on the first pellet encountered and travelled similar distances in comparable times to reach it, regardless of seedbed type. The small number of slugs ignoring pellets before feeding or failing to feed at all was, again, unaffected by seedbed conditions. The only difference in slug activity observed between seedbeds was in the effect they had upon utilisation of available space. Fractal dimension analysis showed that, without metaldehyde pellets, trail complexity was between truly random and constrained on both coarse and fine seedbeds, but trails covered a larger area of the arena on the latter. The presence of pellets significantly reduced trail complexity on both types of seedbed.

Introduction

The extent of cultivation applied to arable land in preparation for sowing is determined by a combination of the soil type and requirements of the crop to be grown. For example, loamy soils may be cultivated to achieve a firm, fine seedbed suitable for oilseed rape, or may be less extensively treated resulting in coarser seedbeds appropriate for wheat. On heavy soils, in contrast, it can be difficult and very costly to produce a reasonable seedbed which limits the choice of crops available to the farmer (Martin & Kelly, 1986). The extent of cultivation, i.e. minimum tillage versus full tillage, has a marked effect on slug numbers and the consequent damage they cause. Cultivations may kill slugs directly by mechanical injury (Hunter, 1967), physically displace individuals (Port, 1989) or bring them to the soil surface where they are exposed to predators and adverse weather conditions (Martin & Kelly, 1986). This latter effect is particularly critical to the survival of eggs as they are highly vulnerable to desiccation (South, 1989a).

The damage caused to seeds and seedlings by slugs remaining after cultivation is related to seedbed conditions. It is generally perceived to be worse in coarse seedbeds with large aggregates because slugs can

easily move between them and reach seed which is not completely covered by soil (Glen *et al.*, 1992; Green *et al.*, 1992). Whilst consolidation may reduce such damage by restricting vertical movement (Gould, 1961), the success of this approach depends on the extent to which soil aggregates can be broken down; in some circumstances, e.g. clay soils, it may, in fact, simply push aggregates deeper into the soil, leaving behind large pockets in which slugs can shelter and move freely, making the situation worse (Stephenson, 1975; Glen *et al.*, 1989; Port, 1989). Molluscicides are, as a result, often routinely applied on fields considered to be at high risk of slug damage (Port & Port, 1986).

Studies of the effect of seedbed conditions on slug activity have largely made indirect assessments by evaluating resultant damage in field conditions (e.g. Gould, 1961; Glen *et al.*, 1992; Green *et al.*, 1992). They therefore take into account both vertical and horizontal movement, but cannot quantify individual behaviour, nor have they considered the response of slugs to molluscicide pellets on different seedbeds.

The experiments described in this paper were designed to complement existing studies by characterising surface activity patterns of individual *Deroceras reticulatum* on coarse and fine seedbeds in the presence and absence of metaldehyde pellets. Although the time-lapse video techniques used do not allow assessment of vertical activity they are a valuable means of making permanent records of horizontal surface movement, which is particularly critical with respect to damage (Hommay *et al.*, 1998). The extent to which slugs used the area available was assessed using fractal dimension analysis. This technique categorises trail paths on a continuum from straight to constrained (i.e. criss-crossing due to some limiting barrier) and assigns them a score such that they may be compared to each other and to a true 'random' path.

Materials and Methods

D. reticulatum (mean weight ± S.E. = 466.13 ± 17.50 mg) used in the experiments were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). There were no significant differences in the weight of slugs between treatments (ANOVA: $F_{3, 156} = 5.07$, *n.s.*). They were maintained at 12°C in ventilated plastic containers (18 x 12 x 7 cm) filled with moist laboratory tissue for up to five days prior to use. This ensured that any slugs in the sample that were unhealthy following collection could be excluded from the experiments. During this time they were provided with Chinese cabbage and carrot *ad libitum*.

The experiments were carried out under controlled conditions at 12 ± 2°C in plastic arenas measuring 57 x 36 x 16 cm, the rims of which were painted with Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) which acts as a barrier to prevent slugs escaping. The arenas were filled with two layers of loamy soil dug from an agricultural plot at Close House Field Station from which any stones, large lumps of organic matter and soil organisms were removed prior to use. The base layer was 8-10 cm deep and its surface was lightly consolidated by packing with the back of a trowel. The top layer consisted of an even covering of fine or coarse aggregates, according to seedbed type. The fine aggregates were obtained by

sieving soil through a 5 mm grade mesh; the coarse aggregates comprised selected large clods of soil as collected from the field with a diameter of approximately 5-6 cm. Fresh soil was used for each replicate and it was watered such that the surface appeared damp at the start of each recording.

Slug activity was recorded using a Panasonic AG-6040 time lapse VHS video recorder. This was set to record 60 times more slowly than normal speed so that a total of 180 hours of activity could be stored on a single cassette. Recordings were played back at normal speed. The camera used was a Sanyo VCB3572 IRP ½" high resolution 570TVL infra-red sensitive camera, fitted with a Computar 8-48mm lens and infra red bandpass filter. The arenas were illuminated at night using a Computar Uniflood LED infra red lamp (serial number CL057787). Light of this wavelength does not appear to disrupt slug activity yet permits recording to take place during darkness (Howling, 1990). Daytime lighting was provided by two 400 Watt halogen lamps suspended 1.55 m above the arena. These were controlled by a timer to match the prevailing sunrise and sunset times, which were updated weekly.

Commercial grade 5% a.i. metaldehyde pellets (De Sangosse, UK) were applied at the manufacturers recommended rate in molluscicide treatments. Slug activity was recorded on each of two seedbed types (coarse and fine) both with and without molluscicide pellets applied, resulting in four experimental treatments (*Table 9.1).*

Table 9.1: Experimental treatments ('+' indicates molluscicide present; '-' indicates molluscicide absent).

Treatment	*Seedbed*	*Molluscicide*
1	Coarse	+
2	Coarse	-
3	Fine	+
4	Fine	-

Pellets were not pre-treated in any way prior to application. They were broadcast equidistant from each other in the arena on the soil surface. Their configuration is shown in *Fig. 9.1*.

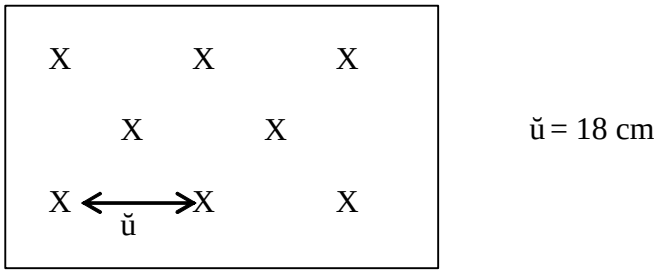


Figure 9.1: Configuration of pellets in the arena (experimental treatments 1 & 3 only).

Eight slugs were used per arena which is equivalent to a field density of 40 slugs per m^2. These were weighed using a Mettler MT5 balance to an accuracy of 0.01 mg. To allow for atypical activity in response to a new environment slugs were placed in the arena for 24 hours prior to the commencement of recording to allow them to acclimatise (Whelan, 1982). During this period they were starved. Following acclimatisation, pellets were applied to the soil surface for molluscicide treatments (*Table 9.1*); for treatments without molluscicide nothing further was added to the arena. Recording then commenced and continued overnight until the following morning. The four treatments, each of which was replicated five times, were recorded in blocks and different slugs were used for each replicate.

Samples of soil aggregates from fine and coarse seedbeds were photographed using a Canon G3 Powershot digital camera, 4 mega pixel resolution. The images were then analysed using a computer programme called Image-J to obtain data on aggregate size. Video recordings were analysed using a computer programme called Inchworm™, as described in *Paper 8*. Pellets were defined as zones and slug trails were resolved into time and distance data for zoned and non-zoned areas. Counts were made of the number of pellets ignored by slugs between the onset of activity and the first pellet feed. To discern feeding from non-feeding contact with a pellet a threshold contact duration criterion of 0.5 minutes was used. If the slug was in contact with the pellet for more than this time it was assumed to be feeding; if it was in contact with the pellet for less than this time, it was categorised as having ignored the pellet. Feeds on the same pellet separated by less than three minutes were treated as a single meal. If consecutive feeds were on different pellets they were counted as separate meals regardless of the intervening time.

The extent to which slug trails filled the arena space in the different treatments was assessed by calculating the fractal dimension according to the procedure of Katz and George (1995). For each slug trail, distance data from the standard Inchworm™ output were resolved into 50 steps ('sticks') of equal length and the fractal dimension, *D*, was calculated according to *Equation 9.1*:

$$D \mid \frac{\log(n)}{\log(n)\, 2\log(d / L)}$$

Equation 9.1

where n is the number of sticks per trail, L is the total trail length and d is Feret's diameter (i.e. greatest distance between two points on the trail). These calculations were carried out using an in-house computer programme (written by Dr. Mark Shirley).

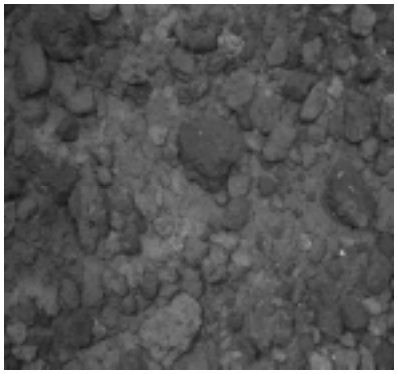
Continuous data were tested for normality and transformed if necessary. Where this resulted in parametric data, independent sample t-tests were used in one-factor analyses; otherwise the non-parametric Kruskal-Wallis test or Mann-Whitney U-test was applied as appropriate. For the single two-factor analysis to compare the total distance travelled by slugs on different seedbeds with and without molluscicide present

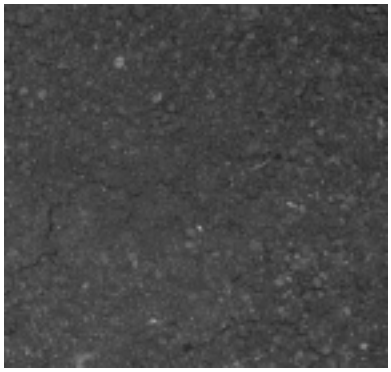
transformation failed to normalise the data and the Scheirer-Ray-Hare test was used. Discrete counts were compared using the chi-square test of association with Yates' correction. All analyses of fractal dimensions were based on log transformed data which conformed to a normal distribution. To assess whether the activity patterns in each treatment differed from that expected if slugs were moving randomly, a t-test was used to compare the mean fractal dimension with that for 501 simulated random walks defined by the same number of sticks (tabulated in Katz & George, 1995). Means were compared between treatments using analysis of variance (ANOVA).

Results

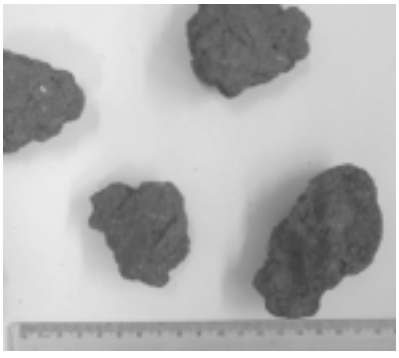
Fig. 9.2 (a)-(d) shows representative coarse and fine seedbeds, along with samples of aggregates from each (with scale bar).

(a) *(b)*





(c) *(d)*



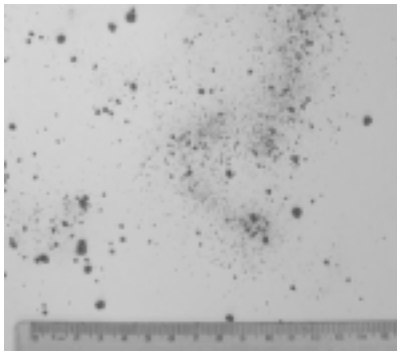


Figure 9.2: Representative seedbeds and soil aggregates (a) coarse seedbed; (b) fine seedbed; (c) coarse aggregates; (d) fine aggregates. Scale bar is in cm.

There were significant differences between the mean Feret's diameter of aggregates from each of the two seedbeds as judged by the Mann-Whitney U-test (*Table 9.2*).

Table 9.2: Results of Mann-Whitney U-tests to compare the Feret's diameter (mm) of soil aggregates from coarse and fine seedbeds.

Seedbed	*Number of samples*	*Number of aggregates measured*	*Mean ± S.E. (mm)*	*Z*	*P-value*
Coarse	5	22	64.84 ± 2.52	-8.139	*< 0.001*
Fine	2	6618	0.68 ± 0.01		

The diameter of aggregates from different samples of a given seedbed did not differ significantly for coarse seedbeds (Kruskal-Wallis: N = 22, df = 4, H = 1.15, *n.s.*) or fine seedbeds (Kruskal-Wallis: N = 6618, df = 1, H = 0.95, *n.s.*).

Analyses of the influence of seedbed on activity onset time are based on treatments where no molluscicide was applied to the arena (i.e. treatments 2 and 4, *Table 9.1*). On each type of seedbed some slugs commenced activity before darkness whereas others did not become active until afterwards. The time interval between darkness and the onset of activity was, therefore, compared separately for these two sets of slugs; in neither case were the differences between coarse and fine seedbeds significant (*Table 9.3*). The proportion of individuals active before and after darkness did not differ between seedbed types either; the majority of slugs began activity after darkness (N = 79, df = 1, G^2_c = 0.195, *n.s.*) (*Fig. 9.3*).

Table 9.3: Results of t-tests to compare the mean time between the onset of activity and darkness for Deroceras reticulatum *on coarse and fine seedbeds.*

Variable	*Seedbed*	*N*	*Mean ± SE (mins)*	*df*	*t*	*P-value*
Time between onset of activity and darkness (slugs active before darkness)	Coarse	12	128.40 ± 21.60	19	-1.35	*n.s.*
	Fine	9	167.65 ± 17.13			
Time between darkness and onset of activity (slugs active after darkness)	Coarse	28	81.49 ± 21.51	56	-1.38	*n.s.*
	Fine	30	100.53 ± 17.42			

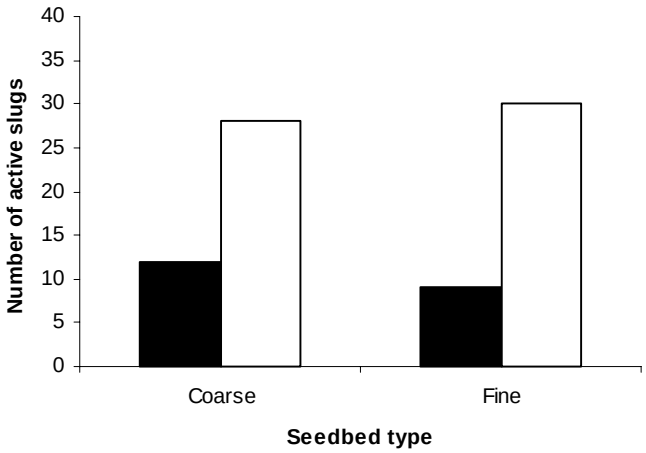


Figure 9.3: Numbers of active Deroceras reticulatum *on coarse and fine seedbeds (black bars = before darkness; white bars = after darkness).*

Results of the influence of seedbed and molluscicide presence on total distance travelled are summarised in *Fig. 9.4* and *Table 9.4*. The presence of pellets significantly reduced the mean distance travelled per night on coarse and fine seedbeds; seedbed type alone had no effect, however, and there was no interaction between seedbed and pellets, i.e. slugs did not respond differently to pellets according to seedbed type.

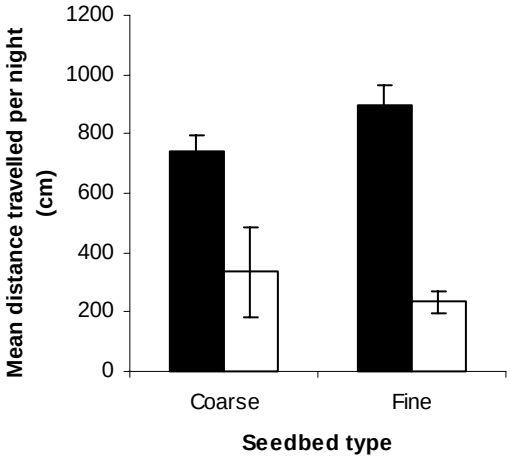


Figure 9.4: Mean distance travelled per night (± S.E.) on coarse and fine seedbeds with and without 5% metaldehyde pellets present (black bars = pellets absent; white bars = pellets present).

Table 9.4: Results of Scheirer-Ray-Hare test to compare the effect of seedbed type and presence of molluscicide on mean total distance travelled per night by Deroceras reticulatum.

Factor/Interaction	*SS/MS $_{total}$*	*df*	*P-value*
Seedbed	0.36	1	*n.s.*
Pellets	19.17	1	*< 0.001*
Interaction	0.04	1	*n.s.*

For both types of seedbed, the majority of slugs fed on at least one pellet during the night. Although the proportion of slugs that failed to feed was higher on coarse than fine seedbeds, this difference was not significant (Chi-square test: N = 80, df = 1, G^2_c = 1.792, *n.s.*) (*Fig. 9.5*).

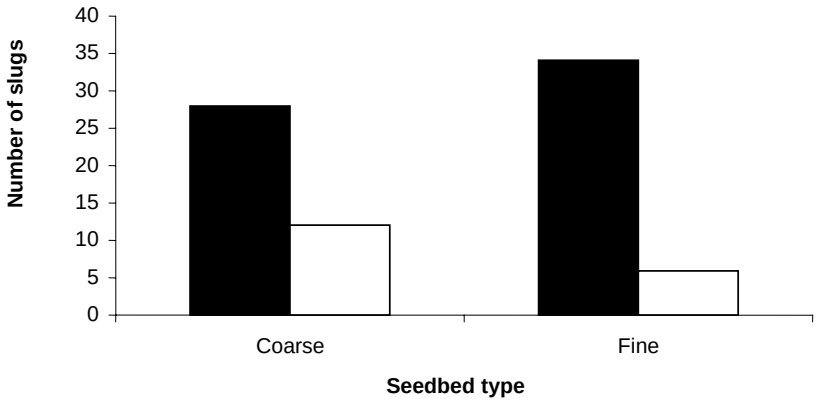


Figure 9.5: Numbers of Deroceras reticulatum *feeding on at least one pellet during the night on coarse or fine seedbeds compared to numbers that did not feed at all (black bars = slugs that fed; white bars = slugs that did not feed).*

For those slugs that fed, most did so on the first pellet they encountered. Where any pellets were ignored, this occurred on coarse seedbeds, but again, differences between seedbed types were not significant (Chi-squared test: N = 62, df = 1, G^2_c = 1.855, *n.s.*) (*Fig. 9.6*).

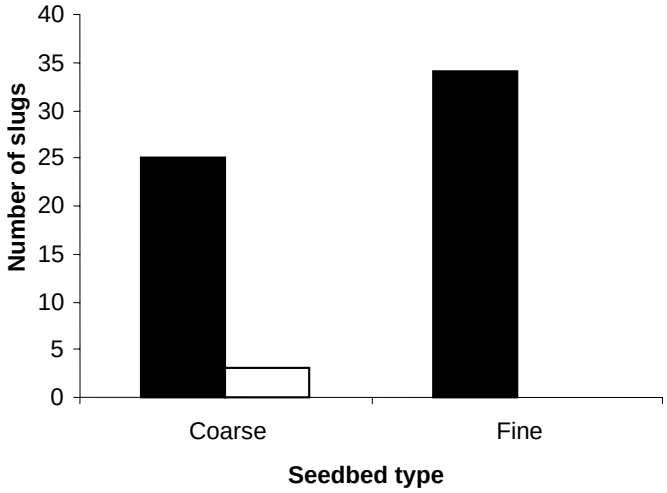


Figure 9.6: Number of pellets ignored before the first feed on coarse and fine seedbeds (black bars = zero pellets ignored; white bars = one or more pellets ignored).

The distance travelled before the first pellet feed did not differ with seedbed type (Independent sample t-test: N = 62, df = 60, t = -0.467, *n.s.*). This was also the case for the time between the onset of activity and the

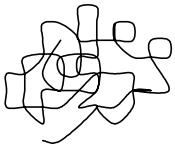
first pellet feed (Independent sample t-test: N = 62, df = 60, t = -0.499, *n.s.*). Data are summarised in *Table 9.5*.

Table 9.5: Mean distance travelled (± S.E.) and time taken between the onset of activity and the first pellet feed.

Seedbed	*Mean values (± S.E.) between onset of activity and first pellet feed*	
	Distance (cm)	*Time (mins)*
Coarse	84.0 ± 20.4	64.3 ± 23.5
Fine	109.2 ± 24.1	76.9 ± 17.6

To investigate the influence of seedbed and molluscicide on trail patterns the fractal dimension, *D* was used; this measures spatial patterns of movement within a defined area. As applied in the current experiments it denotes the extent to which slug trails fill the arena. Theoretically D can take any value from one to infinity, but in practice it usually ranges from one to three. These values may be used to indicate the characteristics of the trail path (*Table 9.6*).

Table 9.6: Classification of Deroceras reticulatum *trail paths using their fractal dimension (after Katz & George, 1995).*

Fractal Dimension, D	1	2	3
Path Characteristic	Straight	Random	Constrained
Example Path			

Since, however, *D* depends upon the number of sticks per trail (*n*) (*Equation 9.1*,) and is a continuum the values in *Table 9.6* are only considered a general guide. To test specifically whether experimental trails differ from a 'true' random path, mean fractal dimension values must be compared to the tabulated mean value for simulated random paths with a similar number of sticks (Katz & George, 1995, Table 1). Since *D* follows a lognormal distribution, standard parametric statistics may be applied and the detailed methodological procedure may be found in this paper.

From *Table 9.6*, it can be seen that the fractal dimension of a random walk approaches two. Thus, inspection of the mean fractal dimensions for each of the experimental treatments shown in *Table 9.7* would suggest that in the absence of metaldehyde pellets, slugs follow trails that can broadly be described as between random and constrained on coarse and fine seedbeds whereas in the presence of metaldehyde pellets they

may be categorised as between straight and random. Comparisons with the mean fractal dimension for simulated random paths of a similar number of sticks (Katz & George, 1995) confirmed these trends (*Table 9.8*). All trails were significantly different from a 'true' random path; when the untransformed mean fractal dimensions for the experimental treatments (*Table 9.7)* are contrasted with that of the comparable simulated random paths (1.81) it can be seen that the differences are in the direction initially indicated.

Table 9.7: Mean fractal dimensions for coarse and fine seedbeds in the presence and absence of metaldehyde pellets.

Treatment			*Mean Fractal*
Seedbed	*Pellets*	*N*	*Dimension (± S.E.)*
Coarse	-	38	2.04 ± 0.07
	+	38	1.40 ± 0.04
Fine	-	35	2.37 ± 0.10
	+	37	1.48 ± 0.06

(-)Metaldehyde absent; (+) Metaldehyde present

Table 9.8: Results of t-tests to compare mean fractal dimensions of Deroceras reticulatum *on coarse and fine seedbeds in the presence and absence of metaldehyde pellets (Table 9.7) with the mean fractal dimension of 501 simulated random paths of a similar number of sticks (Katz & George, 1995, Table 1).*

Treatment			*Log10 (Fractal Dimension)*				
				Standard			
Seedbed	*Pellets*	*N*	*Mean*	*Deviation*	*df*	*t*	*P-value*
Coarse	-	38	0.30	0.09	537	13.88	*< 0.001*
	+	38	0.14	0.06	537	21.84	*< 0.001*
Fine	-	35	0.36	0.11	534	10.46	*< 0.001*
	+	37	0.16	0.09	536	20.38	*< 0.001*

For simulated random paths with 49 sticks: N = 501, mean log_{10} fractal dimension = 0.59, standard deviation log_{10} fractal dimension = 0.13. ('-' indicates metaldehyde absent; '+' indicates metaldehyde present).

When experimental treatments were compared with each other it was seen that on a given seedbed, the presence of metaldehyde significantly reduced the trail complexity, as indicated by a lower fractal dimension (ANOVA: Coarse seedbed: $F_{1, 94} = 96.54$, $P < 0.001$; Fine seedbed: $F_{1, 90} = 90.60$, $P < 0.001$) (*Table 9.7*). In the absence of metaldehyde slug trails on fine seedbeds filled more space than on coarse seedbeds (ANOVA: $F_{1, 71} = 6.49$, $P < 0.05$) (*Table 9.7*) whereas when it was present seedbed type had no effect (ANOVA: $F_{1, 73} = 1.30$, *n.s.*).

Discussion

The primary aim of the experiments presented in this paper was to characterise the surface activity patterns of *D. reticulatum* on coarse and fine seedbeds both with and without molluscicide present. The aspects of surface activity assessed were those related to the time spent in locomotion, distance travelled and the extent to which slugs use the available arena space, i.e. temporal and spatial activity. The effect of seedbed conditions on the initiation of feeding could be evaluated in treatments using molluscicide pellets; it was not, however, intended to examine feeding and post feeding behaviour on molluscicides *per se*.

In order to ensure that the soil aggregates used in this study were representative of fine and coarse arable seedbeds, advice was taken from a local farmer prior to commencing experiments (S. Vernon, *pers. comm.).* There were significant differences in the aggregate size of samples from these two seedbed types, verifying that the conditions being tested were genuinely different from each other, the coarse aggregates being markedly larger than the fine. Samples of aggregates from a given seedbed type did not differ significantly from one another, confirming reproducibility of experimental conditions between replicates.

It has been reported that coarse seedbeds provide more shelter for slugs than fine seedbeds as they can rest in moist pockets between large soil aggregates (Martin & Kelly, 1986). It might be expected, therefore, that slugs on such seedbeds would commence activity later than those on fine seedbeds as the transition between light and darkness, which usually stimulates activity (Rollo, 1991) would be detected less directly. The current experiments did not, however, find any evidence to support this; there was no difference in the interval between darkness and the onset of activity for *D. reticulatum* on coarse and fine seedbeds.

Daily activity cycles are thought to result from an interaction between endogenous rhythms and external conditions (Lewis, 1969b), e.g. photoperiod (Wareing & Bailey, 1985) and temperature (Crawford-Sidebotham, 1972). In the words of Grimm *et al.* (2000), '…whereas endogenous rhythms determine when slugs are ready to become active, it is exogenous conditions which determine whether this readiness is expressed'. As the only variable 'exogenous condition' in the current experiments, it would seem that the extent of shelter is not a strong determinant of activity when conditions are otherwise favourable. It may be that in a harsher environment activity onset behaviour may differ, e.g. field conditions where temperatures fluctuate and air movement is greater.

Overall levels of activity were high; this is not surprising since *D. reticulatum* is a surface dwelling species (Duval, 1970) and individuals were motivated to feed following a short period of starvation. Although activity was essentially nocturnal, a small proportion of slugs commenced activity before artificial sunset. This may partly be due to individuals differing in the time required to acclimatise to the imposed photoperiod, i.e. adapt the endogenous rhythm; whilst 24 hours is thought to be sufficient for most (Whelan, 1982), some may take a little longer and hence emerge from their resting sites when it would previously have become dark. Daytime activity has also been shown to increase with temperature and day-length for *D.*

reticulatum (Wareing & Bailey, 1985). In the current experiment, the day-length matched that of the prevailing conditions (approximately 17L:7D), i.e. long days, which could have induced earlier activity onset. That this was only the case for a small proportion of slugs could be because the temperature, at 12 ± 2°C, was relatively low compared to the long day optimum of 17°C reported by Wareing and Bailey (1985); this may have suppressed the 'willingness' of some slugs to express the endogenously determined readiness to become active. Certainly, in another study of *D. reticulatum* activity where the photoperiod was still relatively long at 10-12 hours and temperatures were higher (15-18°C) the onset of activity more closely corresponded with the start of the dark period (Hommay *et al.*, 1998).

D. reticulatum travelled similar total distances per night on both coarse and fine seedbeds when pellets were absent suggesting that soil aggregate size had little influence on overall mobility. Presumably, provided the soil surface is moist enough for activity, slugs will travel equally willingly around or between large aggregates as directly across a flat surface. In the field situation, greater air movement than in the current experiments may mean that conditions on fine seedbeds are more drying than on coarse seedbeds where slugs can rest in sheltered pockets between large aggregates and conserve moisture (Martin & Kelly, 1986). Contact rehydration is a mechanism used to replenish water loss, for example that due to the production of mucus trails during locomotion, whereby individuals pause foraging, assume a flattened posture on a moist substrate and uptake water through the integument of the foot (Prior, 1989). It could be postulated that if conditions on fine seedbeds are, indeed, more drying than coarse seedbeds, pauses for rehydration during foraging would be more frequent on the former. If so, the distances travelled may be shorter and hence, the results could differ from those of the laboratory based study described in this paper. Confirmation requires further investigation.

The total distances travelled per night in the current experiment when pellets were absent (mean ± S.E. was 7.4 ± 5.7 m on coarse seedbeds and 9.0 ± 6.7 m on fine seedbeds) were longer than those reported in studies where food was provided, albeit that overall food abundance was shown to have little effect, for example Bailey and Wedgwood (1991), where mean ± S.E. = 4.6 ± 3.1 m and Hommay *et al*.,(1998), where mean ± S.E. = 4.0 ± 2.9 m. This is likely simply to reflect the continued foraging due to hunger not being satiated. The presence of metaldehyde pellets significantly decreased the total distance travelled by slugs on both coarse and fine seedbeds. This is not surprising given the action of this active ingredient; as a stomach or contact poison it induces immobilisation through disruption of the nervous system central pattern generator (Mills *et al.*, 1989) which ensues within approximately 45 minutes of feeding (Bailey & Wedgwood, 1991) and, even with moderate doses, may persist for over twenty four hours (Cragg & Vincent, 1952). There was no interaction between seedbed type and pellets, indicating that *D. reticulatum* were responding to pellets in a similar way, regardless of seed bed conditions and that, by implication, aggregate size does not interfere with pellet encounters provided they are accessible.

Most *D. reticulatum* fed at least once during the night regardless of seedbed conditions and of these most did so on the first pellet encountered, supporting the findings of Bailey (1989) and Howlett and Port (2003); this is not surprising since individuals had been starved for 24 hours prior to recordings to ensure they were motivated to forage. Too few slugs fed on a second pellet to allow statistical analysis. As discussed above, within about 45 minutes of ingesting metaldehyde slugs exhibit clear symptoms of poisoning (Bailey & Wedgwood, 1991). Individuals in the current experiment evidently consumed a sufficient quantity of active ingredient to effectively prevent further feeding for the remainder of the night.

The mean distance travelled before the first pellet feed did not differ between seedbeds and was broadly comparable with the distances recorded for *D. reticulatum* feeding on broadcast metaldehyde reported in *Paper 8* (87.6 ± 18.6 cm). Similarly, the time between the onset of activity and the first feed did not differ on coarse and fine seedbeds and, again, agreed with recorded values for broadcast metaldehyde in *Paper 8* (89.0 ± 29.3 min). These findings support the suggestion that olfaction does not play a strong role in attraction to food items over long distances and that slugs tend to encounter them by random (S.E.R. Bailey in Howling, 1991). Were long distance olfaction to be important then it might be that slugs would have located pellets sooner on fine than coarse seedbeds where the larger soil aggregates could act as baffles, dampening olfactory cues, but this was not observed.

Where any pellets were ignored, i.e. slugs were in contact with the pellet for less than 0.5 minutes, this occurred slightly more frequently on coarse than fine seedbeds possibly because only part of some pellets are exposed on coarse seedbeds, the remainder being hidden in a soil crack or under a clod of earth. These differences, however, were not statistically significant. Similarly the numbers of *D. reticulatum* that failed to feed were slightly higher on coarse than fine seedbeds, but again, the difference was not significant. These results suggests that, whilst it may be harder for slugs to locate broadcast pellets in between the large soil aggregates of coarse seedbeds compared to fine seedbeds, this does not reduce overall efficacy. This situation contrasts with that in the study of Glen *et al*. (1992) where damage to seeds was assessed on coarse and fine seedbeds. In this case, the seeds were covered in soil on fine seedbeds, but were more accessible to slugs on coarse seedbeds where they fell in between large aggregates; slugs fed more readily on the latter. Taken together, this suggests sowing seeds into fine seedbeds, followed by a broadcast application of molluscicide affords a high level of protection.

The extent to which slug trails filled the space in the arena was assessed using fractal dimensions analysis. This measure is easy to interpret and was originally developed to categorise cell growth patterns (Katz & George, 1995), but has since been very usefully applied to studies of animal movement (Erlandsson & Kostylev, 1995; Bascompte & Vilà, 1997). It does not give information on angular deviations so cannot be used to infer whether animals are orienting in response to particular stimuli in their environment, e.g. a pellet, unlike other measures of movement such as correlated random walk. It does, however, take account

of the total structure of the trail, even if it spans an entire day, rather than being limited to small sub-trails (Erlandsson & Kostylev, 1995).

In the absence of metaldehyde pellets slug trails on both coarse and fine seedbeds were more constrained than a 'true' random path. i.e. they criss-crossed over each other. This is likely to reflect the arena size. Whilst it has been shown that arena size does not affect total distance travelled (Bailey, 1989; Howling, 1991), it is not surprising that it influences spatial movement patterns; the arenas were small compared to the mean total distance travelled by *D. reticulatum* per night of approximately four to six metres (Bailey, 1989; Hommay *et al.*, 1998) and in the absence of factors that arrest activity it is almost inevitable that an individual will re-cross earlier parts of its trail. It may be speculated that in larger arenas the fractal dimensions of trails may approach that of a truly random path. Trails filled significantly more of the available space on fine than coarse seedbeds in the absence of metaldehyde. This may reflect a tendency of slugs to travel between as opposed to over larger soil aggregates; if so, parts of the soil surface beneath large aggregates in coarse seedbeds are unavailable to the slugs which may explain the lower fractal dimension of trails.

Metaldehyde pellets significantly reduced the space filled by *D. reticulatum* trails. Spatial patterns tended to be straighter than a 'true' random path. It is assumed that further movement is prevented after slugs feed on a pellet and so this 'straighter than random' pattern relates to movement before the first feed. As stated previously, the fractal dimension cannot indicate whether slugs begin to orient differently when they approach stimuli in their environment and therefore it is not possible to say whether these relatively straight movement patterns are due to pellet attraction. As described earlier, however, other studies suggest that this is unlikely to be the case since olfaction is believed to play a role in detection of food only at very close range (Bailey, 1989; Howling, 1991) and in the current experiments *D. reticulatum* travelled reasonably far before first contacting a pellet (~80–100 cm). It may instead be that slugs were moving in an undirected manner, but because pellets were easy to locate, being broadcast and evenly spaced, they encountered them without covering a large area which would result in the lower fractal dimension and this result is, therefore, interpreted with caution. It would be interesting to see whether paths became more random if pellets were more widely spaced. An alternative non-toxic food source may also critically affect spatial movement patterns as slugs would be able to continue foraging after feeding.

In conclusion, it was found that:

1. The activity onset time of *D. reticulatum* did not differ between coarse and fine seedbeds.
2. The total distance travelled per night by *D. reticulatum* was comparable between coarse and fine seedbeds; in both cases this was significantly reduced when metaldehyde pellets were present, but there was no interaction between seedbed and pellets.

3. The initiation of feeding on metaldehyde pellets was not affected by seedbed conditions; on both coarse and fine seedbeds *D. reticulatum* travelled similar distances in comparable times before the first feed and most slugs fed on the first pellet encountered.
4. The number of *D. reticulatum* ignoring metaldehyde pellets or failing to feed altogether did not differ between coarse and fine seedbeds.
5. *D. reticulatum* trails filled significantly more of the available space on fine than coarse seedbeds in the absence of metaldehyde pellets; in both cases trail complexity was between a 'truly random' and constrained path.
6. The fractal dimension was reduced on coarse and fine seedbeds in the presence of metaldehyde pellets; trail complexity was between a straight and random path and there were no significant differences between seedbed types.

In field conditions, seedbeds are less uniform than those in the present experiment, consisting of aggregates of mixed sizes and several species of slugs are present simultaneously. This may result in more complex behaviours than those observed in this study (Glen *et al.*, 1992). It would be difficult to discern different species on monochromatic video recordings, unless they were markedly different sizes, but experiments could be repeated using variable proportions of fine and coarse soil aggregates in the seedbed. The current results would suggest that this would have minimal effect on the timings of activities, but it may alter trail patterns, which would be reflected in the fractal dimension calculations. Recovery from poisoning was not assessed in this study. It is reported that under moist, humid conditions, such as would be found between large soil aggregates on coarse seedbeds, slugs frequently recover from metaldehyde poisoning (Cragg & Vincent, 1952; Crawford-Sidebotham, 1970). It would be useful from the perspective of control to know whether there was significantly greater recovery rates of slugs on coarse seedbeds compared to fine. Fractal dimension analysis has great potential to analyse the effects of a host of factors on spatial movement patterns; an alternative food source has already been suggested. It could, for example, also be used to assess the effects of different shelter materials or novel repellents on surface activity.

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PAPER 10 – *Objective 1.5*

The Effects of Heavy Rainfall on the Visibility and Distribution of Slug Pellets Based on Durum Wheat

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Abstract

Following exposure to simulated heavy rainfall (35 mm in 30 minutes), 53% of slug pellets based on durum wheat remained remain visible on the soil surface on coarse soil compared with 92% on fine soil. Although pellets on fine textured soil remained more visible, they were more likely to be moved from their original positions because of soil flooding. We discuss the implications of these findings in the light of other results from this project and we conclude that, despite the loss of pellet visibility on coarse seedbeds, pellet applications should not be delayed when heavy rain is forecast just after drilling.

Introduction

The aim of this study was to make an initial assessment, under controlled conditions, of the effects of heavy rainfall on the visibility and distribution of slug pellets based on durum wheat, which are known to retain their integrity when exposed to rainfall.

Materials and methods

Twenty-four seed trays (each 330 mm length x 195 mm width x 50 mm deep) were filled with air-dried soil to 40 mm depth. 12 trays were filled with fine soil aggregates (< 6 mm) and 12 trays were filled with coarse aggregates (12-24 mm). Six trays of each type were placed in trays of tap water for 2 h to wet the soil, then left to drain for 15 h, to obtain soil close to field capacity.

The next day, 24 wet-extruded slug pellets base on durum wheat (Metarex Green) were placed in a regular grid, 60 mm apart, on the soil surface of each tray and left for 6 hours, so that the pellets on soil at field capacity could become well hydrated. All trays were then exposed for 30 min to heavy rainfall provided by an indoor rain-tower (70 mm/h). Rainfall was split into two 15 min periods so that the effects of rain on

pellets and soil surface texture could be carefully recorded after 15 min and 30 min. In addition, observations were made at 5 min intervals during rainfall.

Results

The seed trays with fine soil at field capacity became waterlogged after 5 min exposure to rain, while seed trays with coarse air-dried soil showed loss of structure due to the impact of heavy droplets. After 10 min, a cap had formed on the soil surface of all trays with fine aggregates and the trays were flooded. As a result, all pellets were moved from their original positions by the sheet of water covering the surface. Pellets on coarse soil were mostly static, although a few were moved by rain. Soil surface texture was more affected by rainfall in trays with air-dried coarse aggregates than in trays with coarse aggregates at field capacity.

During the second 15 min of exposure, all seed trays with fine soil aggregates continued to be completely waterlogged with pellets floating on water at each assessment. Also, coarse air-dried soil developed a surface cap, which was not present on coarse soil that had been at field capacity initially. Flooding was observed in trays with coarse soil, but less than in trays of fine soil and most pellets remained in their original positions on coarse soil.

Table 10.1: Mean numbers of slug pellets remaining visible (out of 24 pellets initially placed there) on fine or coarse textured soil after 15 and 30 minutes exposure to simulated heavy rainfall.

Soil texture	Period of exposure	
	15 minutes	*30 minutes*
Fine	22.1	22.1
Coarse	18.2	12.8
Least significant difference (P = 0.05)	2.54	

There was evidence that the numbers of pellets remaining visible were influenced by soil texture ($P < 0.001$, 1 df) and period of exposure ($P = 0.002$, 1 df), with a significant interaction between texture and period of exposure ($P = 0.002$, 1 df) (Table 10.1). Fewer pellets remained visible after 15 and 30 min on coarse than on fine soil. There was no change in numbers visible per tray (22) on fine soil after 15 and 30 min, but numbers on coarse soil declined from 18 to 13 over this period. The initial moisture content of the soil did not significantly influence the number of pellets remaining visible after exposure to rainfall.

Discussion and conclusions

Slug pellets were less likely to remain visible following exposure to simulated heavy rainfall on coarse soil than on fine soil. We know that these pellets, based on durum wheat, retain their integrity and do not disintegrate when exposed to such rainfall. Therefore, it is likely that the pellets were simply covered with

soil as a result of rain splash. Even in the extreme conditions of this test, with exposure to 35 mm rainfall in a 30-minute period, just over 50% of the pellets still remained visible. This indicates that pellets based on durum wheat should remain effective against slugs when heavy rainfall follows shortly after application of pellets to the soil. Moreover, studies of the behaviour of slugs (Paper 8, this report) have shown that soil contamination of pellets did not influence the time taken by slugs to their first feed on pellets and had no effect on pellet efficacy. Although pellets on fine textured soil remained more visible than those on coarse soil, they were more likely to be moved from their original positions because of soil flooding.

We conclude that the efficacy of rain-resistant slug pellets based on durum wheat is unlikely to be reduced by exposure to heavy rainfall shortly after application. However, it is sensible to check on pellet distribution after rainfall because pellet distribution could be influenced by rainfall, especially on sloping fields. If conditions warrant a slug pellet application as described in Papers 16 & 17 (this report), pellets should be applied a soon as possible after drilling and rolling (if rolling is possible), even if heavy rain is forecast, because where seedbed conditions are suitable for slugs, they start to feed on seeds almost immediately and a single slug can kill up to about 50 seeds in the first week after drilling (Paper 6, this report). Furthermore, experience in autumn 2002 to 2004 shows that forecasts of heavy rain are often inaccurate in the timing or quantity of rainfall.

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PAPER 11 – *Objective 1.5*

The Performance of Slug Pellets Broadcast on Stubble Compared with Pellets Broadcast after Drilling for Control of Slug Damage to Winter Wheat and Oilseed Rape

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Abstract

In a series of replicated field experiments from 2002 to 2004, we compared the efficacy of molluscicides (slug pellets containing metaldehyde or methiocarb) applied to stubble, up to 42 days before drilling, with the same molluscicides applied just after drilling winter wheat (6 experiments) or oilseed rape (2 experiments). In 2002 and 2003, dry weather in the late summer and early autumn was unfavourable for slugs, slug damage was relatively slight and there were no significant differences in the protection provided at crop establishment by slug pellets applied to stubble compared to pellets applied after drilling and rolling. Under these conditions, it appeared that slug populations were unable to recover from stubble treatments during the period when crops were at risk. In autumn 2004, however, wet weather from early August onward was highly favourable to slugs. Slug pellets applied to stubble reduced slug surface activity in the stubble (as measured by baited refuge traps) but soil sampling showed that the large reservoir population of slugs in the soil was largely unaffected. By the time that winter wheat crops were at risk, slugs had resumed surface activity, following stubble treatments, to levels similar to those on untreated plots and such treatments were much less effective, and provided protection for a shorter time during the critical period of crop establishment, than pellets applied after drilling and rolling. These findings show clearly the role of autumn weather and the importance of applying slug pellets just before damage is expected in a wet autumn.

Introduction

The most reliable control of slug damage to winter wheat is generally obtained by applying bait pellets shortly before sowing, when slugs are active on the soil surface, and then avoiding further tillage for at least three days after treatment (Gratwick, 1992). However, because of the importance of timely sowing in maximising crop establishment, growers are not recommended (Gratwick, 1992) to delay sowing simply to apply bait pellets before drilling. In most situations it is recommended that the best practical option is to broadcast bait pellets on the soil surface at or immediately after drilling (Gratwick, 1992). However, control

of slug damage by treatments applied at this time is sometimes unsatisfactory. The reasons for this are unknown, but heavy rain just after pellet application has been suggested as a possible cause (Hass *et al.*, 1999). We have also shown that, following ploughing, surviving slugs move back to the soil surface 10 cm layer over a period from 10 days to four weeks or more afterward ploughing (Glen *et al.*, 2003; Paper 7, this report). Thus, if sufficient slugs survive ploughing to be a risk to the following crop, there is the potential for a substantial proportion of them to live at a depth where they do not come into contact with slug pellets applied at around the time of drilling. These slugs could then move closer to the surface and damage emerging wheat crops after slug pellets have ceased to be effective. This in turn suggests that there may be benefits from pellet applications to stubble, before ploughing at a time when conditions are suitable for slug activity and for the activity of slug pellets, as a means of controlling slug damage to winter wheat.

Three investigations have been published on the possible value of slug pellet applications to the stubble of the previous crop before cultivation for winter wheat. In two trials in 1970, Gould & Webley (1972) tested broadcast applications of bait pellets to stubble, 19 or 37 days before drilling, in comparison to treatments broadcast one or three days after drilling. There was little slug damage in the dry autumn of that year and, although slug traps on treated plots recorded significantly fewer slugs than traps on untreated plots, there were no significant differences between treatment timings and there were no significant differences in damage, recorded as plant establishment. Rogers-Lewis (1977) summarised the results of a trial (but did not provide details) in which slug pellets were broadcast on stubble seven or four weeks before drilling winter wheat, or slug pellets were mixed with the seeds at drilling in autumn 1974. He described slug damage as severe on the untreated plots. Pellets applied seven weeks pre-drilling showed no significant difference from the untreated plots, whereas there was a significant increase in plant stand on the other treatments. Port *et al.* (1992) reported that their unpublished data from field trials on slug pellet timing showed variable results, with significant differences between treatment timings only where there were large numbers of slugs and ideal weather conditions. They highlighted one trial in which treatment after harvest (pre-ploughing) did not significantly reduce slug activity as recorded by traps in wheat whereas activity was reduced in plots treated with pellets after ploughing or after sowing. These later treatments were also reported to be more effective in reducing damage.

Thus, the results of four trials have been published comparing slug pellet treatments applied to stubble with applications after drilling, but these trials are described only briefly or in summary. There is a need to compare the treatment timings in more detail in relation to patterns of slug population in soil, slug activity and damage under different weather conditions. With this in mind, we established a series of trials in fields going into both winter wheat and oilseed rape from autumn 2002 to 2004.

Materials and methods

Each year from 2002 to 2004, we established one to three experiments in fields to be sown with winter wheat as shown in Table 11.1, to compare the efficacy of molluscicides applied to stubble with molluscicides applied just after drilling. One experiment was also set up each year in 2002 and 2003 in fields to be sown with winter oilseed rape (Table 11.1). It was not possible to establish any experiments in fields to be sown with winter oilseed rape in 2004 because stubble applications were not feasible before drilling oilseed rape due to wet weather delaying wheat harvest to the extent that there was a very short interval between harvesting wheat and drilling oilseed rape. In total, there were six experiments in fields to be sown with wheat and two experiments in fields to be sown with oilseed rape.

Table 11.1: Details of field experiment sites and dates of molluscicide pellet applications in relation to the dates of drilling either winter wheat or oilseed rape.

Year	*Crop drilled*	*Field*	*County*	*Date of pellet application to stubble*	*No. days before drilling*	*Drilling Date*	*Date of pellet application after drill.*
2002	Oilseed rape	Cowley's	Somerset	9 August	33	11 Sept.	12 Sept.
2003	Oilseed rape	Glebe	Somerset	21 August	4	25 Aug.	28 Aug.
2002	Winter wheat	Bulldozer	Somerset	9 August	43	21 Sept.	23 Sept.
2002	Winter wheat	Paddocks 4,5 & 6	Somerset	30 August	41	10 Oct.	15 Oct.
2003	Winter wheat	Billingsley	Shropshire	17 Sept.	13	30 Sept.	7 Oct.
2004	Winter wheat	Billingsley	Shropshire	19 Sept.	10	30 Sept.	1 Oct.
2004	Winter wheat	Glebe	Somerset	20 August	40	29 Sept.	1 Oct.
2004	Winter wheat	Green Triangle	Wiltshire	16 August	52	7 Oct.	12 Oct.

In each experiment, there were five replicates of five treatments: 1) metaldehyde pellets broadcast on stubble after harvest of the previous crop and before cultivation; 2) methiocarb pellets broadcast on stubble after harvest of the previous crop and before cultivation; 3) metaldehyde pellets broadcast as soon as possible after drilling and rolling; 4) methiocarb pellets broadcast as soon as possible after drilling and rolling; and 5) no molluscicide. The treatments were arranged in a randomised Latin Square design, with a different randomisation for each experiment. Plots were each 12 m x 12 m and all sampling was done in the central 4 m x 4 m area of each plot to reduce the likelihood of the results being influenced by slugs moving between plots. The outer buffer width of 4 m in each plot was chosen based on the finding of Glen *et al.* (1991) that there was no evidence of slug movement between untreated and molluscicide-treated plots at distances of 2

m or more into plots over a period of several months. Both fields sown with oilseed rape were cultivated by non-inversion tillage before drilling. All fields sown with winter wheat were ploughed before drilling, except for Green Triangle in 2004, which was scratch cultivated immediately before direct drilling. In Bulldozer Field, Somerset, in 2002, plots 16, 17 18, 21, 22 & 23 were flooded during establishment of the winter wheat crop and for this reason the experiment in this field was analysed for three replicates only (plots 1-15) as a randomised block design.

All molluscicides were applied at recommended rates. The metaldehyde pellets (De Sangosse UK) were Metarex Green (all sites in 2002, 2003 and at Billingsley, 2004) or Metarex Amba (Glebe Field and Green Triangle, 2004), both containing 5% a.i. and applied at 8kg product/ha. The methiocarb pellets (Bayer CropScience) were Draza (4% a.i., applied at 5.5 kg product/ha) in 2002 and Decoy Wetex (2% a.i., applied at 7.5 kg product/ha) in 2003 and 2004. The molluscicide pellets were weighed out separately for each experimental plot and applied by hand, with 50% broadcast whilst walking at a steady pace in one direction and the remaining 50% broadcast whilst walking at right angles to the original direction.

In all trials, slug activity was assessed at intervals, using traps baited with non-toxic food, as described in Paper 14, this report: upturned flowerpot saucers, terracotta coloured, 25 cm diameter, with 20 ml of chicken layers' mash placed in a small heap on the soil in the middle of the area to be covered by the trap. At least one trap was placed in each plot, in the afternoon or early evening on days when the soil surface was visibly moist, and temperature was in the range 5 – 25^{o}C, indicating that soil conditions were suitable for slug activity on the soil surface (Young *et al.* (1991). Traps were examined the following morning.

In two field experiments in 2004 (Glebe Field and Green Triangle), slug populations living in the upper 10 cm of soil were also estimated by soil sampling and flooding, as described by Glen *et al.* (1989). On each occasion, one soil sample 25 cm x 25 cm x 10 cm deep was dug from each plot and the samples were transported to a glasshouse where they were steadily flooded over a period of 8-10 days. The samples were examined daily and all slugs were identified and weighed, fully hydrated.

Slug damage to winter oilseed rape was assessed by counting the numbers of damaged and undamaged plants in five quadrats (each 0.5 m x 0.5 m) placed at random, then lined up with drill rows before counting, per plot. Slug damage to winter wheat was normally assessed by counting the numbers of damaged and undamaged plants in five double lengths of 0.5m drill row, selected at random, per plot. However, in experiments where it was difficult to identify drill rows, plants were counted in quadrats.

The results were analysed using analysis of variance, with transformations of percentages to angles, weights to $\log_{10}$ and numbers to square roots or $\log_{10}$ if necessary to stabilise the variance. Evidence for significant differences was sought by orthogonal contrasts: (1) comparing treated with untreated, (2) testing whether there was any evidence of differences between the stubble or after drilling timing of application, (3) between

molluscicides and (4) interactions between these factors and assessment dates. The repeated measurements on different dates were investigated by separate analyses on different dates and by treating different dates of assessment as if they were subplots within the main plots in an overall analysis.

Results

In all but one trial, there was no evidence of any significant interaction between the different treatment timings (stubble or after drilling) and the type of molluscicide used (metaldehyde or methiocarb). Therefore, it was possible to pool the results for both active ingredients, giving ten replicates for each, so increasing the precision of the comparison of treatment timings. Weather data are presented in Fig. 1, for Yeovilton, the meteorological station closest to the field sites in Somerset and Wiltshire, as an example of the weather pattern each year in the period from August to mid November when treatments and most of the assessments were made.

2002, Cowley's Field, Bulldozer Field and Paddocks 4, 5 & 6

In three trials of slug pellet timing in Somerset in 2002, dry weather considerably constrained the number of dates on which the conditions were suitable for slug trapping. Trapping was possible on only one date in the experiment in Cowley's Field, on 9-10 August. This was before oilseed rape was drilled and therefore before the post-drilling pellet application had been made. Only *Deroceras reticulatum* (Müller) was present (7.4/trap on untreated plots) @@and there was evidence of a significant reduction in slug activity only on the plots where pellets had been applied to the stubble ($P < 0.05$; Fig. 2a).

In the trial in winter wheat in Bulldozer field (Fig. 2b) trapping was done on three dates, including two dates after the stubble treatment but before the post-drilling treatment. The trap catch consisted of *D. reticulatum* (61-77%) and *Deroceras laeve* (Müller) (23-38%). For total numbers of both species, there was no evidence of an overall significant difference between treated and untreated plots, but strong evidence of an interaction between treatment timing and date of sampling ($P < 0.01$). As expected, on 10 August and 12 September (before drilling) there was significantly less slug activity on the plots with stubble applications compared to plots that were to be treated with slug pellets after drilling but had not yet been treated. Untreated plots were intermediate and not significantly different from either extreme. On the final trapping date on 1 November, plots with stubble treatment and plots with treatment after drilling showed significantly less slug activity than untreated plots. Similar differences were significant for *D. reticulatum* ($P <0.05$) but for *D. laeve* there was an overall significant difference between treatment timings ($P < 0.01$) with significantly fewer on the stubble treatment than on the after-drilling treatment and intermediate numbers on the untreated plots (square root mean numbers/trap 0.61, 1.05 and 0.79, respectively; LSD 0.32/0.39 for comparisons of treatment timings and treated and untreated respectively). In the winter wheat trial in Paddocks 4, 5 & 6, trapping on 30-31 October (Fig. 2c) recorded only *D. reticulatum* with a mean of 2.2/trap on untreated plots and significantly fewer (0.4/trap) on plots treated with pellets after drilling ($P = 0.05$). The number of slugs per trap in the stubble-treated plots was not significantly different from the other two treatments.

There were no significant differences in plant numbers at establishment in any of the field experiments in 2002. However, there were significant effects on plant damage by slugs. In the oilseed rape at Cowley's Field (Fig. 3a), slug pellet treatment had a significant influence on the number of damaged plants/m^2 ($P < 0.05$) with a significant interaction between treatment and date of damage assessment ($P < 0.05$), such that there were no significant differences in damage at the time of first examination (20 September, cotyledon stage), but by the second examination (9 October, 3-4 true leaves) both the stubble-treated plots and the plots treated after drilling showed significantly fewer plants with slug damage than the untreated plots, with no significant difference between the treatment timings. The percentages of plants with damage showed the same trends, with similar levels of statistical significant.

In the winter wheat in Bulldozer Field (Fig. 3b), slug pellet treatment significantly influenced the number of damaged plants/m^2 ($P < 0.05$) but there were no significant differences between treatment timings and no significant interaction between treatment and date of damage assessment. (The mean square root number of plants damaged was 4.04/m^2 on treated compared with 5.51/m^2 on untreated plots (LSD = 1.43)). There were no statistically significant differences in the percentages of plants with damage. In the winter wheat in Paddocks 4, 5 & 6 (Fig. 3c), the slug pellet treatments significantly reduced the number of damaged plants/m^2 ($P < 0.01$) but there was no significant interaction between treatment timings, nor between treatment and date of damage assessment. The percentages of plants with damage showed similar, statistically significant differences.

2003, Glebe Field and Billingsley

In the experiment drilled with oilseed rape in Glebe Field in 2003, the weather was dry through August and September (Fig. 1), and it was not possible to put out traps to assess slug activity. Slug damage was slight in this experiment and there was no overall significant difference between treatments. However, the difference between treated and untreated plots changed with date of assessment ($P < 0.05$) with no initial differences on 4 September but by 12 September both treatment timings showing significantly less damage compared to the untreated plots ((mean 1.8% and 4.6%, respectively). By 29 September, 6.8% of plants on untreated plots were damaged and the treated plots were similar to this (Fig. 4).

In the experiment drilled with winter wheat at Billingsley, Shropshire in 2003, trapping was done during or around suitable rainfall. Traps were examined after 20 mm rain on 23 September, on five dates post-drilling between 30 October and 26 November, then on a further two dates in February (Table 11.2). *Deroceras reticulatum* comprised 75-93% of trap catches. Numbers of *Arion* spp. were initially low but increased (especially *A. distinctus*) between 11 November and 13 February. Slug activity increased steadily as the soil moistened during the autumn with a peak of activity on 17 November. There was also a high incidence of slug activity on 3 and 13 February, during a period of mild, moist weather following frosty conditions in January 2004.

Table 11.2. Mean square root number of slugs per plot (per 6 refuge traps) for pre-drilling assessment on 23 September and for 7 post-drilling occasions between 30 October 2003 and 13 February 2004, Billingsley.

Treatment	*Mean square root number of slugs per plot (per 6 traps)*							
	23 Sep	*30 Oct*	*3 Nov*	*11 Nov*	*17 Nov*	*26 Nov*	*3 Feb*	*13 Feb*
Pellets on stubble	3.23	0.20	0.67	2.52	2.79	1.68	4.92	3.22
Pellets after drilling	6.26	0.54	1.02	2.21	2.70	1.56	4.74	3.34
Untreated	6.81	1.32	2.29	3.56	3.87	2.15	5.71	3.79
LSD (*P*=0.05)	0.68 (between treated & untreated) 0.55 (between treatment timings)							

Pellets after drilling had not been applied at the time of assessment on 23.09.2003.

For total numbers of slugs, there was an overall significant difference between treated and untreated plots, with strong evidence of an interaction between treatment timing and date of sampling ($P < 0.001$). Stubble pellet treatments provided significant ($P<0.001$) reductions in slug numbers compared with the untreated mean of 47.2 slugs per 6 traps (7.9/trap) on 23 September. On this assessment date, the post-drilling treatments had not been applied and slug activity on these treatments was similar to the untreated mean. Significant reductions in mean slug numbers were obtained for the stubble and post-drilling treatments on 30 October, 3, 11 and 17 November (Table 11.2). There were no significant differences on 26 November, when slug activity had declined with the onset of colder weather with overnight frosts. Winter trapping on 3 February 2004 showed a high incidence of slug activity, with small but significant reductions in mean number of slugs trapped on both stubble-applied and post-drilling treatments. No significant differences were obtained for the final period of trapping on 13 February.

Table 11.3. Mean percentage of wheat plants damaged by slugs on 17 November 2003 and on 13 February 2004, Billingsley.

Treatment	*Mean % plants damaged*	
	17 Nov. 2003	*13 Feb. 2004*
Pellets on stubble	14.3	12.3
Pellets after drilling	14.8	13.7
E. untreated	35.6	17.6
LSD (p=0.05)	5.1 between treated & untreated	
	4.2 between treatment timings	

Damage was assessed on 17 November 2003 at GS 12 and 13 February 2004 at GS 13/21, to coincide with periods of increased slug activity and the onset of visible damage. There were no significant differences in

plant numbers. The mean percentage of plants damaged by slugs (Table 11.3) was influenced by a significant interaction between treatment and date ($P<0.001$) with damage significantly reduced by both stubble and post-drilling applications of slug pellets on 17 November compared with the untreated mean. A lower incidence of slug damage was recorded in mid February by when many of the leaves that had previously shown evidence of damage had been lost, with little evidence of fresh damage. The mean percentages of plants damaged by slugs were not significantly different between treatments by mid February. There were no significant differences between treatment timings on both dates of assessment.

2004, winter wheat Billingsley

For winter wheat at Billingsley, Shropshire, in autumn 2004, four traps were set out and examined on each plot on six dates (Table 11.4). The trap catch was 65-72% *D. reticulatum*, 27-34% *Arion* spp. and about 1% *Tandonia budapestensis*. For total numbers of slugs, there was an overall significant difference between treated and untreated plots, with strong evidence of an interaction between treatment timing and date of sampling ($P < 0.001$). On 23 September (three days after stubble treatment), stubble applications gave significant (87%) reductions in mean number of slugs per plot compared with the untreated mean 31.4 slugs per plot, i.e. 7.9 per trap. These reductions were significant ($p<0.001$) for both *D. reticulatum* and *Arion* spp. compared with untreated means of 21.4 and 10.0 per four traps respectively. On 8 October (8 days after drilling and before crop emergence), the effects of the stubble-applied treatments were declining, with 40% reduction in activity compared with the untreated mean of 19.6 slugs per plot. For the treatments applied after drilling, the reduction (76%) resulted in significantly fewer slugs than for the stubble treatments, with significant reductions ($P<0.01$) for both *D. reticulatum* and *Arion* spp. compared with untreated means of 14.2 and 5.4 per four traps, respectively. On 15 October (15 days after drilling) numbers were lower, but the effects of the stubble-applied treatments were similar to 8 October, with 49% reductions compared to the untreated mean 12.2 slugs per plot (4 traps). For the treatments applied after drilling, there was a 75% reduction in activity, again resulting in significantly fewer slugs than the stubble treatments. This reduction was significant ($p<0.001$) for *D. reticulatum* but not for *Arion* spp. ($p=0.630$) compared with untreated means of 10.4 and 1.8 per four traps respectively.

Table 11.4. Mean square root number of slugs per plot for traps examined between 23 September and 17 November 2004, Billingsley.

Treatment	Mean number of slugs per plot (4 traps)					
	23.09	*08.10*	*15.10*	*03.11*	*09.11*	*17.11*
Pellets on stubble	1.84	3.40	2.47	2.50	4.37	3.51
Pellets after drilling	5.13	2.09	1.63	2.48	3.81	3.11
Untreated	5.53	4.39	3.43	2.32	4.56	3.46
LSD ($P = 0.05$)	0.74 between treated and untreated, 0.60 between treatment timings					

Pellets after drilling had not been applied at the time of assessment on 23.09.2004.

There was little activity in the periods ending on 3 November and 17 November, with no significant differences between treatments. However, during mild, damp conditions in the period ending 9 November, slug activity reached the highest incidence since drilling. The effects of the stubble-applied treatments continued to decline with slug numbers on these treatments were similar to and not significantly different from the untreated mean 21.4 slugs per plot (5.4 per trap). Only the post-drilling treatment provided a significant reduction in slug numbers (Table 11.4).

Table 11.5. Angular percentages of wheat plants damaged by slugs on 3 November 2004, Billingsley.

Treatment	*Mean angular % plants damaged by slugs*
Pellets on stubble	73.0
Pellets after drilling	61.0
Untreated	81.1
LSD (P = 0.05)	7.2 between treated & untreated 5.9 between treatment timings

The plant population was not significantly affected by any treatment on two dates of assessment (3 November and 12 December 2004). A high incidence of slug damage was visible on 3 November during the early stages of crop emergence (Table 11.5). Treatment after drilling was the most effective timing (P < 0.001), providing a significantly greater reduction than pellets applied to stubble. A visually obvious crop vigour effect was recorded at GS 21 on 5 December. Mean crop vigour score for the post-drilling application of methiocarb was significantly higher than the untreated mean vigour score 2.8.

2004, winter wheat, Glebe Field

In Glebe Field, slug populations in the soil (83% *D. reticulatum*, 7.4% each of *A. distinctus* and *Milax gagates* (Draparnaud)) were sampled on four dates from early August to mid October 2004 (Fig. 11.5), with the first two dates (2 and 20 August) being before any treatments were applied. Slug numbers and biomass/m^2 changed significantly over time (P < 0.001), peaking in mid September then falling, as expected, after cultivation. There were no significant effects of the slug pellet treatments on biomass of slugs in the soil. However numbers showed a significant interaction between treatment timing and date (P = 0.01), with significantly fewer slugs on stubble-treated plots in mid September (26 days after treatment) than the other plots, which were untreated at that time (60% reduction in numbers). No other differences were significant, including the final sampling in mid October, 18 days after the post-drilling treatment.

Traps were put out on this experiment at intervals from early August to late November 2004 (Fig. 11.6). Trap catches were predominantly *D. reticulatum* (76-100%) with smaller numbers of *A. distinctus* (0-3%) and *M. gagates* (0-22%). There was a significant interaction between treatment timing and date of assessment ($P < 0.001$). On the first date (4 August), before any treatments had been applied, there were no significant differences, with about five slugs/trap on all treatments. Just after stubble treatment (21 August) numbers per trap were low on all treatments, with no significant differences. There was a substantial increase in activity in mid September, and the effect of the stubble treatment showed as significantly lower numbers per trap compared with the other treatments. By 7 October, shortly after drilling, there was significantly less slug activity on the plots treated after drilling, but not on the stubble-treated plots, compared to the untreated plots. The same situation was found on 18 October, by which time activity on the untreated and stubble-treated plots had increased significantly. By November 2004, there were no significant differences in slug activity between the treatments. Both *D. reticulatum* and *M. gagates* showed similar trends to the overall trap catches ($P < 0.01$). *Arion distinctus*, which was presented in lowest numbers showed significantly fewer slugs on treated compared with untreated plots ($P < 0.05$).

Slug damage to wheat plants in Glebe Field in October showed differences between treatment timings ($P < 0.001$) that changed with dates ($P < 0.05$) (Fig. 11.7); on 18 October, there was significantly less damage on the plots treated after drilling compared to both the stubble-treated and the untreated plots. Stubble-treated plots had an intermediate level of damage, significantly less damage than the untreated plots. By November, slug damage had increased on all treatments. Only the plots treated after drilling showed significantly less damage than the untreated plots.

2004, winter wheat Green Triangle

In Green Triangle, slug populations in soil were sampled on three dates from mid August to early November 2004 (Fig. 11.8). Three slug species were common in soil samples: *D. reticulatum*, *A. distinctus* and *M. gagates*, representing 17%, 25% and 56%, respectively of all slugs recorded. As in Glebe Field, numbers and biomass peaked in mid September. However the changes in numbers and biomass with time were less marked than for Glebe Field. There was no evidence, for all species combined or each of the three main species, of any significant differences between the treatment timings in either numbers or biomass of slugs.

Traps were put out on each occasion when soil samples were taken (Fig. 11.9), with catches consisting of *D. reticulatum* (49-70%), *A. distinctus* (5-19%) and *M. gagates* (21-41%). Traps were put out just after stubble treatment on 16 August and examined the next morning. There was relatively little slug activity, but total numbers/trap were significantly reduced on the stubble-treated plots compared to the untreated plots. By mid September, there was a substantial increase in activity to a mean of about 10 slugs per trap, but no significant differences between the treatments. Activity declined by early November, and again there were no significant differences between treatments. The numbers of *D. reticulatum* in traps were significantly influenced by treatment timing ($P < 0.05$), with significantly fewer on stubble-treated plots on 17 August

than on plots to be treated after drilling. However, there were no significant differences on later dates. There were no significant differences for the other individual species.

The numbers of wheat plants showed significant differences between treated and untreated plots and between treatment timings ($P = 0.01$), but no interactions between treatment timing and date of assessment. There were significantly fewer plants on the untreated plots compared with plots treated after drilling (Fig. 10). Stubble-treated plots were intermediate, with significantly fewer plants than the after-drilling treatment and not significantly more than the untreated. The percentage of plants damaged by slugs showed a significant interaction between treatment timing and date of assessment ($P < 0.001$). On 1 November, the pattern of plant damage was similar to plant numbers, with significantly less damage on plots treated after drilling compared to both stubble-treated and the untreated plots. Stubble-treated plots had a level of damage that was intermediate and not significantly different from the untreated. By 1 December, slug damage had reached almost 100% on all treatments, with no significant differences between treatments.

Discussion

Dry weather from August into early autumn in 2002 and 2003 (Fig. 1) was unfavourable for slug activity, reproduction and growth. Under these conditions, it appeared that slug populations were unable to recover from stubble treatments during the period when autumn-sown crops of wheat or oilseed rape were at risk, so that there were no significant differences in the protection provided at crop establishment by slug pellets applied to stubble compared to pellets applied after drilling and rolling. There was only evidence of a recovery in slug activity in one field site, Paddocks 4, 5 and 6 in 2002, (Fig. 1c), 60 days after stubble treatment. However, even on this field site, there was no evidence of greater slug damage on the stubble treatment compared to the treatment after drilling, when damage was assessed up to 75 days after stubble treatment (36 days after drilling).

In contrast, wet weather from early August onward in 2004 (Fig. 1) was highly favourable to slugs. Under these conditions, slug pellets applied to stubble reduced slug surface activity in the stubble, as recorded by baited refuge traps, but soil sampling showed that the large reservoir population in the soil was largely unaffected by slug pellet treatment. Glen & Wiltshire (1986), Wiltshire & Glen (1989) and Glen *et al*. (1991) studied the effects of molluscicide applications to wheat crops on the slug population resident in the upper 10 cm of soil. Such applications resulted in only about a 50% reduction in the slug population compared to untreated plots, with a slightly greater reduction in biomass of *ca.* 60%. Even a 50% reduction leaves a substantial residual population. By the time that winter wheat crops were at risk in our experiments in 2004, slugs had resumed surface activity following stubble treatments and had reached levels similar to those on untreated plots. Thus, stubble treatments were much less effective, and provided protection for a shorter time during the critical period of crop establishment, than pellets applied after drilling and rolling.

The intervals between stubble treatment and drilling were relatively short in 2003 (4 and 13 days). However, these intervals were rather longer in 2002 (33-43 days) and 2004 (10-52 days). Despite the wide range of intervals in 2004, the results of comparisons of treatment timings were similar at all three field sites in that year. We believe that our findings show clearly the role of autumn weather and the importance of applying slug pellets just before damage is expected in a wet autumn. In essence, stubble treatments were most effective and gave up 6 weeks protection of crops at establishment in 2002 and 2003 when damage was relatively slight and when it could be argued that molluscicide treatments were not needed at most field sites. However, the stubble treatments were relatively ineffective in a wet autumn when slug attack was severe and long-lasting protection was important. Application of pellets just after drilling also has the considerable advantage that it allows a proper assessment of damage risk to be made, based on soil and weather conditions at drilling (this report, Paper 16), whereas this is not possible for pellets applications to stubble, which, thus, may be made unnecessarily. It is best practice to assess soil & weather conditions at and after drilling, then treat if necessary.

Previous authors (Gould & Webley, 1972; Rogers-Lewis, 1977; Port *et al.*, 1992) who have compared stubble treatments with applications at around the time of drilling did not provide full weather details, but the brief information in their papers supports our conclusions. Gould & Webley (1972) reported that there was little slug damage in the dry autumn of the year of their trials and that slug traps on all treated plots recorded significantly fewer slugs than traps on untreated plots, with no significant differences between treatment timings and no significant differences in damage. Rogers-Lewis (1977) described slug damage as severe on untreated plots in a trial where pellets applied seven weeks pre-drilling showed no significant difference from the untreated plots, in contrast to a significant increase in plant stand on other treatments. Port *et al.* (1992) stated that there were significant differences between treatment timings only where there were large numbers of slugs and ideal weather conditions. They showed results of one trial where pellets applied to stubble did not significantly reduce slug activity in wheat, in contrast to plots treated with pellets after ploughing or after sowing.

The one field site drilled with winter wheat that was not ploughed (Green Triangle, 2004) showed the most severe slug damage of all fields, with significant loss of plant stand on untreated plots and with almost 100% of wheat plants damaged by slugs. This is typical for direct-drilled crops (Glen & Moens, 2002; Glen & Symondson, 2003). However, it is important to note that the responses of the slugs to the different treatment timings in this experiment were similar to those in the two experiments where the soil was ploughed before crop establishment in 2004. Thus, it is unlikely that the effect of ploughing in burying slugs (Paper 7, this report) was having any significant effect on the pattern of damage over time in these experiments.

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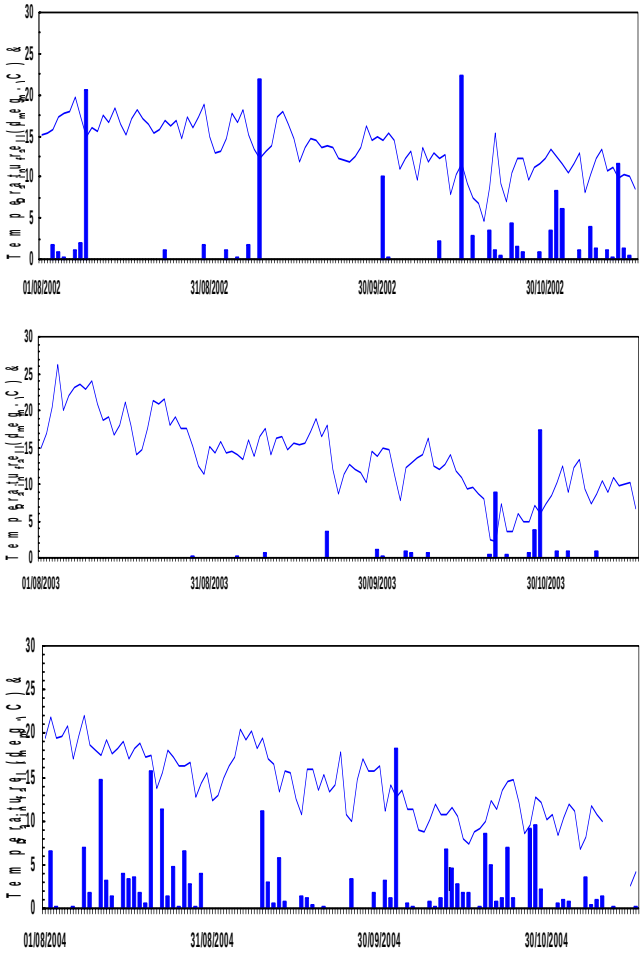

Figure 11.1. Daily mean temperature and rainfall recorded at Yeovilton meteorological station, from August to mid November, 2002-2004.

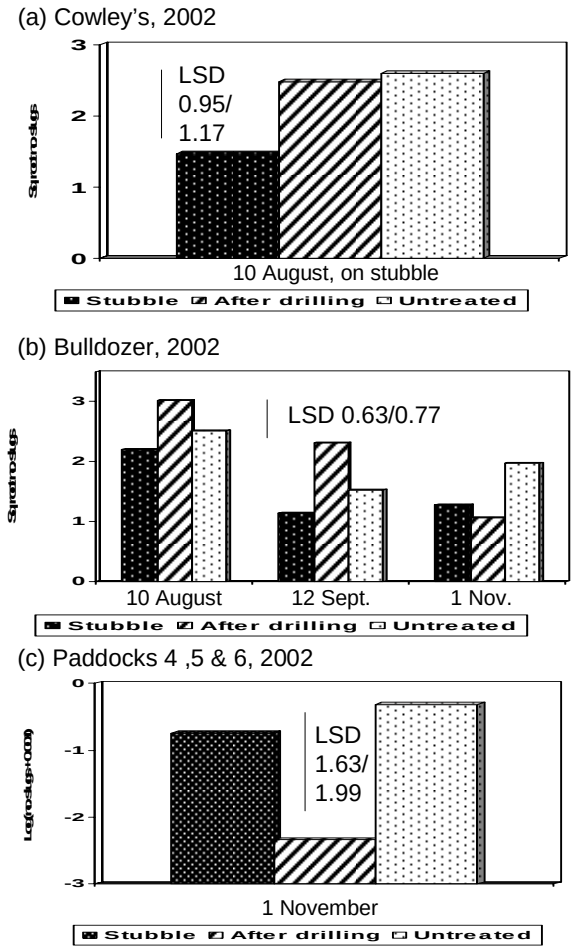

Figure 11.2. Mean numbers of slug recorded per trap baited with chicken layers' mash on plots with slug pellets applied to stubble, or pellets applied after drilling or untreated, at three field sites in 2002 . The vertical bar shows the approximate value of the LSD, followed by the numerical values for comparisons between treatment timings and between treated and untreated.

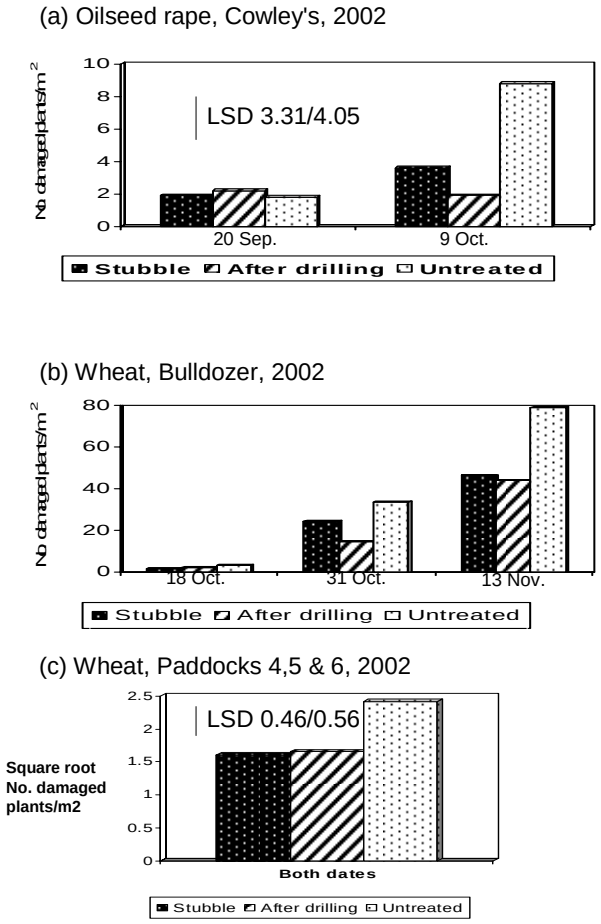

Figure 11.3. Numbers of plants damaged by slugs at establishment on plots with slug pellets applied to stubble or pellets applied after drilling or untreated, at three field sites in 2002. The vertical bar shows the approximate value of the LSD, followed by the numerical values for comparisons between treatment timings and between treated and untreated. In Paddocks 4, 5 & 6 the dates of damage assessment were 1 and 15 November.

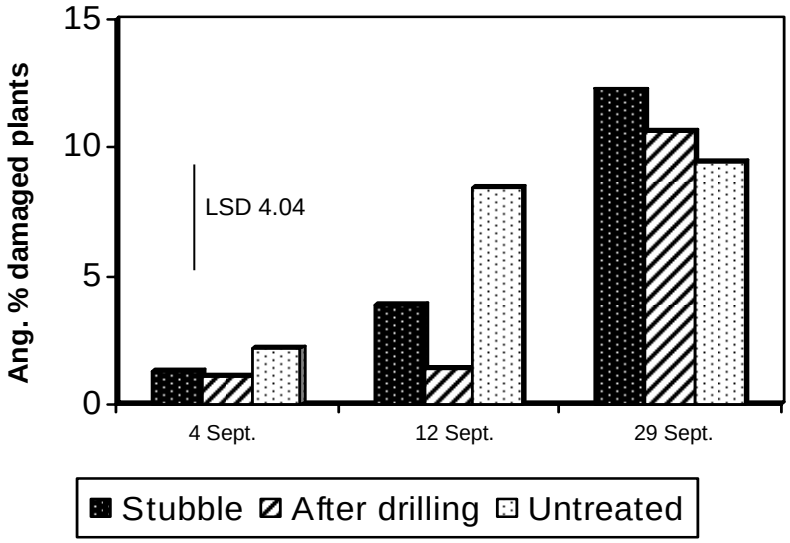

Figure 11.4. Percentage of oilseed rape plants damaged by slugs at establishment on plots with slug pellets applied to stubble, or pellets applied after drilling or untreated, at Glebe Field in 2003. The vertical bar shows the approximate value of the LSD with its numerical value for comparison between treated and untreated.

Slug population development in soil samples, Glebe Field, Higher Clapton Farm, 2004 (mainly *D. reticulatum*)

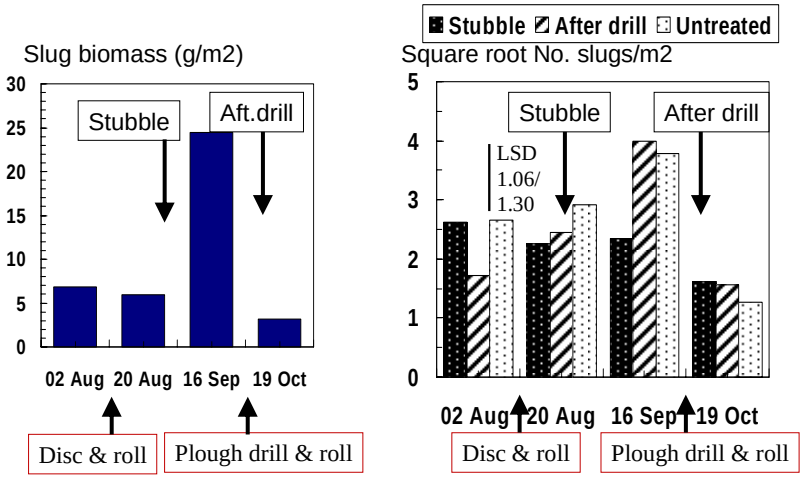

Figure 11.5. Biomass and numbers/m^2 of slugs in soil, at Glebe Field in 2004.Where there were no significant differences, the mean values for each date are shown. . The vertical bar shows the approximate value of the LSD, followed by the numerical values for comparisons between treatment timings and between treated and untreated.

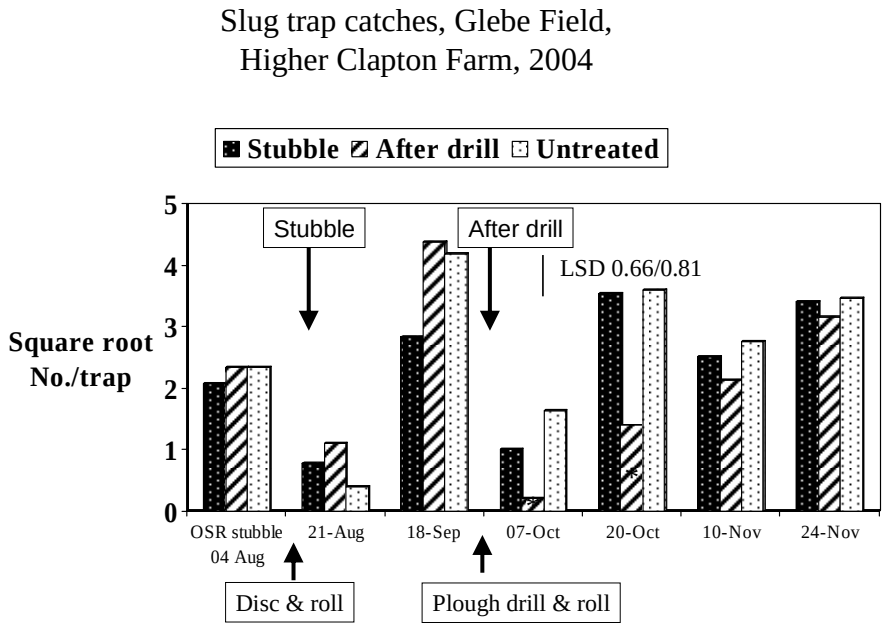

Figure 11.6. Mean numbers of slug recorded per trap baited with chicken layers' mash on plots with slug pellets applied to stubble, or pellets applied after drilling or untreated, Glebe Field, 2004 . The vertical bar shows the approximate value of the LSD, followed by the numerical values for comparisons between treatment timings and between treated and untreated.

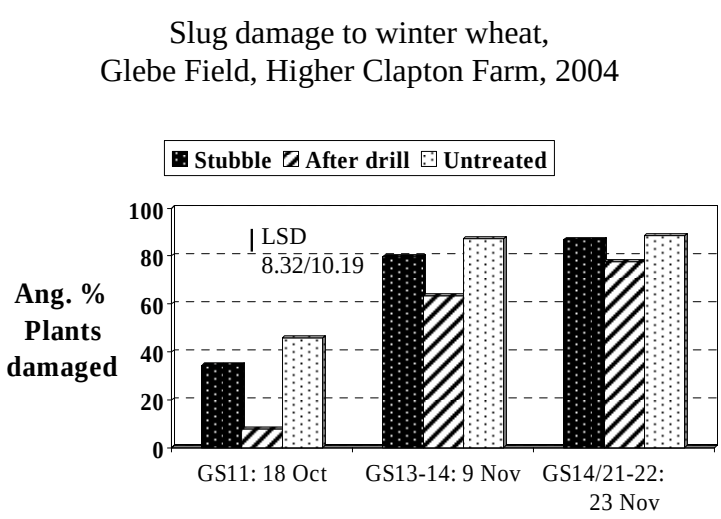

Figure 11.7. Percentage of winter wheat plants damaged by slugs at establishment on plots with slug pellets applied to stubble, or pellets applied after drilling or untreated, Glebe Field, 2004. The vertical bar shows the approximate value of the LSD, followed by the numerical values for comparisons between treatment timings and between treated and untreated.

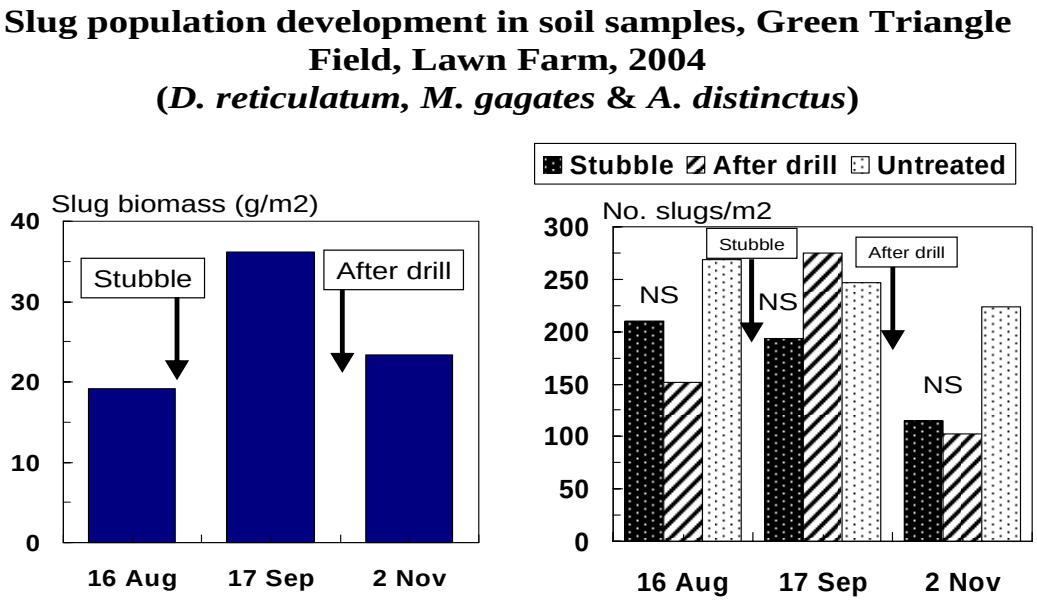

Figure 11.8. Biomass and numbers/m^2 of slugs in soil. Green Triangle, 2004.Where there were no significant differences, the mean values for each date are shown.

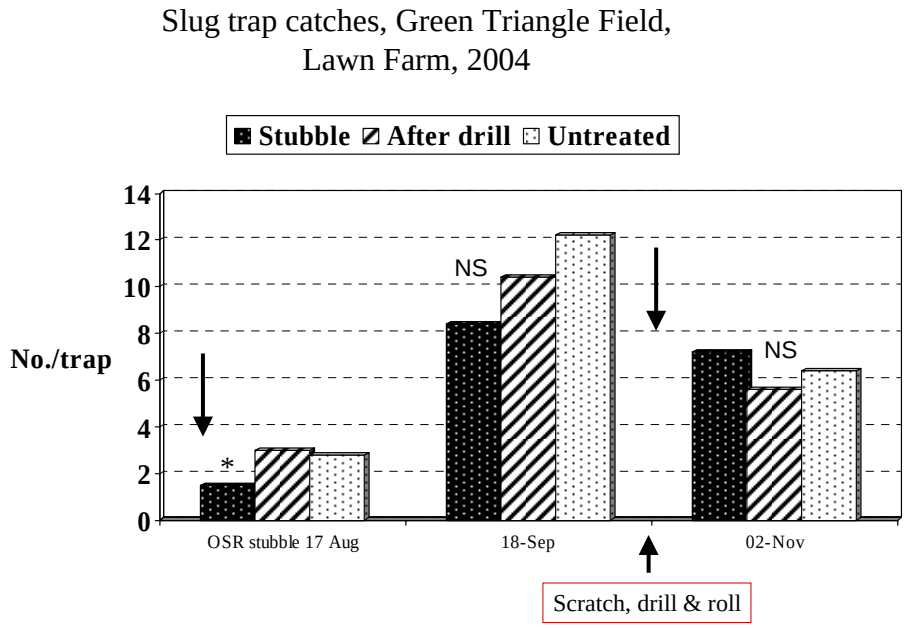

Figure 11.9. Mean numbers of slug recorded per trap baited with chicken layers' mash on plots with slug pellets applied to stubble, or pellets applied after drilling or untreated, Green Triangle, 2004 .

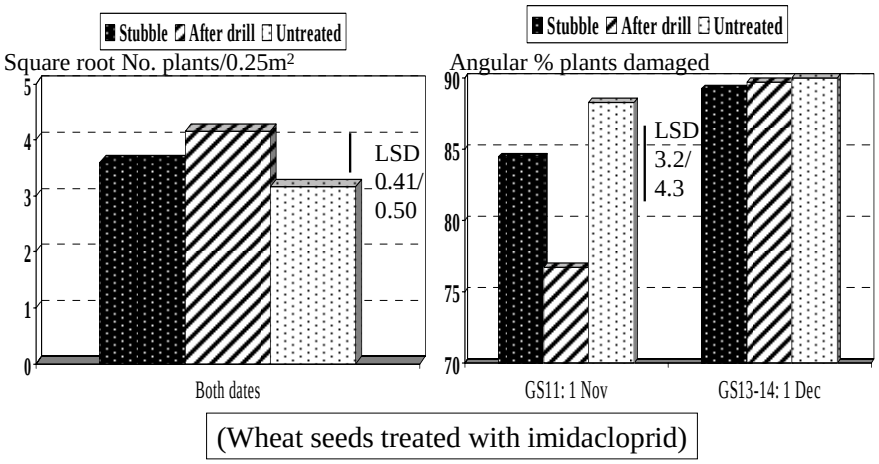

Figure 11.10. Numbers of winter wheat plants/m of drill row and percentage of plants damaged by slugs at establishment on plots with slug pellets applied to stubble, or pellets applied after drilling or untreated, Green Triangle, 2004.

PAPER 12 - Objective 1.6

A Long-Term Field Experiment on Slug Activity and Slug Damage in Relation to Seedbed Preparation and Slug Pellet Application

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Abstract

A long-term replicated factorial field experiment was established on clay soil at ADAS, Boxworth, Cambridgeshire in 2001, to compare slug activity and slug damage on consolidated seedbeds and loose seedbeds, with slug pellets applied pre-emergence, post emergence or both pre- and post-emergence. Winter wheat was sown in autumn 2001 and 2002, followed by oilseed rape in autumn 2003 and winter wheat in autumn 2004. Imidacloprid seed treatment (Sibutol Secur, Bayer CropScience) was also included as a factor in autumn 2001. Stubble-applied treatments of slug pellets were compared in autumn 2002, 2003 and 2004. Minimum tillage was compared with ploughing in autumns 2003 and 2004.

Year 1: For winter wheat, drilled on 19 October 2001, slug pellets applied pre-emergence or both pre- and post-emergence significantly reduced the percentage of plants damaged by slugs in December 2001. The reduction in damage after the post-emergence treatment was not significant compared with the untreated mean (40%). Seedbed consolidation had no significant effect in December, but slug activity, predominantly *Deroceras reticulatum,* continued during the late-winter period, peaking in January. On three dates in November and December, slug activity was significantly higher on the consolidated compared with the loose seedbed but in January and March, no differences were recorded. On 7 March, the mean percentage of plants damaged by slugs was significantly lower on the consolidated seedbed compared with the loose seedbed (means of 5.9% and 15.5%, respectively). The effects of imidacloprid seed treatment were not significant compared with the non-insecticide treated seed (Sibutol).

Year 2: In autumn 2002, significant reductions in slug activity were recorded during November for the pre-emergence treatment applied after drilling winter wheat on 24 October. However, there was considerably less slug damage than in 2001 and no significant reductions in slug

damage were obtained from any treatments in autumn/early winter 2002. During November, significant reductions in slug activity were obtained from stubble-applied pellets. On two dates in November 2002, there was significantly greater slug surface activity on consolidated than on loose seedbeds but, by March 2003, this difference was reversed and slug damage was significantly lower on consolidated than on loose seedbeds. Thus, as in the previous year there was initially greater surface activity on consolidated seedbeds, which probably reflected the difficulty that slugs had in moving through the soil and was not a predictor of slug damage. This supports the view that, after crop emergence, crop damage is a better indicator of the need for control measures than trap catches. In both 2001-02 and 2002-03, the reduction in damage from seedbed consolidation extended beyond the period of protection obtained from molluscicide, demonstrating the value of consolidation for controlling slug damage. The results for 2001-02 also clearly demonstrate the benefits of integrated control, with initial protection provided by slug pellets and later protection from consolidation.

Year 3: Winter oilseed rape was drilled on 3 September 2003 but establishment was initially poor in dry conditions. Slug activity increased during late October and was significantly lower on ploughed compared with direct-drilled plots. Slug activity was significantly reduced on 5 November by pre-emergence, post-emergence and pre + post-emergence pellet applications compared with the untreated (0.8 slugs/three traps). Significant reductions were also obtained on 6 January from pellets applied post-emergence or pre + post-emergence compared with the untreated mean (0.7 slugs/three traps). Although the incidence of damage was slight, the percentage of oilseed rape plants damaged was significantly lower where plots were established by ploughing compared with direct drilling. These results demonstrated a lower incidence of slug activity after ploughing and emphasised a possible benefit from reduction of slug damage to oilseed rape.

Year 4: Winter wheat drilling was delayed by wet weather until 13 November 2004. Pre-drilling slug activity was higher than in autumn 2001-2003 and was significantly lower on plots that had been established in the previous year by ploughing compared with direct drilling. A stubble application of slug pellets on 21 October provided significant reductions in slug activity compared with untreated plots on 25 October and 1 November. Post drilling slug activity declined and no significant differences were recorded for post-drilling applications of slug pellets or for mean plant populations or percentages of wheat plants damaged by slugs.

Introduction

The objectives of this 4-year study were to compare the effects of integrated measures for slug control, based on different combinations of cultural and chemical treatments:

- Comparison of tight (consolidated) seedbeds with loose seedbeds (power harrowed) (Years 1 & 2)
- Comparison of reduced, non-inversion tillage with ploughing and discing (Years 3 & 4)
- Seed treatment comparison, between seed not treated with insecticide (Sibutol) or treated with imidacloprid (Sibutol Secur) (Year 1)
- Slug pellets applied to stubble (Years 2-4) compared with untreated
- Slug pellets applied (1) post drilling, pre--emergence, or (2) post-emergence or (3) pre+post-emergence , compared with untreated(Years 1, 2 ,3 & 4).

YEAR 1: 2001-02

Objectives (winter wheat)

- Comparison of tight (consolidated) seedbed achieved by rolling compared with a loose seedbed (power harrowed).
- Seed treatment comparison between seed not treated with insecticide (Sibutol) or treated with imidacloprid (Sibutol Secur).
- Slug pellets (methiocarb, as Draza) applied at 5.5 kg/ha pre-emergence, post-emergence or pre+post-emergence, compared with untreated controls.

Methods and site details, 2001-02

Two pre drilling soil samples (dimensions 25 x 25 cm taken to a depth of 20 cm) were collected from each of the main plots on 18 October 2001. The soil surface in the trial area was rough, but weathered at the time of sampling, following ploughing of oilseed rape stubble during September. Soil samples were returned to a flooding facility at ADAS Wolverhampton where slugs were extracted over a period of 14 days (Figure 12.1). Slug totals by species, weight and length were recorded (Tables 12.2 and 12.3).

The trial was drilled with winter wheat cv. Claire at 300 seeds/m² on 19 October 2001. Treatment details are summarised in Table 12.1. Plot sizes were: main plots 48x12 m; sub plots 12x12m. The trial was sown using a Reco 3m combination drill with power harrow raised for the tight (consolidated) seedbed and lowered for the loose. The tight (consolidated) seedbed areas were rolled on 30 October 2001. Post-drilling treatments of methiocarb pellets (as Draza, Bayer CropScience, at 5.5 kg product/ha) were applied at pre-emergence or post-emergence timings on 29 October or 4 December 2001 respectively. Pre+post emergence treatments were applied on 29 October and 4 December. Slug pellet applications were by hand pepper-potting over plots in two directions at right angles. At the time of drilling, the surface of the soil was dry but moist at depth. Seed rates were 170 kg/ha for the Sibutol seed batch (TGW 48.6g) and 157 kg/ha for the Sibutol Secur batch (TGW 44.9g). Drilling was soon followed by heavy rain which totalled 105 mm at Boxworth in the period 20-31 October. First crop emergence was noted on 29 October; 50% emergence on 5 November and crop at GS 10 on 13 November.

Slug trapping was conducted on each of the 48 sub-plots using three chicken-layers' mash-baited traps, 25 cm in diameter, on eight occasions between 5 November 2001 (6 days after drilling) and 7 March 2002 (Tables 4a-4c; Figure 2). For each trapping occasion, traps were moved to a new trapping location and reset using fresh bait (approximately 20 ml per trap).

Plant populations were assessed from 5 paired 0.5 m lengths of drill per plot on 4 and 17 December 2001 and 7 March 2002 (Tables 5a-5c). Data were converted to number of plants per m². Numbers and percentages of plants damaged by slugs were recorded for assessments made on 17 December and 7 March (Tables 6a-6c).

The seedbed across the trial area was ploughed in September. The tight seedbed was achieved by rolling on 30 October (11 days after drilling on 19 October). The loose seedbed was power-harrowed at the time of drilling with the power harrow raised when the consolidated plots were drilled. Seed treatments were applied as commercial treatments to winter wheat seeds as bitertanol + fuberidazole (Sibutol) or bitertanol + fuberidazole + imidacloprid (Sibutol Secur),(both Bayer CropScience).

The trial was analysed as a split block design with three factors, using Genstat 5.

Table 12.1: Summary of treatments for winter wheat trial in harvest year 2002.

Factor 1 Seedbed	*Factor 2 Seed treatment*	*Factor 3 Post-drilling molluscicide treatments*
1. Tight (consolidated) rolled on 30 October.	1. Sibutol	1. untreated
2. Loose (not rolled)	2. Sibutol Secur	2. pre-emergence applied 29.10.2001
		3. post-emergence applied 04.12.2001
		4. pre + post emergence 29.10 + 04.12.2001

Results, 2001-02

Pre-drilling slug extraction

Two soil samples 25x25 cm taken to a depth of 20 cm were collected from each of 12 main plots on 18 October 2001. Plots at the time of sampling were in a roughly-ploughed condition. Samples were collected from western and eastern sides of the main plot; one from each area. Slugs were extracted from the samples

by a slow flooding technique over the next 14 days. A total of 38 slugs, equivalent to a population of 25.3/m² was extracted comprising 12 *Deroceras reticulatum* and 26 *Arion distinctus.* The population of slugs on the main plots across the trial area ranged from 0-112/m². The highest slug numbers were extracted from samples from main plot 1 (Figure 12.1) on which the majority were small (< 20 mg) *A. distinctus* extracted during the final stages of flooding. Sizes of slugs extracted were as summarised in Table 12.2.

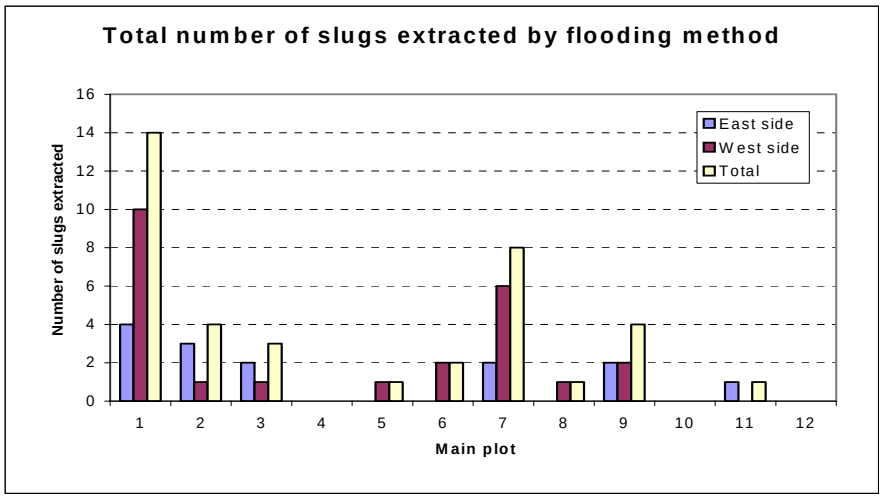


Figure12.1: Number of slugs extracted by flooding method from each of 12 main plots. Main plot 1 was on the northern side of the study field.

Table 12.2. Mean length of slugs extracted after flooding by species and percentage of slugs by size grouping. Sampling date 18 October 2001.

	Deroceras reticulatum	Arion distinctus
Total number extracted by flooding method	12	26
Mean length mm	16.1 mm	12.4 mm
Percentage in each of 3 size categories		
< 10 mm	16.7%	38.5%
10-20 mm	33.3%	46.1%
> 20 mm	50.0%	15.4%

Table 12.3. Mean weight of slugs extracted by species and percentage of slugs by weight category. Sampling date 18 October 2001.

	Deroceras reticulatum	Arion distinctus
Total number extracted by flooding method (number weighed)	12 (10)	26 (22)
Mean weight mg	134.1 mg	70.4 mg
Percentage in each of 9 weight categories		

< 10 mg	10%	18.2%
10-20 mg	10%	31.8%
21-30 mg	0	9.1%
31-40 mg	0	18.2%
41-50 mg	0	0
51-100 mg	20%	4.5%
101-200 mg	30%	4.5%
201-300 mg	30%	4.5%
> 300 mg	0	9.1%

It was possible to weigh only 32 from the total of 38 slugs extracted. Overall, 50% of the *Arion distinctus* extracted by flooding were in the size categories < 10 mg and 10-20 mg and indicative of recent egg hatch. 20% of the *Deroceras reticulatum* extracted weighed 20 mg or less.

Tables 12.4a-12.4c. Mean number of slugs per 3 traps sited in winter wheat – harvest year 2002.

Table12. 4a. Factor 1. Seedbed (tight (consolidated) seedbed achieved by rolling).

Seedbed	*5.11.01*	*13.11.01*	*19.11.01*	*26.11.01*	*10.12.01*	*17.12.01*	*18.01.02*	*07.03.02*
1. Tight	0.08	0.88 *	0.96	1.88	1.54 *	2.21 *	1.96	1.29
2. Loose	0.04	0.25 *	1.04	1.08	0.42 *	1.00 *	2.17	1.38
SED (df 28(2))	0.073	**0.217**	0.334	0.613	0.355	0.427	0.544	0.446
LSD	**0.150**	0.443	0.681	1.252	0.725	0.872	1.111	0.910
cv %	**406.4**	134.1	115.5	143.3	125.3	92.3	91.4	115.9
F pr treatment	**0.573**	0.006	0.804	0.204	0.003	0.007	0.703	0.852

Table 12.4b. Factor 2. Seed treatment.

Seed treatment	*5.11.01*	*13.11.01*	*19.11.01*	*26.11.01*	*10.12.01*	*17.12.01*	*18.01.02*	*07.03.02*
1. Sibutol	0.04	0.71	0.96	1.29	0.79	1.67	2.42	1.13
2. Sibutol Secur	0.08	0.42	1.04	1.67	1.17	1.54	1.71	1.54
SED (df 28(2))	0.073	**0.217**	**0.334**	**0.613**	0.355	0.427	0.544	0.446
LSD	**0.150**	0.443	0.681	1.252	0.725	0.872	1.111	0.910
cv %	**406.4**	134.1	115.5	143.3	125.3	92.3	91.4	115.9
F pr treatment	**0.573**	0.186	0.804	0.544	0.297	0.771	0.200	0.355

No significant effects on slug activity were obtained from imidacloprid (Sibutol Secur) seed treatment for any of the assessment dates.

Table 12.4c. Factor 3. Post-drilling slug pellet application (pre-emergence, post-emergence or pre+post emergence timings).

Pellet treatment	*5.11.01*	*13.11.01*	*19.11.01*	*26.11.01*	*10.12.01*	*17.12.01*	*18.01.02*	*07.03.02*
1. untreated	0.08	0.42	1.17	2.00	0.92	2.00	3.00	1.92
2. pre-em	0	0.42	1.00	1.17	1.08	1.33	2.50	1.25
3. post-em	0.08	0.75	0.92	1.58	1.00	1.92	1.67	1.50
4. pre+post em	0.08	0.67	0.92	1.17	0.92	1.17	1.08 *	0.67

SED (df 28(2))	0.104	0.306	0.471	0.865	0.501	0.603	0.768	0.629
LSD	0.212	0.625	0.962	1.767	1.023	1.231	1.568	1.285
cv %	406.4	134.1	115.5	143.3	125.3	92.3	91.4	115.9
F pr treatment	0.809	0.601	0.945	0.737	0.985	0.425	0.076	0.263

Post-drilling refuge trapping

Three refuge traps baited with poultry layers mash were established immediately after drilling on each of the 48 sub plots. Checks for slugs were made on 5, 13, 19, 26 November; 10 and 17 December 2001, 18 January 2002 and 7 March 2002 (Tables 12.4a-12.4c). Slug activity post-drilling was initially low but increased substantially as the seedbed moistened during late October. On three dates in November and December, slug activity was significantly higher on the consolidated compared with the loose seedbed but in January and March, no differences were recorded. At the peak of slug activity recorded on 18 January 2002, a significant reduction in slug activity was obtained from the pre + post-emergence application of methiocarb (Draza) slug pellets compared with the untreated mean 3.0 slugs per three traps. However, with the F value for treatment significant only at p=0.076, these data should be interpreted with caution.

Slug activity increased steadily post-drilling, peaking between 17 December 2001 and 18 January 2002 (Figure 12.2).

There were initially only weak and non significant regressions between numbers of slugs under refuge traps for the five trapping periods 5, 13, 19, 26 November and 10 December 2001. A significant regression was obtained for total slug numbers in refuge traps on 10 and 17 December (R^2 40.7%, $p<0.001$).

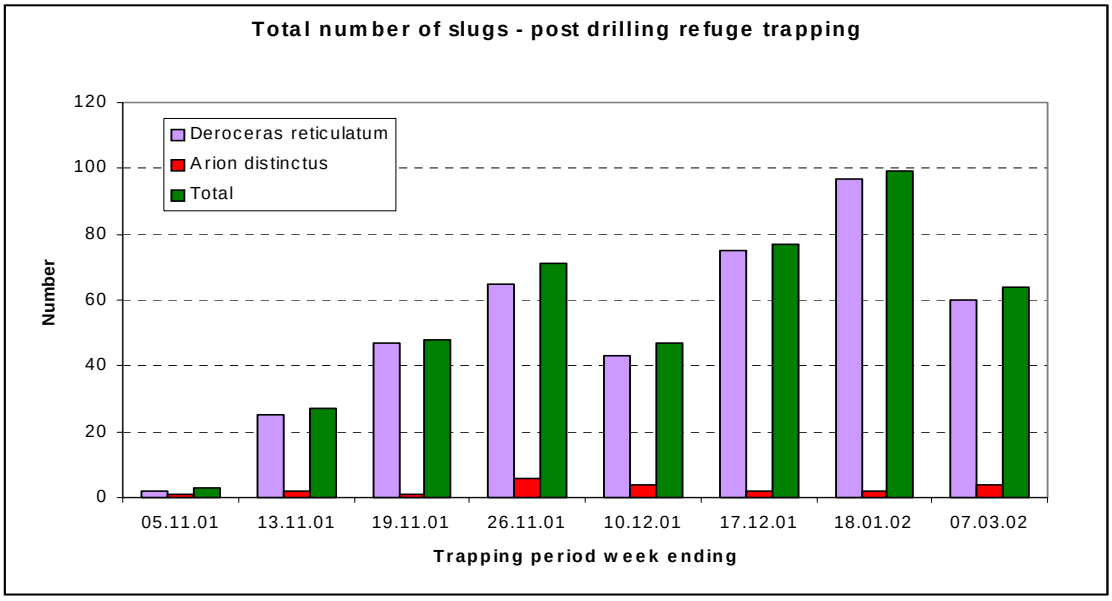


Figure 12.2. Total number of slugs under refuge traps on eight occasions in autumn 2001 and winter 2002.

Figure 12.2 shows that slugs, predominantly *Deroceras reticulatum,* remained active over-winter. For all trapping periods between 13 November and 7 March, *D. reticulatum* comprised the majority of slugs recorded under traps, for example 98% and 94% of the totals for slugs under traps on 18 January and 7 March respectively. These data contrasted with those obtained from the soil flooding extractions which had shown a higher proportion of *Arion distinctus* than *D. reticulatum* (68% and 32% of total extract respectively.

Plant numbers and plant damage

Plant populations were recorded for three assessment dates. Mean number of plants across the trial area at the first assessment was 70.8/m² representing an establishment percentage of 23.4%. At the second assessment, establishment had increased slightly to provide a mean plant population across the trial area of 78.8/m² falling overwinter to a mean of 36.5/m² at the third assessment on 7 March 2002. Plant population in March was significantly ($p<0.001$) correlated with the plant population on 17 December (R^2 56.4%). No significant differences were recorded for any treatment or treatment interactions for plant population assessments made on 4, 17 December 2001 or 7 March 2002.

Tables 12.5a-5c. Mean percentage of winter wheat plants damaged by slugs on 17 December 2001 and 7 March 2002.

Table 12.5a. Seedbed (Factor 1).

Factor 1. Seedbed	*17.12.2001*	*07.03.2002*
Tight (consolidated)	23.9	5.9
Loose	29.9	15.5
SED (df 28(2))	4.72	3.46
LSD	9.64	7.06
cv %	60.8	108.8
F pr treatment	0.219	0.012

Table12. 5b. Seed treatment (Factor 2).

Factor 2. Seed treatment	*17.12.2001*	*07.03.2002*
Sibutol	29.0	10.4
Sibutol Secur	24.9	11.0
SED (df 28(2))	4.72	3.46
LSD	9.64	7.06
Cv %	60.8	108.8
F pr treatment	0.392	0.886

Mean percentages of plants damaged by slugs were not affected by seed treatment with similar values obtained for Sibutol and Sibutol Secur treatments.

Table12. 5c. Molluscicide treatment (Factor 3).

Factor 3. Slug pellet treatment	*17.12.2001*	*07.03.2002*
untreated	40.1	18.5
Pre-em	21.7	10.3
Post-em	29.2	6.2
Pre and post em	16.7 *	7.8
SED (df 28(2))	6.68	4.88
LSD	13.64	9.97
cv %	60.8	108.8
F pr treatment	0.009	0.103

On 17 December, there was a tendency for slug damage to be less severe on the consolidated seedbed compared with the loose seedbed, although differences were not significant (Table 12.5a). Slug activity, predominantly *Deroceras reticulatum,* continued over-winter and peaked in mid January (Figure 12.2). The mean percentage of plants damaged by slugs on 7 March was significantly ($p = 0.012$) lower on the consolidated seedbed compared with the loose seedbed (means of 5.9% and 15.5% respectively) (Table 12.5a).

Comparison of molluscicide treatments showed significant ($p<0.009$) reductions on 17 December from methiocarb pellets applied at pre-emergence and pre + post-emergence timings compared with the untreated mean 40.1% plant damage. The reduction in damage from the post-emergence treatment was not significant compared with the untreated mean. Analysis of the angular transformation for these data sets gave similar results with only the molluscicide pre-emergence and pre+post-emergence treatments giving significant ($p=0.018$) reductions in damage compared with the untreated. Comparisons using Least Significant Difference (LSD) values of the mean percentages of wheat plants damaged by slugs on 7 March (Table 12.6c) indicated a lower incidence of damage (6.2% plants damaged by slugs) where post-emergence methiocarb had been applied, compared with the untreated mean 18.5% damage. An additional reduction in

damage was not obtained where methiocarb was applied at pre and post-emergence timings (mean 7.8% damage).

The data from the March assessments contrasted with those from the 17 December plant damage assessment and indicated a longer-term benefit from seedbed consolidation. The effect extended from drilling through to GS 22-23 and continuing beyond the period of protection obtained from methiocarb pellet treatments. No significant interactions between factors were recorded for either plant population or slug damage at the 7 March assessments. There was, however, a tendency for a lower incidence of damage to be recorded for tight seedbeds and where pre+post-emergence methiocarb treatments had been applied.

YEAR 2: 2002-03

Objectives (winter wheat)

- Comparison of tight (consolidated) seedbed achieved by rolling compared with a loose seedbed (power harrowed).
- Slug pellets (metaldehyde, as Metarex) applied as a pre-drilling stubble treatment at 8.0 kg/ha compared with no stubble treatment.
- Slug pellets (metaldehyde, Metarex) applied at 8.0 kg/ha pre-emergence, post-emergence or pre+post-emergence, compared with untreated controls.

Methods and site details

The trial was drilled into a moist seedbed with winter wheat cv Malacca at a seed rate of 190 kg/ha on 24 October 2002. Plot sizes were: main plots 48x12 m; sub plots 12x12m. Metaldehyde pellets (as Metarex, De Sangosse UK) were applied following heavy rain at a product rate of 8.0 kg/ha to the wheat stubble on 10 September. The trial site was ploughed on 19 September but the weather was then predominantly dry for the next four weeks. Seedbed conditions were initially rough and cloddy. The tight (consolidated) seedbed areas were rolled on 28 October 2002, 4 days after drilling. Early crop emergence (GS 10) was recorded on 13 November; GS 11 was reached on 20 November. Post-drilling treatments of metaldehyde were applied at pre-emergence or post-emergence timings on 28 October or 3 December 2002. Treatment details are summarised in Table 12.6.

Pre-drilling slug trapping was undertaken between 9 and 12 September 2002 in untreated areas of the wheat stubble, using chicken layers' mash-baited traps, 25 cm in diameter, and methiocarb-baited traps, 18 cm in diameter, when conditions were sufficiently moist to favour slug activity. Traps were checked after one or three nights on 10 and 12 September respectively. Molluscicide-baited traps were checked after three nights on 12 September. Soils were wet following rain on 9 September, when the traps were set-out and when they were checked on 10 September. The soil became drier by 12 September following dry, warm weather. The results of a comparison of trap baits are described in Paper 14, this report.

Two pre drilling soil samples (dimensions 25 x 25 cm taken to a depth of 20 cm) were collected the rough-plough on each of the 48 main plots on 18 October 2002. Soil samples were returned to a flooding facility where slugs were extracted over a period of 21 days. Data are not presented as no slugs were extracted by flooding.

Slug trapping was conducted on each of the 48 sub-plots using three chicken-layers mash baited traps on nine occasions between 30 October (6 days after drilling) and 14 March 2003.

Plant populations were assessed from 5 paired 0.5 metre lengths of drill at GS 11-12 on 29 November. A post-winter assessment of plant population was made on 11 March 2003. Data were converted to number of plants per m². Numbers and percentages of plants damaged by slugs were recorded for assessments made on 29 November and 11 March.

Table 12.6. Summary of treatments for winter wheat study in harvest year 2003.

Factor 1 Seedbed	*Factor 2 Stubble treatment*	*Factor 3 Post-drilling molluscicide treatment*
1. Consolidated (tight) Rolled 28.10.2003.	1. untreated	1. untreated
2. Loose. Not rolled.	2. metaldehyde applied 10.09.2002	2. pre-emergence metaldehyde applied 28.10.2002 3. post-emergence applied 03.12.2002 4. pre + post emergence 28.10 + 03.12.2002

The consolidated (tight) seedbed was initially prepared by ploughing on 19 September and followed by power harrowing at the time of drilling on 24 October. The consolidated (tight) seedbed was prepared by rolling on 28 October. The loose seedbed treatment was prepared in the same way with the exception that plots were not rolled to leave an open seedbed.

Slug pellet applications comprised metaldehyde (Metarex, De Sangosse Ltd.) at a product rate of 8.0 kg/ha. Application was by hand pepper-potting over plots in two directions at right angles.

The trial was analysed as a split block design with three factors using Minitab.

Results

Pre-drilling trap assessments

Trap catches by block are summarised in Figure 12.3. Figure 1a plots the distribution of slug catch by block. Factorial analysis showed no significant factor-related effects after one night's trapping using mash-baited traps. There was some evidence for a block-related effect with a higher incidence of slug activity in block 1. Mean numbers of slugs per layers' mash-baited trap were 0.68 and 0.19 per trap after one and three nights trapping on 10 and 12 September respectively. The soil surface was wet for the first night's trapping following heavy rain on 9 September (Cambridgeshire rainfall total 28 mm in week ending 10 September

2002). Although the trap catch was lower after three nights, as the soil quickly dried, a significant ($p<0.001$) correlation for mean number of slugs per trap was obtained with 24.3% of variance explained.

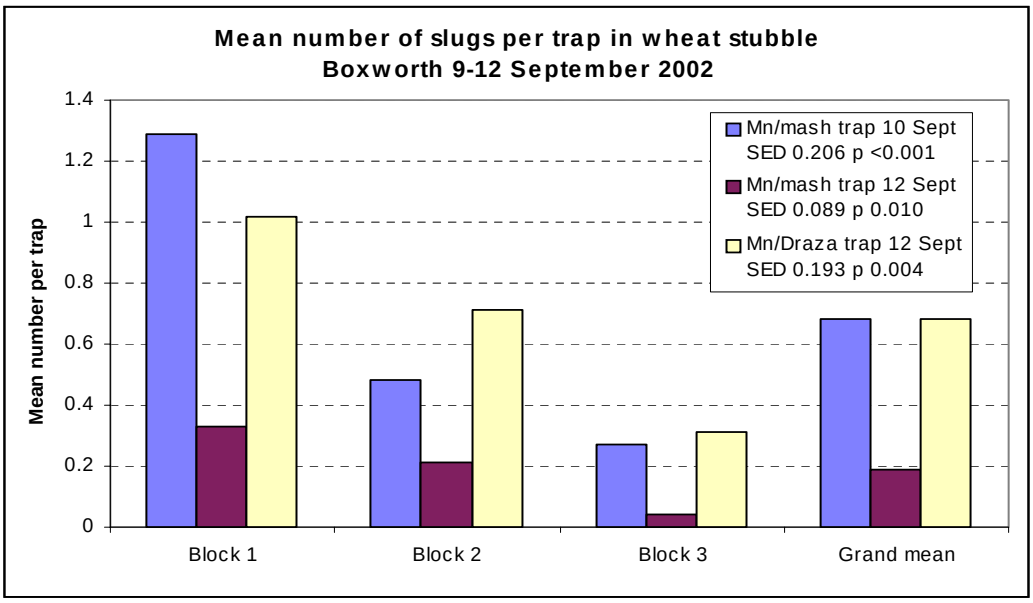


Figure 12.3. Mean number of slugs per mash-baited trap after one and three nights and mean number of slugs per methiocarb-baited trap after three nights.

Catches in both types of traps comprised predominantly *Deroceras reticulatum*, which formed 99% and 96% of the total catch under mash and methiocarb-baited traps respectively. The remainder of the catches comprised 1 *Arion distinctus*, 1 *A. hortensis* agg., and 2 *A. ater*.

Soil flooding – slug extractions

Soil samples of dimensions 25 x 25 cm taken to a depth of 20 cm cultivation depth were collected from each of the 48 sub plots in the trial at ADAS Boxworth on 18 October 2002. Samples were returned to a glasshouse facility at ADAS Wolverhampton where a trickle-irrigation method for slow flooding of soil was adopted over a 10 day period. Samples were checked twice daily for slugs for the duration of the flooding extraction. Despite pre-ploughing evidence for slug activity on the soil surface in the period 9-12 September 2002 and a substantial layer of ploughed-down stubble within many of the samples, no slugs were extracted. It was suspected that deep ploughing of the site on 19 September, followed by dry conditions that persisted for four weeks adversely impacted on the slug population or delayed the return of slugs to the soil surface.

Post-drilling trap assessments

For all post-drilling assessments, refuge trap catches comprised predominantly *Deroceras reticulatum* with low numbers of *Arion* spp. for most assessment dates. Table 12.7 summarises total slug numbers under 3

mash-layers baited refuge traps on each of 48 sub plots (144 traps in total) for nine, post-drilling, trapping events between 30 October and 14 March.

Table 12.7. Total slug numbers under refuge traps and percentage of total catch for Deroceras reticulatum *on nine assessment dates.*

Post-emergence refuge trapping dates	*Total number of* D. reticulatum	*Total number of* Arion *spp*	D. reticulatum *percentage of total slug number*
30.10.02	12	0	100
06.11.02	36	2	95
13.11.02	65	7	90
20.11.02	66	2	97
27.11.02	112	3	97
05.12.02	84	3	97
13.02.03	27	3	90
28.02.03	63	2	97
14.03.03	242	13	95

Arion spp. comprised *A. distinctus*, other *A. hortensis* agg. and *A. subfuscus.*

Table 12.8. Mean number of slugs per three refuge traps for autumn, winter and spring assessment dates.

Assessment date	*Seedbed treatment Factor 1*		*SED (df 30)*	*LSD P=0.05*	*cv %*	*F pr treatment*
	Tight (rolled)	Loose				
30.10.2002	0.38	0.13	0.117	0.240	162.8	0.041
06.11.2002	0.92	0.67	0.348	0.703	151.8	0.476
13.11.2002	1.96	1.04	0.296	0.604	68.3	0.004
20.11.2002	1.58	1.25	0.333	0.680	80.9	0.323
27.11.2002	3.63	1.17	0.636	1.298	91.8	0.001
05.12.2002	2.21	1.42	0.418	0.854	79.6	0.067
13.02.2003	0.83	0.42	0.280	0.572	155.2	0.146
28.02.2003	1.42	1.29	0.386	0.788	99.0	0.748
14.03.2003	4.13	6.50	0.909	1.856	59.3	0.014

Slug activity was initially low after the test crop of winter wheat was drilled. On 30 October, the mean number of slugs (all *D. reticulatum*) was significantly (p=0.041) higher on the tight (consolidated) seedbed compared with the loose, unrolled, plots (mean 0.38 and 0.13 slugs per three traps respectively, Table 12.8).

Slug surface activity gradually increased during the autumn as soils moistened. Mean number of slugs were significantly higher on consolidated compared with loose plots for assessments made on 13 and 27 November. As in the first year of the study, higher slug activity was recorded during the autumn and winter on the tight (rolled) treatment compared with the loose seedbed (Table 12.8) and this may have resulted from the difficulty that slugs had in moving through consolidated soil.

A low incidence of slug activity was recorded during warmer weather following frosts in mid February, followed by increased activity on 28 February. Slug activity continued to increase and on 14 March, mean number of slugs per three traps was significantly (p=0.014) higher on the loose compared with the tight seedbed (Table 12.9). This contrasted with the results obtained in the autumn. Slug totals for 14 March were higher than slug totals for any trapping period during the autumn and winter.

There was a trend towards higher activity on plots that were not treated with slug pellets on the stubble on 10 September (Table 12.9). Slug activity during the autumn peaked in late November or early December as noted earlier for the effects of seedbed treatment. On 27 November, mean number of slugs per three refuge traps was significantly (p=0.05) higher on the untreated compared with the treated plots. An increase in slug activity was recorded post-winter, peaking on 14 March. No further significant differences in slug activity were recorded.

Table 12.9. Mean number of slugs per three refuge traps for autumn, winter and spring assessment dates.

Assessment date	*Stubble treatment*		*SED (df 30)*	*LSD (p=0.05)*	*cv %*	*F pr treatment*
	Untreated	Treated				
30.10.2002	0.21	0.29	0.117	0.240	162.8	0.483
06.11.2002	1.08	0.50	0.348	0.703	151.8	0.102
13.11.2002	1.67	1.33	0.296	0.604	68.3	0.268
20.11.2002	1.63	1.21	0.333	0.680	80.9	0.219
27.11.2002	3.04	1.75	0.636	1.298	91.8	0.051
05.12.2002	2.04	1.58	0.418	0.854	79.6	0.279
13.02.2003	0.50	0.75	0.280	0.572	155.2	0.378
28.02.2003	1.17	1.54	0.386	0.788	99.0	0.339
14.03.2003	5.54	5.08	0.909	1.856	59.3	0.617

Effect of pre and post-emergence molluscicide treatments

Table 12.10 shows the effects of pre and post-emergence slug pellet treatments applied on 28 October and 3 December 2002 respectively.

Table 12.10. Mean number of slugs per three refuge traps for autumn, winter and spring assessment dates.

Assessment date	*Molluscicide treatment*				*SED (df 30)*	*LSD p=0.05*	*cv %*	*F pr treatment*
	Untreated	Pre-em.	Post-em.	Pre+ post-em				
30.10.2002	0.33	0.08	0.50	0.08	0.166	0.339	162.8	0.045
06.11.2002	1.50	0.08	1.17	0.42	0.491	1.002	151.8	0.025
13.11.2002	1.92	0.92	1.75	1.42	0.419	0.855	68.3	0.106
20.11.2002	2.25	0.75	1.75	0.92	0.471	0.962	80.9	0.010
27.11.2002	2.67	1.83	2.42	2.67	0.899	1.836	91.8	0.766
05.12.2002	2.75	1.83	1.33	1.33	0.590	1.205	79.6	0.072
13.02.2003	0.17	1.00	0.33	1.00	0.396	0.809	155.2	0.082
28.02.2003	1.50	1.25	1.08	1.58	0.547	1.117	99.0	0.787
14.03.2003	5.33	5.75	5.00	5.17	1.285	2.624	59.3	0.944

Low slug activity at the first assessment on 30 October was significantly (p=0.045) reduced by the pre-emergence metaldehyde treatments applied two-days earlier on 28 October. Significant reductions in mean number of slugs per three traps were recorded for assessments made on 6 and 20 November, compared with the untreated means 1.50 and 2.25 slugs per three refuge traps respectively. On 5 December, the post-emergence treatment applied two days earlier resulted in reduced slug activity compared with the untreated mean 2.75 slugs per three traps (p=0.072). By this date, the effects of the pre-emergence treatments had declined. No further significant reductions in slug numbers were recorded and on 28 February and 14 March, slug numbers were similar irrespective of treatment.

Plant numbers and damage

Mean number of plants/m² was not significantly affected by seedbed or molluscicide treatments. The overall mean plant population was 233.6/m² for the second plant count compared with 254.6/m² for assessments made on 29 November 2002.

The mean percentage of plants damaged by slugs was slight with an incidence lower than in harvest year 2002 (year 1 of the study) and with no evidence for significant treatment-related effects or of a significant correlation between percentages of plants damaged by slugs and plant population.

Significant correlations were obtained between mean plant population and slug totals under refuge traps at peak slug activity on 27 November ($p=0.028$; R^2 10.1%). The equation y (for mean number of plants/m²) = 262.0 – 3.20x (total slugs on 27 November) indicated a reduction in plant population of 1.15% for each additional one slug per plot (0.33 per trap). The mean percentage of plants damaged by slugs was significantly correlated with slug totals for 27 November ($p=0.033$; R^2 9.5%) and which indicated an increase in plant damage of 1.08% for each additional slug per three traps (y=0.95+0.132 x (total slugs on 27 November)).

The incidence of slug damage remained low during the winter. At the second damage assessment made on 11 March 2003, an overall mean of 1.97% damage was recorded compared with 1.27% at the first damage check on 29 November 2002. In March, the mean percentage of plants damaged by slugs was significantly ($p = 0.012$) lower on the tight seedbed compared with the loose seedbed. A similar longer-term effect of seedbed consolidation was also noted in the first year of the experiment. No significant differences were recorded for stubble treatments or pre and post emergence metaldehyde applications.

Table 12.11. Mean percentage of plants damaged at second damage assessment on 11 March 2003

Table 12.11a. Seedbed (Factor 1)

Factor 1	*Tight (rolled)*	*Loose (not rolled)*
	1.30	2.64
SED 0.501 (df 30)		
LSD 1.023		
Cv 87.9%		
F pr 0.012		

Table 12.11b. Stubble treatment (Factor 2).

Factor 2	*Untreated*	*Treated*
	2.30	2.64
SED 0.501 (df 30)		
LSD 1.023		
Cv 87.9%		
F pr 0.203		

Table 12.11c. Molluscicide treatment (Factor 3).

Factor 3	*Untreated*	*Pre-em.*	*Post-em.*	*Pre + post emergence*
	1.94	1.84	2.20	1.90
SED 0.706 (df 30)				
LSD 1.442				
Cv 87.9%				
F pr 0.961				

Significant correlations were obtained for mean plant populations at the first and second assessments ($p < 0.001$; R^2 35.2%) and between mean percentages of plants damaged by slugs at the first and second damage assessments ($p < 0.001$; R^2 39.4%). Plant damage at the second assessment made on 11 March 2003 was significantly correlated with mean number of slugs per three refuge traps on 14 March ($p < 0.008$; $R^2$14.2%) indicating that treatment effects had persisted over-winter.

YEAR 3: 2003-04

Objectives (winter oilseed rape)

- Establishment using a minimum-cultivation, direct-drilling technique compared with seedbed preparation by ploughing, power harrowing and rolling.
- Slug pellets (metaldehyde, as Metarex) applied as a pre-drilling stubble treatment at 8.0 kg/ha compared with no stubble treatment.
- Slug pellets (metaldehyde, as Metarex) at 8.0 kg/ha applied pre-emergence, post-emergence or pre and post emergence, compared with untreated controls.

Methods and site details:

Treatment details are summarised in Table 12.12. Plot sizes were: main plots 48x12 m; sub plots 12x12m. The planned late August drilling date was delayed by very dry soil, so winter oilseed rape cv Winner was drilled on 3 September 2003 at a seed rate of 6.0 kg/ha using a Moore Direct disc drill. Due to dry seedbed conditions in September and October, plant establishment was poor, particularly on the inverted plots on which ploughing and cultivating probably resulted in loss of soil moisture. Plants on direct drilled plots were at GS 1,02-1,03 on 24 October contrasting with a very small number of plants which were at only cotyledon stage on the ploughed plots. As a result, it was necessary to broadcast additional oilseed rape seed cv Winner at a seedrate of 8.0 kg/ha on 30 October 2003 (following 7 mm of rain).

Soil sampling, planned for late August, had been delayed as soil conditions were dry. Samples (one sample per plot of dimensions 25 x 25 cm taken to a depth of 20 cm) were collected on 12 November from each of the 48 main plots when the soil was moist. Slugs were extracted from the soil samples over a period of 14 days using a trickle-irrigation, slow flooding technique. Slug trapping was conducted on each of the 48 sub-plots using three chicken-layers mash baited traps, 25 cm in diameter. Slug trapping on the wheat stubble was undertaken in the period 15-18 August, following slight rain, and after the application of metaldehyde. This had been applied as a stubble treatment on 11 August. No slugs were trapped and these data are therefore not presented.

Trapping was conducted post-drilling, on nine occasions between 11 September (8 days after drilling) and 6 January 2004. Trapping on 23 September was preceded by 13 mm rain but, as the weather subsequently remained dry until late October, no suitable periods for further monitoring were presented until late October.

Plant populations were assessed from 5 x 0.25 m² quadrats per plot on 15 January 2004. Typical growth stages at the time of assessment were 1,04 on the direct-drilled plots and 1,01 on the ploughed plots. Data were converted to number of plants per m². Numbers and percentages of plants damaged by slugs were recorded.

Table 12.12. Summary of treatments for winter oilseed rape trial in harvest year 2004.

Factor 1 Seedbed	*Factor 2 Stubble treatment*	*Factor 3 Post-drilling molluscicide treatments*
1. Minimum cultivation (direct drilled 03.09.2003)	1. untreated	1. untreated
2. Plough, power-harrow, roll on 18.08.2003)	2. metaldehyde applied 11.08.2003	2. pre-emergence metaldehyde applied 10.09.2003 3. post-emergence applied 28.11.2003 4. pre + post emergence 10.09.2003 + 28.11.2003

The inverted-seedbed (Factor 1) treatment was prepared prior to drilling by ploughing, power harrowing and rolling on 18 August. The minimum cultivation plots were established using a direct drilling method which resulted in a non-inverted seedbed. Slug pellet applications comprised metaldehyde slug pellets (Metarex, De Sangosse UK) at a product rate of 8.0 kg/ha. Application was by hand pepper-potting over plots in two directions at right angles.

The trial was analysed as a split block design with three factors, using Minitab.

Results

Soil flooding – slug extractions

Sampling had been postponed from that planned pre-drilling to mid November when trapping indicated evidence of slug activity on the soil surface. Despite moist soil at the time of sampling and a substantial layer of ploughed-down stubble within many of the samples, only one *Arion distinctus* was extracted. This was equivalent to an overall slug population of only 0.33/m². As in the previous season, it was suspected that dry soil conditions between early August and late October had an adverse effect on the slug population.

Pre-drilling refuge trapping

A total of 3 refuge traps per plot were set-out on 15 August following light rain. Traps were checked on 18 August but no slugs were caught. Ploughing and cultivation treatments were applied to the inverted plots immediately after completion of the refuge trapping. During the predominantly dry conditions during August, it proved difficult to select further occasions for slug trapping when conditions were sufficiently moist to favour slug activity.

Post-drilling refuge trapping

A low total of 76 slugs was recorded from refuge trap catches for the 9 trapping periods between 11 September and 6 January. The slug total comprised 65 *Deroceras reticulatum* and 11 *Arion* spp. (overall 86% *D. reticulatum).*

Table 12.13. Effects of cultivation on the mean number of slugs per three refuge traps for nine, post-drilling assessments.

Assessment date	*Seedbed treatment*		*SED*	*LSD*	*cv %*	*F prob.*
	Factor 1		*(df 30)*	P=*0.05*		*treatment*
	Min. cult. direct drill	Plough, power-harrow				
11.09.2003	0.04	0	0.042	0.086	69.3	0.325
23.09.2003	0.08	0.08	0.092	0.188	382.1	1.000
29.10.2003	0.33	0	0.149	0.305	309.8	0.033
03.11.2003	0.13	0	0.094	0.193	523.1	0.195
05.11.2003	0.50	0.04	0.181	0.370	231.4	0.017
14.11.2003	0.38	0	0.144	0.295	266.7	0.014
21.11.2003	0.46	0.17	0.177	0.361	195.6	0.109
02.12.2003	0.08	0.21	0.119	0.243	285.7	0.307
06.01.2004	0.29	0.38	0.197	0.401	204.2	0.674

Slug activity was initially low in dry seedbeds until heavier rainfall events occurred during and from late October 2003. Mean numbers of slugs per three traps were significantly lower on ploughed plots compared with direct-drilled (minimally cultivated) plots for assessments made on 29 October; 5 and 14 November (Table 12.13). No other differences were significant.

Table 12.14. Effects of stubble treatment with slug pellets on the mean number of slugs per three refuge traps for nine, post-drilling assessments.

Assessment date	*Stubble treatment*		*SED*	*LSD*	*cv %*	*F pr*
	Factor 2		*(df 30)*	*(p=0.05)*		*treatment*
	Untreated	Treated				
11.09.2003	0.04	0	0.042	0.086	69.3	0.325
23.09.2003	0.17	0	0.092	0.188	382.1	0.080
29.10.2003	0.25	0.08	0.149	0.305	309.8	0.272
03.11.2003	0.08	0.04	0.094	0.193	523.1	0.662
05.11.2003	0.29	0.25	0.181	0.370	231.4	0.819
14.11.2003	0.29	0.08	0.144	0.295	266.7	0.159
21.11.2003	0.42	0.21	0.177	0.361	195.6	0.247
02.12.2003	0.21	0.08	0.119	0.243	285.7	0.307
06.01.2004	0.25	0.42	0.197	0.401	204.2	0.403

Although no slugs were recorded under refuge traps established on the wheat stubble (trapping period 15-18 August); activity increased post drilling as the soil progressively moistened during the autumn. There was a tendency for lower slug activity to be recorded where the stubble treatment had been applied until the trapping period ending 2 December. However, differences were not significant for any assessment period, probably as slug activity on the soil surface had been negligible or zero in dry conditions during August when the stubble treatment had been applied.

Table 12.15. Effects of post-drilling treatments with slug pellets on the mean numbers of slugs per three refuge traps for nine assessments.

Assessment date	*Post-drilling molluscicide treatment* *Factor 3*				*SED (df 30)*	*LSD* P=*0.05*	*cv %*	*F prob. treat-ment*
	Untreated	Pre-em.	Post-em.	Pre+ post em.				
11.09.2003	0	0.08	0	0	0.059	0.120	69.3	0.406
23.09.2003	0.17	0	0.17	0	0.130	0.265	382.1	0.366
29.10.2003	0.25	0.08	0.33	0	0.211	0.430	309.8	0.388
03.11.2003	0.25	0	0	0	0.133	0.272	523.1	0.177
05.11.2003	0.83	0	0.25	0	0.256	0.522	231.4	0.008
14.11.2003	0.42	0	0.17	0.17	0.204	0.417	266.7	0.257
21.11.2003	0.58	0.08	0.42	0.17	0.249	0.508	195.6	0.190
02.12.2003	0.08	0.17	0.08	0.25	0.168	0.343	285.7	0.726
06.01.2004	0.67	0.58	0.08	0	0.277	0.567	204.2	0.046

Initially low slug activity increased as the soil moistened during the autumn, notably following periods of heavier rainfall in late October. Mean numbers of slugs per three refuge traps were significantly (p=0.008) lower for metaldehyde applied pre-emergence, post-emergence or pre+post-emergence compared with the untreated mean 0.83 slugs per three traps on 5 November, during the highest period of slug activity recorded in autumn 2003. Significant (*P*=0.046) reductions were obtained on 6 January from metaldehyde pellets applied post-emergence or pre+post-emergence compared with the untreated mean 0.67 slugs per three traps.

Although slug activity and slug totals under traps were small, significant interactions were obtained between the seedbed (Factor 1) and post-drilling application of metaldehyde pellets (Factor 3) for assessments made on 5 and 21 November (P = 0.045 and 0.021, respectively). Mean number of slugs per three traps was significantly higher on direct drilled plots that received no post-drilling slug pellet application compared with slug totals on plots established by ploughing or where a post drilling application of slug pellets had been made (Tables 12.16 and 12.17).

*Table 12.16. Factor 1 seedbed*Factor 3 post-drilling molluscicide: mean number of slugs per three refuge traps, 5 November 2003 assessments.*

Factor 1 Ź / *Factor 3 Ÿ*	*untreated*	*Pre-em*	*Post-em*	*Pre and post-em*
Tight (direct drill)	1.50	0	0.5	0
Loose (plough, power-harrow, roll)	0.17	0	0	0
SED 0.362 LSD 0.739				
P=0.045				

*Table 12.17. Factor 1 seedbed*Factor 3 post-drilling molluscicide: mean number of slugs per three refuge traps, 21 November 2003 assessments.*

Factor 1 Ź / *Factor 3 Ÿ*	*untreated*	*Pre-em*	*Post-em*	*Pre and post-em*
Tight (direct drill)	1.17	0	0.67	0
Loose (plough, power-harrow, roll)	0	0.17	0.17	0.33
SED 0.355 LSD 0.724				
P=0.021				

A significant interaction was identified between the seedbed (Factor 1) and the stubble treatments (Factor 2) for assessments made on 21 November (table 12.18). Significantly higher slug numbers were recorded on direct-drilled, stubble-untreated plots compared with direct-drilled stubble treated plots. On stubble-untreated plots, slug numbers on direct-drilled plots were significantly higher than on ploughed plots.

*Table 12.18. Factor 1 seedbed*Factor 2 stubble treatment: mean number of slugs three refuge traps, 21 November 2003 assessments.*

Factor 1 Ź *Factor 2 Ÿ*	*untreated*	*Stubble-treatment applied*
Tight (direct drill)	0.83	0.08
Loose (plough, power-harrow, roll)	0	0.33
SED 0.249 LSD 0.509		
P=0.005		

Plant population and damage assessments.

The mean numbers of damaged plants/m² and mean percentages of plants damaged by slugs are presented in Tables 12.19-12.21. Note that due to the failure of the oilseed rape test crop to establish on the cultivated (ploughed, power-harrowed and rolled) plots during the dry conditions in autumn 2003, it was necessary to broadcast additional seed (cv Winner at 8.0 kg/ha) on 30 October as the soil moistened. The mean number of plants/m² were significantly higher on ploughed plots compared with the minimally-cultivated plots (Table 12.19). Although the incidence of damage was slight (due to dry conditions from early August to late October 2003), the mean percentage of plants damaged by slugs was significantly lower where plots were established by ploughing compared with direct drilling (Table 12.19). These results demonstrated a lower incidence of damage after ploughing and emphasised a possible benefit from reduction of slug damage to oilseed rape.

Table 12.19. Mean number of plants/m² and mean percentage of plants damaged by slugs on 15 January 2004. Effects of seedbed treatments. Factor 1.

Seedbed treatment	*Mean no. plants/m²*	*Mean no. damaged plants/m²*	*Mean % plant damage*
Direct drill	49.6	1.43	3.08
Plough	58.9	0.63	1.08
SED (30 df)	3.96	0.359	0.765
LSD (p=0.05)	8.09	0.734	1.562
Cv %	25.3	120.4	127.5
F probability treatment	0.025	0.034	0.014
F probability blocks	0.346	0.005	0.008

Although there was a tendency towards higher plant numbers and a lower incidence of plant damage on plots where the stubble had been treated with slug pellets compared to plots untreated at this time, differences between stubble-treated and stubble-untreated were not significant (Table 12.20).

Table 12.20. Mean number of plants/m² and mean percentage of plants damaged by slugs on 15 January 2004. Effects of stubble treatment. Factor 2.

Stubble treatment	*Mean no. plants/m²*	*Mean no. damaged plants/m²*	*Mean % plant damage*
Stubble untreated	51.8	1.20	2.48
Stubble treated	56.7	0.87	1.67
SED (30 df)	3.96	0.359	0.765
LSD (p=0.05)	8.09	0.734	1.562
Cv %	25.3	120.4	127.5
F probability treatment	0.232	0.361	0.297
F probability blocks	0.346	0.005	0.008

On plots treated with slug pellets post-drilling, a significant reduction in mean number of slugs in refuge traps had been obtained at the peak of slug activity on 5 November. However, no significant effects were recorded for mean number of plants/m², mean number of damaged plants/m², or for mean percentage of plants damaged by slugs for pre, post or pre+post-emergence pellet treatments compared with untreated means (Table 12.21).

No significant interactions were identified for any of the plant population or damage assessments.

Table 12.21. Mean number of plants/m² and mean percentage of plants damaged by slugs on 15 January 2004. Effects of pre and post-emergence pellet treatments. Factor 3.

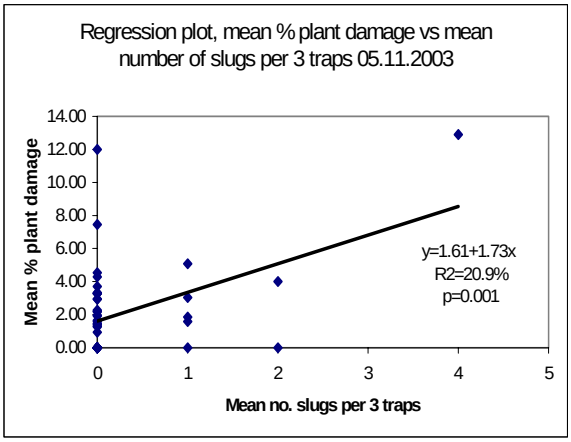
Molluscicide treatment	*Mean no. plants/m²*	*Mean no. damaged plants/m²*	*Mean % plant damage*
untreated	57.7	1.27	2.36
Metarex pre em	54.4	1.20	2.23
Metarex post em	53.1	0.67	1.48
Metarex pre+post em	51.8	1.00	2.24
SED (30 df)	5.60	0.507	1.080
LSD (p=0.05)	11.43	1.036	2.206
Cv %	25.3	120.4	127.5
F probability treatment	0.753	0.643	0.839
F probability blocks	0.346	0.005	0.008

Various regressions were tested between percentages of plants damaged by slugs at assessment on 15 January against slug activity recorded under refuge traps. The regressions shown in Table 12.22 were obtained between plant damage and slug numbers for trapping periods between 29 October – 6 January 2004.

Table 12.22. Test regressions between percentage plants damaged (y) and slugs under refuge traps (x) for seven assessment dates between 29 October and 6 January 2004.

Test regression between percentage plants damaged (y) and slugs under refuge traps (x) for assessment dates:	*p value*	*R²*	*Equation*
29 October 2003	0.186	3.8%	y=1.90+1.04x
3 November 2003	0.001	21.8%	y=1.82+4.07x
5 November 2003	0.001	20.9%	y=1.61+1.73x
14 November 2003	<0.001	34.2%	y=1.50+3.07x
21 November 2003	0.055	7.8%	y=1.75+1.04x
2 December 2003	0.158	4.3%	y=1.87+1.40x
6 January 2004	0.305	2.3%	y=1.86+0.64x

The 'best fit' relationships between plant damage and slug activity were obtained for the trapping periods from 3 to 14 November which all resulted in more than 20% of the variance explained (Table 12.22). This coincided with increased slug activity and moister soil following rainfall events in late October. These data suggest that the timing for post emergence pellet treatments could be more effectively managed with treatments being applied at the first indication of substantially increased slug activity. The regressions for 5 and 14 November are shown in Fig. 14a and 14b, respectively.



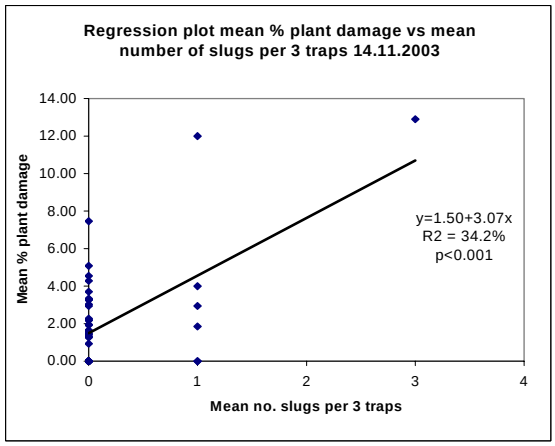


Figure 12.4a. Regression plot for mean percentage of plants damaged by slugs (y) and mean number of slugs per three traps on 5 November (x).	*Figure 124b. Regression plot for mean percentage of plants damaged by slugs (y) and mean number of slugs per three traps on 14 November (x).*

At the peak of slug activity recorded in the autumn on 5 November, the untreated mean catch was 0.83 slugs per three refuge traps where a post-drilling molluscicide application was not made. The regression equation y=1.61+1.73x, relating damage on oilseed rape plants to slug activity on 5 November, indicates 3.0% plant damage from 0.83 slugs per three traps. A doubling of slug number to a mean of 1.66 per three traps indicates that plant damage would be likely to increase to 4.5%. A low incidence of damage of 1.6% was indicated even if no slugs were trapped. A similar result was obtained for data obtained on 14 November. Examination of data for individual plots shows that, in a number of cases, slug damage was recorded when no slugs were trapped. On 14 November, a similar result was obtained. For this date, the untreated mean catch was 0.42 slugs per three refuge traps where a post-drilling molluscicide application was not made. The regression equation y=1.50+3.07x indicates 2.8% damage to oilseed rape plants from a mean of 0.42 slugs per three traps.

YEAR 4: 2004-05

Year 4 Objectives (winter wheat)

- Factor 1 comparisons: establishment using a reduced-cultivation, non-inversion technique compared with ploughing and discing.
- Slug pellets (metaldehyde, as Metarex) at 8.0 kg/ha applied as a pre-drilling treatment to oilseed rape stubble compared with no stubble treatment.
- Slug pellets (metaldehyde, as Metarex) at 8.0 kg/ha applied pre-emergence, post-emergence or pre and post emergence, compared with untreated controls.

Methods and site details

Treatment details are summarised in Table 12.23. Plot sizes were: main plots 48x12 m; sub plots 12x12m.Winter wheat cv Robigus was drilled into a moist seedbed on 13 November. The previous crop in harvest year 2004 was winter oilseed rape which followed winter wheat in harvest years 2002 and 2003 and preceded by oilseed rape in 2002.

Pre-drilling trapping on stubble, remaining from the previous oilseed rape crop, was undertaken in suitably moist condition between 31 August-1 September and 23-24 September 2004. Two traps were set-out on each of the molluscicide-untreated sub-plots 2, 15, 20, 29, 35, 46 (12 traps set in total). Slug trapping was conducted on each of the 48 sub-plots using three chicken-layers mash baited traps, 25 cm in diameter, on two occasions (25 October and 1 November) in the pre-drilling period and after the application of the stubble-applied metaldehyde treatments. Post-drilling slug trapping was conducted on each of the 48 sub-plots using three chicken-layers mash baited traps, 25 cm in diameter, on three occasions (18 November, 22 December and 18 January 2005, post-drilling winter wheat on 13 November.

Plant populations were assessed from 5 x 0.25 m^2 quadrats per sub-plot at GS 12 on 19 January 2005 and converted to number of plants/m^2 (Table 6). A quadrat method was used as the rows were not well defined. Data were converted to number of plants per m^2. Numbers and percentages of plants damaged by slugs were recorded (Tables 7 and 8 respectively).

The reduced, non-inversion tillage (Factor 1) treatment was undertaken on 1 November 2004 prior to drilling the test crop of winter wheat on 13 November 2004. The inversion treatment was achieved by ploughing and discing on 1 November. Drilling was done using a Moore drill with half the coulters removed to enable drilling to be possible into wet soil. An appropriate adjustment to the seed rate was made.

Slug pellet applications comprised metaldehyde slug pellets (Metarex, De Sangosse Ltd.) at a product rate of 8.0 kg/ha. Application was by hand pepper-potting over plots in two directions at right angles. The post-emergence treatment was applied on 22 December, at wheat growth stage 11, following overnight rain and warmer weather (maximum temperature up to 11 °C) following cold weather with slight overnight frosts).

The trial was analysed as a split block design with three factors, using Minitab.

Table 12.23. Summary of treatments for winter wheat trial in harvest year 2005.

Factor 1. Seedbed treatments	*Factor 2. Stubble treatment*	*Factor 3. Post-drilling molluscicide treatments*
1. Reduced cultivation (non-inversion tillage ('Tinemaster') on 01.11.2004.	1. untreated	1. untreated
2. Ploughing to invert soil followed by discing on 01.11.2004.	2. metaldehyde applied 21.10.2004.	2. pre-emergence metaldehyde applied 17.11.2004 (4 days after drilling)
		3. post-emergence metaldehyde applied 22.12.2004 at GS 11.
		4. pre + post metaldehyde applied on 17.11.2004 + 22.12.2004

Results

Pre-drilling refuge trapping

Trapping on all sub-plots was undertaken on two occasions (25 October and 1 November) between the application of the stubble-applied treatments and drilling the winter wheat crop. A summary of the effects on mean numbers of slugs from the seedbed treatments and the stubble-applied treatments of metaldehyde are summarised in Tables 12.24 and 12.25 respectively. A total of 345 and 267 slugs were recorded from refuge trap catches for trapping events on 25 October and 1 November respectively. The slug total comprised 500 *Deroceras reticulatum* and 112 *Arion* spp. (overall 82% *D. reticulatum).*

Table 12.24. Effects of seedbed treatments on the mean number of slugs per three refuge traps in the period before drilling.

Seedbed treatment	*Mean number of slugs per three refuge traps.*	
Factor 1	25.10.2004	01.11.2004
Reduced cultivation	8.88	6.21
Ploughed	5.50	4.92
SED (30 df)	1.292	0.703
LSD (p=0.05)	2.638	1.436
F prob. treatment	0.014	0.076
Cv%	62.2	43.8

Slug activity was initially low in dry seedbeds until heavier rainfall events occurred during October 2004. Although the pre-drilling cultivations were not undertaken until 1 November, mean numbers of slugs per three traps were significantly lower on the plots to be ploughed, compared to reduced-cultivation plots for the assessment made on 25 October (Table 12.24). Lower mean slug numbers were recorded on plots to be ploughed compared with the reduced cultivation on 1 November, but differences then were not significant at p=0.05 (see also Figure 1). Ploughing and cultivations were undertaken on 1 November on completion of the refuge trapping. These data suggest a longer-term effect persisting from the previous harvest year 2004. In this year, significantly lower numbers of slugs had been recorded on plots prepared by ploughing, compared with minimally-tilled and direct-drilled plots for assessments made on three dates in autumn 2003. This was prior to drilling the test crop of oilseed rape in the third year of the study. These data had shown a substantial effect in terms of reduction in slug activity on the soil surface as a result of seedbed preparation by ploughing, that had been done in dry conditions on 18 August 2003. With a financially-driven trend towards oilseed rape establishment using minimal-tillage or direct-drilling techniques, our data suggest that increased slug problems to subsequent crops may result from this change in cultivation practice.

Table 12.25. Effects of stubble treatments on the mean number of slugs per three refuge traps, in the period before drilling.

Stubble treatment	*Mean number of slugs per three refuge traps.*	
Factor 2	25.10.2004	01.11.2004
Untreated	10.83	7.88

Treated (metaldehyde)	3.54	3.25
SED (30 df)	1.292	0.703
LSD (p=0.05)	2.638	1.436
F prob. treatment	<0.001	<0.001
Cv%	62.2	43.8

The stubble treatment of metaldehyde was applied on 21 October 2004.

Significant reductions in mean number of slugs were obtained for assessments made on 25 October and 1 November 2004 on plots where metaldehyde had been applied to the stubble (Table 12.25), with slug activity was reduced by 67.3% and 58.8% respectively compared with the untreated means 10.8 and 7.9 slugs per three refuge traps. At this time (pre-drilling), there were no significant differences in slug numbers for the post-drilling treatments.

A significant regression was obtained for slug numbers under refuge traps (3 per plot) on 25 October and 1 November. Slug totals for the trapping event completed on 25 October showed a higher incidence of activity compared with activity on 1 November as shown in Figure 12.5.

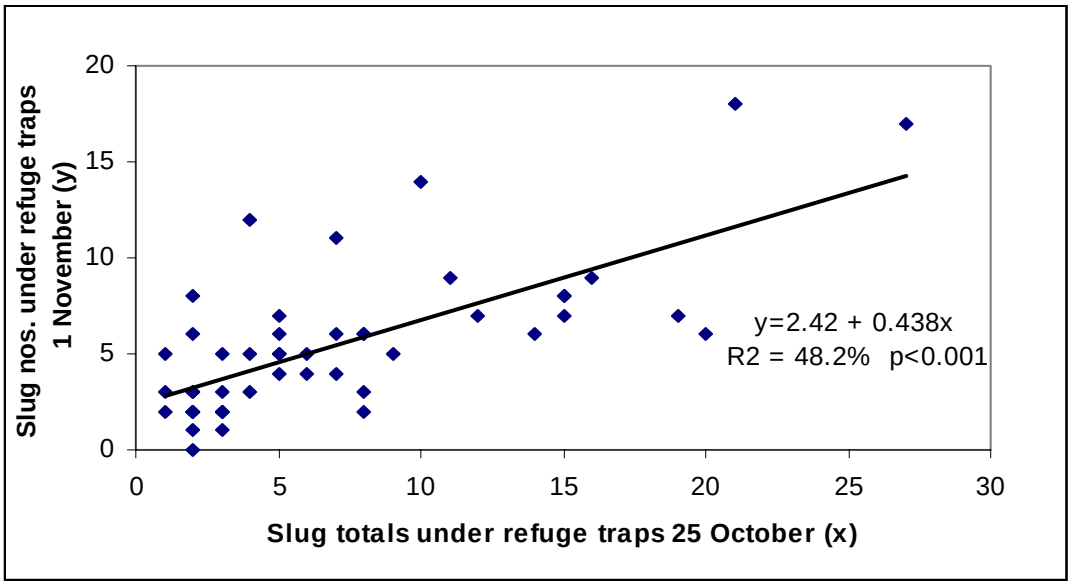


Figure 12.5. Regression plot for total slug numbers on 1 November (y) against numbers on 25 October (x)

Post-drilling refuge trapping:

Refuge trapping on the 48 sub plots was undertaken on three occasions, post-drilling winter wheat on 13 November and following application of the pre and post-emergence metaldehyde pellet treatments. Trapping periods were 17-18 November (during mild (maxima up to 11-12 °C), wet weather and preceding frosts); 21-22 December (a brief period of mild (maximum temperature up to 11 °C), wet weather after frosts) and 17-18 January 2005 (mild weather (maximum up to 10 °C) when the soil was moist. Slug activity was low and no treatment-related differences were obtained (data not presented). No slugs at all were recorded under 144 traps (3 per sub-plot) on 18 November; additional assessments in the week ending 3 December also showed a low incidence of slug activity (1 slug under 20 traps). On 18 January, a total of 6 slugs were recorded under 144 traps.

Plant population and damage assessments.

Plant populations for each of the 48 sub plots were assessed on 19 January 2005 (GS12) by counting winter wheat plants in five quadrats, each of area 0.25 m². Numbers of plants damaged by slugs were recorded. Data were expressed as mean number of plants/m², mean number of damaged plants/m² and mean percentage of plants damaged by slugs (Tables 12.26-12.28). Despite a substantial incidence of slug during late October and early November, slug damage to wheat plants was relatively slight.

Although significant regressions between plant population and percentages of plants damaged by slugs were obtained (Tables 9 and 10), this did not translate into a high incidence of slug damage to the wheat.

Table 12.26. Mean number of plants/m², mean number of slug-damaged plants/m² and mean percentage of plants damaged by slugs at assessment on 19 January 2005. Effects of seedbed treatments.

Seedbed treatment Factor 1	*Mean number of plants/m²*	*Mean number of slug-damaged plants/m²*	*Mean percentage of plants damaged by slugs*
Reduced cultivation	97.8	3.7	4.1
Plough	101.4	3.6	3.7
SED (30 df)	3.002	0.704	0.851
LSD (p=0.05)	6.130	1.437	1.737
F prob. treatment	0.244	0.963	0.658
Cv%	10.4	66.8	75.7

Table 12.27. Mean number of plants/m², mean number of slug-damaged plants/m² and mean percentage of plants damaged by slugs at assessment on 19 January 2005. Effects of stubble treatments.

Stubble treatment Factor 2	Mean number of plants/m²	Mean number of slug-damaged plants/m²	Mean percentage of plants damaged by slugs
Untreated	99.6	3.7	4.0
Metarex treated	99.6	3.6	3.8
SED (30 df)	3.002	0.704	0.851
LSD (p=0.05)	6.130	1.437	1.737
F prob. treatment	0.991	0.814	0.754
Cv%	10.4	66.8	75.7

Table 12.28. Mean number of plants/m², mean number of slug-damaged plants/m² and mean percentage of plants damaged by slugs at assessment on 19 January 2005. Effects of pre and post crop emergence pellet treatments.

Molluscicide treatment Factor 3	Mean number of plants/m²	Mean number of slug-damaged plants/m²	Mean percentage of plants damaged by slugs
Untreated	96.9	3.9	4.6
Pre emergence	98.0	5.1	5.4
Post emergence	101.5	2.7	2.8
Pre and post emergence	102.1	2.8	2.8
SED (30 df)	4.239	0.994	1.201
LSD (p=0.05)	8.656	2.030	2.452
F prob. treatment	0.552	0.071	0.101
Cv%	10.4	66.8	75.7

Various regressions were tested between percentages of plants damaged by slugs at assessment on 19 January against slug activity recorded under refuge traps for two autumn-trapping periods and between plant population on 19 January against slug activity under refuge traps. Significant relationships between plant damage and slug activity were obtained for the trapping periods completed on 25 October and 1 November,

although the percentage variance explained was only 16.8% and 15.5% respectively (Table 12.29). These trapping events coincided with increased slug activity and moister soil following rainfall during October.

Table 12.29. Test regressions between percentage plants damaged (y) and slugs under refuge traps (x) for two assessment dates on 25 October and 1 November 2004.

Test regression between percentage plants damaged (y) and slugs under refuge traps (x) for assessment dates:	p value	R^2	Equation
25 October 2004	0.004	16.8%	y=2.16 + 0.240x
1 November 2004	0.006	15.5%	y=1.85+0.366x

Significant relationships between plant population and slug activity were obtained for the trapping periods completed on 25 October and 1 November, although the percentage variance explained was only 6.5% and 11.3% respectively (Table 12.30).

Table 12.30. Test regressions between numbers of plants/m² (y) and slugs under refuge traps (x) for two assessment dates on 25 October and 1 November 2004.

Test regression between plant population (y) and slugs under refuge traps (x) for assessment dates:	p value	R^2	Equation
25 October 2004	0.080	6.5%	y=103 - 0.501x
1 November 2004	0.020	11.3%	y=105 – 1.04x

An improved relationship with 27.9% of the variance explained was obtained between plant population and percentage of plants damaged by slugs (Figure 12.6). The equation ($y = 106 - 1.77x$ ($p<0.001$)) could indicate that an increase in slug damage from 0% to 10% resulted in a 16.7% decrease in plant population.

Alternatively, however, this regression could indicate that, where there were fewer plants initially, a greater percentage of plants were damaged as a consequence. In either case, this is indicative of the potentially high risk of slug damage on a late-drilled (13 November) winter wheat crop assuming that conditions at and after drilling were favourable for slug activity.

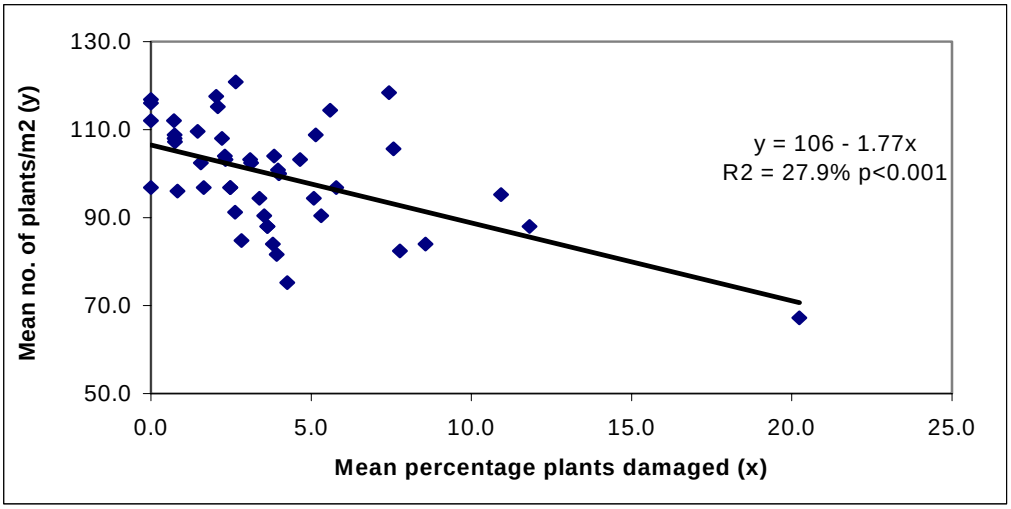


Figure 12.6. Regression plot for plant population (y) and percentage of plants damaged by slugs (x).

No significant interactions or differences were recorded for plant population, damaged plants per m² or mean percentage of plants damaged.

Acknowledgements

The work described here is part of a Defra-sponsored project in the Sustainable Arable LINK Programme. The industry partners are Bayer CropScience Ltd, CropTech, De Sangosse UK, Godfrey Farms Ltd, the Home-Grown Cereals Authority and Lonza Ltd. The academic partners are ADAS UK Ltd, Rothamsted Research and the University of Newcastle. DEFRA is the government sponsor.

PAPER 13 – *Objective 2*

Forecasting slug populations in arable crops using individual-based simulation models

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Abstract

Two individual-based simulation models were used to predict *Deroceras reticulatum* populations and the outputs were compared with the dynamics of actual populations in arable fields. The first model was used to make predictions over a series of 6-week periods (considered to be sufficient for practical pest forecasting) and these were compared with the numbers of slugs recorded on an arable field in set-aside in north-east England. Using the daily weather for the period of study, the model predictions closely matched the measured population levels. However, when using predicted weather (based on averages), the predictions of the model were sometimes different from the measured populations. This finding emphasises the difficulty of predicting slug populations when future weather cannot be accurately forecast more than a few days ahead. The predictions of the first model were also compared in the same way with data on *D. reticulatum* populations from an arable field in south-west England. Even when the model was run using the daily weather for the period of study, the model predictions diverged from the actual population in three out of four six-week periods. Possible reasons for these discrepancies were examined and it was concluded that incorrect estimation of the egg bank by the model was the most likely explanation. A second simulation model was compared with data on *D. reticulatum* populations in cereal fields going into oilseed rape in south-west England. Once again lack of knowledge of the size of the egg bank made it necessary to estimate this. However, by adjusting the estimated egg densities it was possible to obtain good predictions of the densities of *D. reticulatum* up to mid August, but from then on the model overestimated the slug population by a considerable margin. We conclude that the population models can be used, together with field assessments of slug populations to provide useful estimates of *D. reticulatum* activity and population development, up to the time of autumn cultivation, provided that the models are run using actual daily weather data. These models would need further development to be used as decision support tools.

Introduction

Previously it has not been possible to predict accurately the risk of slug damage to wheat (Glen *et al*., 1993), partly because unreliable empirical methods have had to be used, due to a lack of detailed understanding of slug population dynamics. However, short term forecasting (over a few days) is possible as slug activity is known to be considerably influenced by weather conditions (Young *et al*., 1991) and agronomic practices. To tackle the problem of longer term forecasting, a framework for modelling the population dynamics of slugs to explain and predict effects of weather, farming practices and the role of predators, parasites and pathogens in arable crops has been developed (Bohan *et al*., 1997; Shirley *et al*., 1997, 1998). This framework has been used to develop a model which simulates changes in populations of slugs taking into account growth rates, fecundity and mortality together with meteorological data (Shirley *et al*., 2001).

Choi *et al*., (2006) have produced a second, spatially explicit individual based model (IbM) to study the dynamics of a population of *D. reticulatum*. This IbM establishes a virtual field to simulate slug spatial dynamics and changes in abundance, based on the dependence of slug population dynamics on environmental conditions (temperature and moisture) as shown by Choi *et al*. (2004) for a population of *D. reticulatum* in continuous winter wheat.

In this paper we report tests of both models (Shirley *et al.*, 2001; Choi *et al.*, 2006) to assess how accurately they predicted slug populations. An accurate prediction would allow a farmer to sample the slug population at a convenient time and then to produce an estimate of the size of that population at a time when the slugs might be causing damage to a crop and, if necessary, allow the farmer to take pre-emptive action.

Materials and methods

The Slug Population Dynamics Individual-based Simulation Model (SPoD-ISM) (Shirley *et al*., 2001) is a predictive model, designed to "forecast" future slug populations when the model is started with a measured population. The model was run, using as a start point data collected using defined area traps on an arable field in set-aside in north-east England. Successive runs were done for several months using the actual daily weather for the simulations. Each run lasted 6 weeks, and was the mean of 10 replicates. This process was then repeated, but using predicted weather (based on long-term averages). A final set of runs was done using as start points data collected using soil flooding from an arable field in south-west England together with actual daily weather. Some of the results suggested a poor fit of the model output to the field data and to identify the cause of these further analyses were done with sub-sets of the data.

The individual-based simulation model of Choi *et al.* (2006) was also run with data from an arable field in south-west England. With this model the numbers of slugs are simulated in four size classes ((1)eggs, (2) neonates (1-10mg), (3) juveniles (11-100mg) and (4) adults). The numbers of each size class were set at the start of March. There were 20 replicates run for each starting condition.

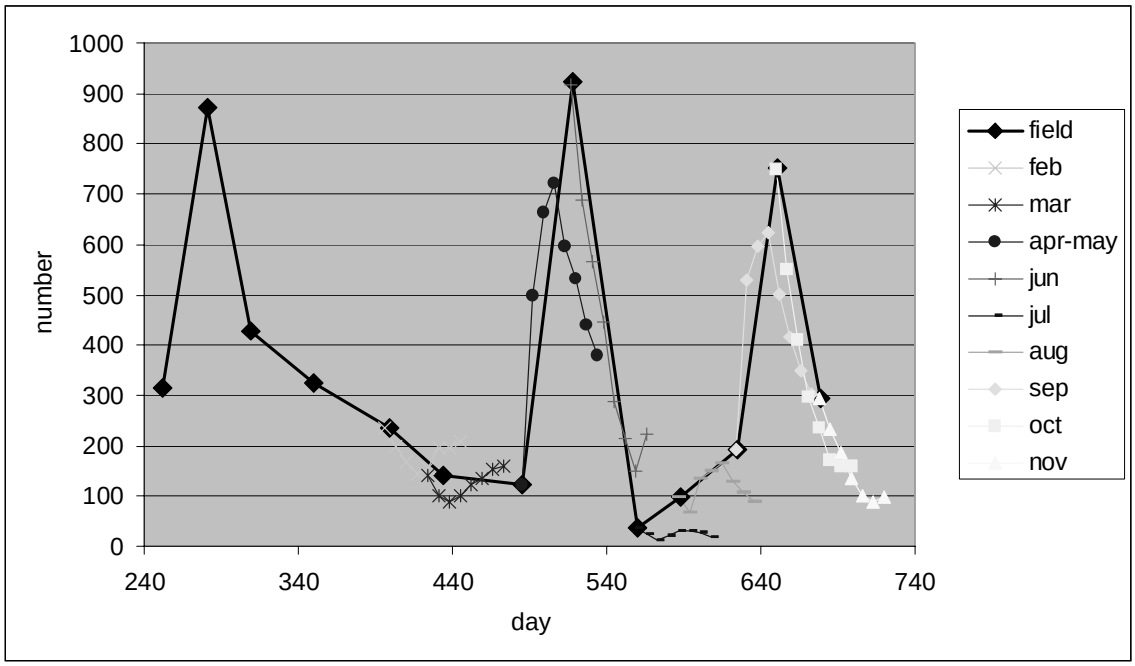


Figure 13.1: Numbers of Deroceras reticulatum *recorded in defined area traps from a field in north-east England together with the populations predicted by the Slug Population Dynamics Individual-based Simulation Model run with recorded weather. Each model output is the average of ten runs for a six week period after starting with the known numbers of slugs sampled from the field.*

Results

When the SPoD-ISM model was run using recorded weather data the predicted slug populations matched closely the actual populations observed in the field (Figure 13.1). When predicted weather (based on long-term averages), rather than actual weather was used to run the SPoD-ISM model the predicted population trends sometimes did not show peaks of abundance when the observed field population did (Figure 13.2).

The SPoD-ISM model was run with data from a site in south-west England, again using recorded weather data. However, the fit of the predictions to the observed populations was poor (Figure 13.3). To examine whether this poor fit was due to recruitment of neonate and juvenile slugs the runs were

repeated, but this time only using large slugs (> 100 mg). The fit between observed and predicted populations was much better (Figure 13.4).

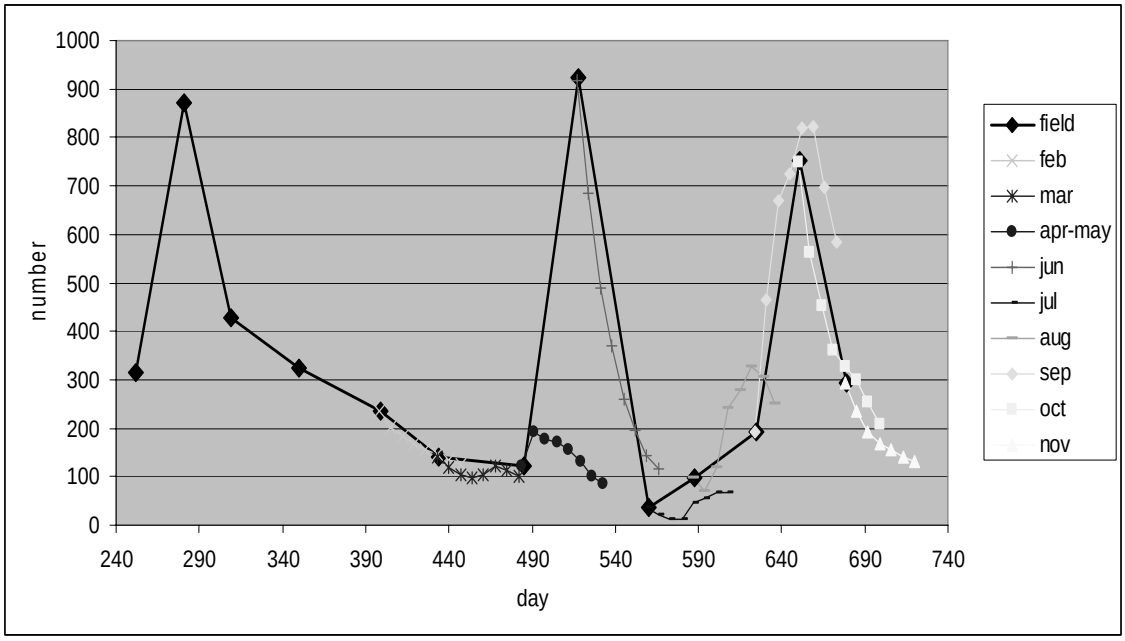


Figure 13.2: Numbers of Deroceras reticulatum *recorded in defined area traps from a field in north-east England together with the populations predicted by the Slug Population Dynamics Individual-based Simulation Model run with predicted weather. Each model output is the average of ten runs for a six week period after starting with the known numbers of slugs sampled from the field.*

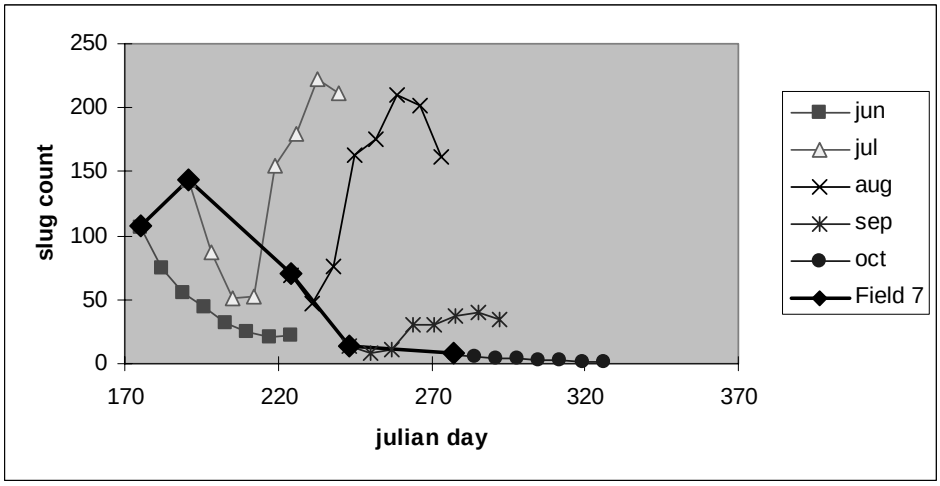


Figure 13.3: Numbers of Deroceras reticulatum *recorded by soil flooding from a field in south-west England together with the populations predicted by the Slug Population Dynamics Individual-based Simulation Model run with recorded weather. Each model output is the average of ten runs for a six week period after starting with the known numbers of slugs sampled from the field.*

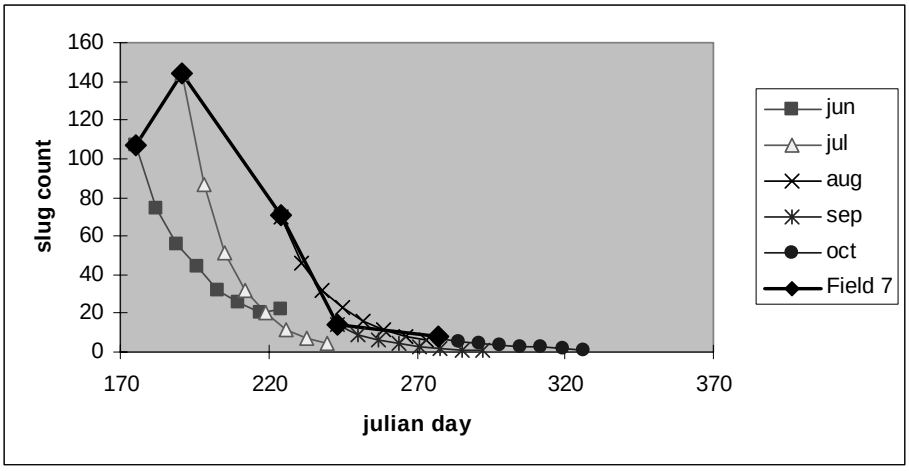


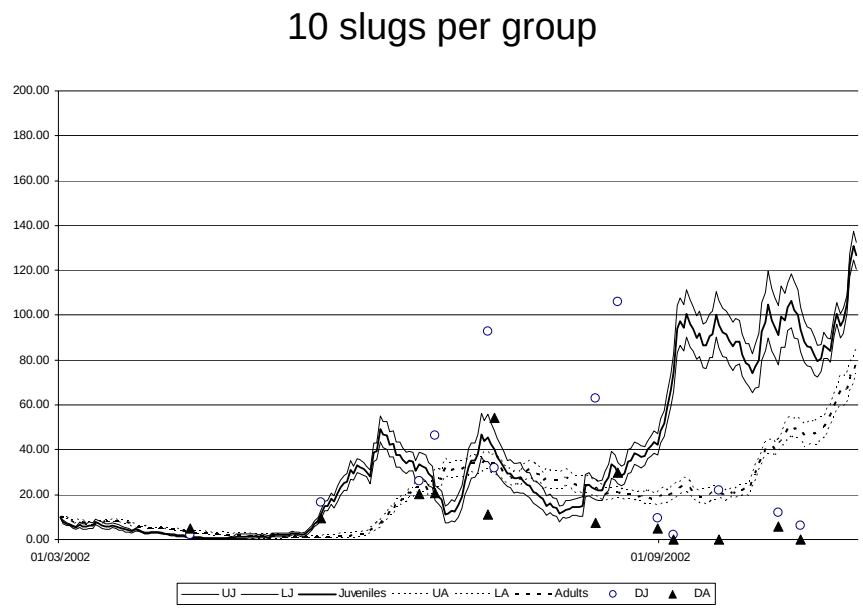
Figure 13.4: Numbers of large (>100 mg) Deroceras reticulatum *recorded by soil flooding from a field in south-west England together with the populations predicted by the Slug Population Dynamics Individual-based Simulation Model run with recorded weather. Each model output is the average of ten runs for a six week period after starting with the known numbers of slugs sampled from the field.*

The simulation model of Choi *et al*. (2006) was started at the beginning of March with a population of 10 slugs in each group/m^2 (eggs, neonatal slugs, juveniles and adults). In this condition, it underestimated the size of the *D. reticulatum* population in summer, then overestimated populion density in autumn (Fig. 13.5a). When the initial number of eggs was increased to 100/m^2 , the model provided a good simulation of slug abundance during the summer but considerably overestimated slug abundance in autumn (Fig. 13.5b).

Discussion

The fit to the observed data found with both models provides some validation of them as good descriptions of slug biology and underscores the utility of such models for describing changes in population abundance over time. The good fit of the SPoD-ISM model to the north-east England field data, when run with recorded weather was expected, as the model was constructed using data from similar sites in the same region. When the model was run with predicted weather data the predictions were not so reliable. This was because the predictions were based on long term averages and did not take account of unusual periods of weather that were favourable to slugs allowing the population to increase in April-May and August of the year used for the test. These periods of weather favourable to slugs are impossible to forecast far in advance and this finding emphasises the difficulty of predicting slug populations more than a few days ahead.

(a)



(b)

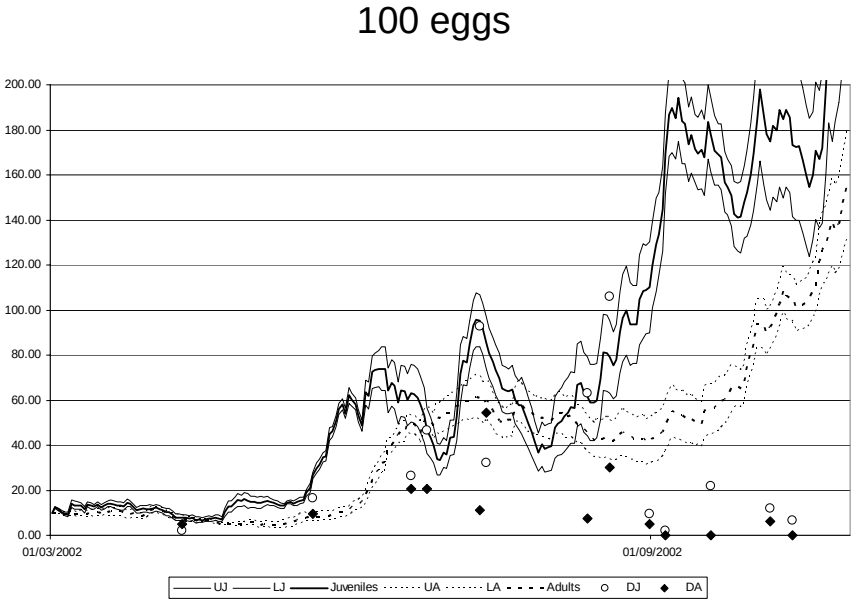


Figure 13.5: Numbers of Deroceras reticulatum *recorded by soil flooding from a field in south-west England together with simulated abundance of the juvenile and adult slug population, using the model of Choi et al. (2006) run with recorded weather. The solid line represents the numbers of simulated juveniles (—), whilst the dotted line is the simulated adults (. . .). Observed juveniles are plotted as open circles (_) and closed triangles (a) or diamonds (b) represent the observed count of adults.*

When the SPoD-ISM model was run with data from the site in south-west England the fit of the predictions to the observed populations was poor. The runs which begin in July, August and to some extent September did not match populations observed at this site. For the last two runs this is explained, in part, by the fact that the field was cultivated during that period and this would have made a very large difference to the numbers of slugs surviving. When only the large slugs were modelled (Figure 13.4) the predicted increases in model population size, which were not matched by the field data, were absent and were therefore due to recruitment by "hatching of eggs" in the model. The models assumptions for recruitment from egg hatch are based on data from north-east England, and these are presumably not applicable for south-west England (which may have a peak in hatching a month earlier than the north-east).

The individual based model of Choi *et al*., (2006), following a simple change in the egg population abundance, but no change in the model structure or assumptions, was found to fit well to data from the south-west England site up until the time of cultivation.

To make either model applicable to different sites it will be important to quantify the recruitment to slug populations from eggs hatching. It is possible to sample the numbers of eggs present in the soil, but the process is extremely laborious and far beyond the capacity of farmers and advisors. One potential solution to the problem is to estimate the egg bank from the numbers of reproductively active slugs in the population, which can be sampled much more easily. In Chapter 4 we have described the relationship between body weight and maturity in slugs and from this analysis it may be possible to use the weight distribution of slugs in traps to calculate potential egg bank size.

We have not built into the models the facility for predicting the impacts of cultivations or other control interventions. This is because the impacts of these interventions on population size are extremely variable, partly depending on method of cultivation and partly on the weather conditions during and after intervention (Papers 5, 7 & 12, this report). Consequently there are currently insufficient data to make reliable estimates of these impacts.

Weather conditions have a dramatic effect on slug populations, as illustrated by the difference between populations estimated from the models run with recorded and predicted weather (Figures 13.1 and 13.2). This makes predicting future populations a risky business and unless precise local forecasts of weather are available for some time in the future this problem will be difficult to overcome. One solution that may be helpful in using these models for decision support would be to use the model to predict future slug populations for three weather scenarios: average weather, consistently good

weather for slugs, and consistently poor weather for slugs. This would give the farmer or advisor the ability to see what future slug populations might be in normal, worst case and best case scenarios.

At present the population models can be used, together with field assessments of slug populations to provide useful estimates of *D. reticulatum* activity and population development, up to the time of autumn cultivation, provided that the models are run using actual daily weather data. These models would need further development to be used as decision support tools.

Acknowledgements

The work described here is part of a Defra-sponsored project in the Sustainable Arable LINK Programme. The industry partners are Bayer CropScience Ltd, CropTech, De Sangosse UK, Godfrey Farms Ltd, the Home-Grown Cereals Authority and Lonza Ltd. The academic partners are ADAS UK Ltd, Rothamsted Research and the University of Newcastle. DEFRA is the government sponsor. We thank all the partners in this project, together with A Craig who helped with field work.

PAPER 14 – *Objective 3*

Using Traps with Non-Toxic Bait to Assess Slug Activity and Damage Risk

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Abstract

Traps baited with molluscicide pellets have, for some years, been used to assess the risk of slug damage to winter wheat; a catch of four or more slugs per trap over three nights in the period before cultivation indicates a potential risk of slug damage to a following crop of winter wheat. However, molluscicide baits are undesirable in traps. Chicken layers' mash is known to be an effective non-toxic bait, but catches in traps with this bait have not been related to damage levels in winter wheat. We compared the numbers of *Deroceras reticulatum* (the commonest slug species in arable crops) in traps baited with chicken layers' mash with the numbers in traps baited with molluscicide pellets to calibrate traps with this non-toxic bait for damage forecasting in winter wheat. We put out traps in standing cereal crops and in stubble, from May to October 2002, in Cambridgeshire, Northumberland and Somerset. When weather was suitable for slug activity (mild temperatures and the soil surface moist throughout the trapping period) and when the numbers in traps baited with molluscicide pellets was around the threshold value of four slugs per traps after three nights, slug catches in traps baited with chicken layers' mash after one night were similar to this number. We conclude that a threshold of four slugs per trap baited with chicken layers' mash, left overnight, can be used as the basis of a system for assessing the risk of slug damage to winter wheat. Thus, traps with this non-toxic bait provide a quicker and safer assessment of slug damage risk than traps baited with molluscicide pellets. When the soil surface dried during the trapping period, the numbers of *D. reticulatum* in traps baited with chicken layers' mash were lower than in pellet-baited traps, but numbers declined in both trap types over a three night period, emphasising the importance of trapping under suitable soil and weather conditions, and the advantage of trapping over a one-night period. In one field, a mean of 1.6 *Deroceras laeve* was recorded in mash-baited traps after one night, whereas significantly fewer (0.3/trap) were recorded in molluscicide-baited traps after three nights. In a second field, a mean of about one *Arion subfuscus* was recorded in mash-baited traps after one night, whereas significantly more (2.7/trap) were recorded in molluscicide-baited traps after three nights. This indicates that the comparative efficiency of traps differs between slug species and this must be borne in mind.

Introduction

Slugs are major pests of winter wheat at establishment and it is important to assess the risk of damage so that control measures can be applied if necessary, before seeds and seedlings have been killed (Glen & Moens, 2002). In a study of the use of slug traps for forecasting slug damage to winter wheat in the United Kingdom (Glen *et al.*, 1993), the best predictor of the percentage of wheat seeds killed was the peak numbers of slugs trapped before cultivation. In that study, traps were baited with molluscicide pellets and left in the field for a period of three nights before examination. The relationship between trap catch and wheat kill was highly significant but also highly variable, so Glen *et al.* (1993) used the upper limit of the relationship between trap catch and the percentage of wheat seeds killed to identify a threshold catch of four or more slugs per trap over a three-night period, which indicated a potential risk of economically significant damage (defined as 10% or more seeds killed by slugs). However, molluscicide pellet baits are undesirable in traps because in concentrated amounts they are a potential hazard to pets and wildlife.

Young *et al.* (1996) compared several different non-toxic materials as bait in slug taps: wheat bran, whole wheat grains, blank molluscicide pellets without active ingredient, chicken layers' mash (commercial poultry food), sliced apple, sliced raw potato, whole cabbage leaves, beer placed in a shallow dish, sliced carrot and meat-based tinned cat food. *Deroceras reticulatum* (Müller) was the main species present and Young *et al.* (1996) concluded that chicken layer's mash was the most effective bait. However, slug number in traps baited with chicken layers' mash have not been calibrated to damage levels in winter wheat. In order to do this, we compared slug catches in traps baited with chicken layer's mash with numbers in traps baited with molluscicide pellets at several sites in both standing crops of cereals before harvest and in the stubble of cereal and oilseed rape crops, with traps examined after one night and three nights.

Materials and methods

Traps baited with molluscicide pellets were as used by Glen *et al.* (1993): upturned flowerpot saucers, terracotta coloured, 18 cm diameter, with 5 ml of methiocarb pellets placed in a small heap on the soil in the middle of the area covered by the traps (Draza, Bayer CropScience, 4% a.i. w/w was used in Cambridgeshire and Somerset; Rivet, Bayer CropScience, 3% a.i. w/w was used in Northumberland). Traps baited with chicken layers' mash were upturned flowerpot saucers, terracotta coloured, 25 cm diameter, with 20 ml of chicken layers' mash placed in a small heap on the soil in the middle of the area to be covered by the trap. This quantity of mash was chosen as it was found in preliminary studies that it was unlikely to be completely consumed overnight even in field sites with large numbers of slugs present. The trap size (25 cm diameter) was chosen as being the largest size that can readily be placed between the stems of standing cereal crops. Traps were placed in the field from May to October 2002, in the afternoon or early evening on days when the soil surface was visibly moist, and temperature was in the range 5 – 25^{o}C, indicating that soil conditions were suitable for slug activity on the soil surface (Young *et al.*, 1991). At the time of trapping, no slug pellet treatments had been applied to any of the study sites.

In four fields in Somerset, at least ten traps of each type were placed in standing cereal crops from May to July 2002 and, in four fields, in the stubble after harvest in August. In Northumberland, 50 traps of each type were placed in the stubble in two fields (Field 1 on 15 October and Field 2 on 29 October 2002). In fields in Somerset and Northumberland, traps were placed in pairs, one trap baited with molluscicide pellets and the other baited with chicken layers' mash, with 2 m between each trap in a pair and 10-20 m between pairs of traps. All traps were examined after one night. Traps baited with molluscicide pellets were always left and examined again after three nights in the field. Traps baited with chicken layers' mash were also normally left *in situ* and examined after three nights.

At ADAS Boxworth, Cambridgeshire, three traps baited with chicken-layers' mash, 25 cm in diameter, and three methiocarb-baited traps, 18 cm in diameter were placed in winter wheat stubble on each of 48 plots within a long-term field experiment on integrated control of slugs (This Report, Paper 12). Traps were put out after heavy rain fell on 9 September 2002. Mash-baited traps were checked after one and three nights on 10 and 12 September respectively. Molluscicide-baited traps were checked after three nights on 12 September. Soils were moist after rain when the traps were checked on 10 September, but became drier by 12 September following subsequent dry, warm weather

In order to assess whether the difference in trap size between traps baited with chicken layers' mash or molluscicide pellets would influence the numbers of slugs recorded, traps of 18 cm diameter and 25 cm diameter were baited with chicken layers' mash and placed in cereal stubble in two fields in Somerset on 1 and 5 August 2002, with six traps of each type in each field in a randomised design.

Results

By far the most abundant slug species in the traps was *Deroceras reticulatum* and, at all but two sites, analyses were confined to this species because only a few individuals of other species were recorded. The exceptions were: Bulldozer Field, Somerset, where *Deroceras laeve* (Müller) was present and Field 1 Northumberland, where *Arion subfuscus* (Draparnaud) was present.

Results for *D. reticulatum* were first analysed separately for each field. For traps placed in standing cereal crops in Somerset, there was no evidence of any difference between dates and field sites, so all sites and dates were analysed together. Similarly, for traps placed in cereal stubble in Somerset, there was no evidence of any difference between field sites for traps put out on the same date, so sites trapped on the same date were analysed together. However, results differed between traps put out on different dates (9 August or 19 August 2002) in cereal stubble, so these dates were analysed separately. The results of regression analyses are summarised in Table 14.1 for all sites and dates when conditions remained suitable for trapping for at least the first overnight period.

Table 14.1: Summary of the parameters for regressions between numbers of Deroceras reticulatum *per trap baited with chicken layers' mash after one night or three nights (Mash 1 or Mash 3, respectively) and per trap baited with molluscicide pellets after one and three nights (Moll 1 or Moll 3, respectively) at different sites when conditions were suitable for tapping in 2002.*

Date(s)	*Site(s) & crop*	*y-value*	*x-value*	*n*	*Prob.*	*Regr. Coeff.*	*S.E.*	*Regr. Const*	*S.E.*	*Prob. <0*	*y-value when x = 4*
May-July	Somerset Cereals	Mash 1	Moll. 1	75	<0.001	1.020	0.098	5.26	1.21	<0.001	
9 Aug	Somerset Stubble	Mash 1	Moll. 1	19	NS	-	-	4.95	2.05	<0.05	
9 Sept	Cambs. Stubble	Mash 1	Moll. 1	0	-	-	-	-	-	-	
15 Oct.	Northumb. Stubble 1	Mash 1	Moll. 1	50	<0.001	0.679	0.168	2.62	0.76	0.001	
29 Oct.	Northumb. Stubble 2	Mash 1	Moll. 1	50	<0.01	0.629	0.192	3.99	0.98	<0.001	
May-July	Somerset Cereals	Mash 1	Moll. 3	75	<0.001	0.589	0.053	2.89	1.22	<0.05	5.3
9 Aug	Somerset Stubble	Mash 1	Moll. 3	19	NS	-	-	6.27	2.14	<0.01	
9 Sept	Cambs. Stubble	Mash 1	Moll. 3	48	0.003	0.445	0.141	1.132	0.398	0.007	2.9
15 Oct.	Northumb. Stubble 1	Mash 1	Moll. 3	50	<0.001	0.586	0.128	2.07	0.79	0.01	4.4
29 Oct.	Northumb. Stubble 2	Mash 1	Moll. 3	50	<0.01	0.259	0.089	2.95	1.38	<0.05	4.0
May-July	Somerset Cereals	Moll. 3	Moll. 1	75	<0.001	1.444	0.103	5.39	1.27	<0.001	
9 Sept	Cambs. Stubble	Moll. 3	Moll. 1	-	-	-	-	-	-	-	
15 Oct.	Northumb. Stubble 1	Moll. 3	Moll. 1	50	<0.001	1.091	0.093	1.16	0.42	<0.01	
29 Oct.	Northumb. Stubble 2	Moll. 3	Moll. 1	50	<0.001	1.512	0.232	7.86	1.19	<0.001	
May-July	Somerset Cereals	Mash 3	Mash 1	75	NS	-	-	-	-	-	
9 Sept	Cambs. Stubble	Mash 3	Mash 1	48	<0.001	0.200	0.052	0.175	0.151	0.255	
15 Oct.	Northumb. Stubble 1	Mash 3	Mash 1	50	0.01	0.150	0.059	1.69	0.37	<0.001	
29 Oct.	Northumb. Stubble 2	Mash 3	Mash 1	50	0.01	0.316	0.118	6.149	0.94	<0.001	

On all but one occasion when trapping was done under conditions suitable for slug activity and when both types of traps were examined after both one night and three nights, the numbers of *D. reticulatum* recorded

overnight in traps baited with chicken layers' mash were strongly correlated with numbers recorded after one night and three nights in traps baited with slug pellets (Fig. 14.1a and Fig. 14.1b, respectively, for traps in standing cereal crops in Somerset; Fig. 14. 2a and Fig. 14.2b, respectively for traps in stubble in Field 1 Northumberland; Fig. 14.3a and Fig. 14.3b, respectively, for traps in field 2 Northumberland). In stubble in Cambridgeshire, where molluscicide-baited traps were examined after three nights only, slug numbers in mash-baited traps after one night were significantly correlated with catches under methiocarb-baited traps after three nights (Figure 14.4).

For each of the regressions between mash-baited traps (y-axis) and molluscicide-baited traps (x-axis), the constant (value of the intercept on the y-axis) was significantly greater than zero (Table 14.1). The regression coefficients were not significantly different from 1 when trap catches were compared after one night for both types of bait. Thus, after one night, traps baited with chicken layers' mash recorded, on average, about three to five slugs per trap when traps baited with molluscicide pellets recorded no slugs after one night (Fig. 14.1a, Fig. 14.2a, Fig. 14.3.a). This relative difference between trap catches was clearly maintained throughout the entire range of trap catches in Somerset (Fig. 14.1a) and there was no significant evidence of a relative change in Cambridgeshire or Northumberland. However, when numbers after one night in mash-baited traps were compared with numbers in molluscicide-baited traps after three nights (Fig. 14.1b, Fig. 14.2b, Fig. 14.3b, Fig. 14.4a) the regression coefficients were significantly less than 1 ($P < 0.001$ for Cambridgeshire, Somerset and Field 2, Northumberland; $P < 0.01$ for Field 1 Northumberland). Thus, as slug numbers increased, the trap catch increased more gently in mash-baited traps after one night compared to traps baited with molluscicide after three nights and, at high numbers, the latter traps caught more than mash-baited traps. However, the comparison of high slug numbers in traps of different types is less relevant to damage forecasting than the comparison at lower threshold numbers. The regression lines indicate that a threshold trap catch of four *D. reticulatum* in the traps baited with molluscicide pellets after three nights was equivalent to a catch of three to five slugs overnight in the traps baited with chicken layers' mash (Table 14.1).

For traps baited with molluscicide pellets, there were strong linear relationships between numbers of *D. reticulatum* in traps numbers after three nights (y-axis) and after one night (x-axis) at the three sites where this was measured (Table 14.1). This relationship is shown in Fig. 14.5a for traps in standing cereal crops in Somerset. The regression constants were highly significant in all cases. The regression coefficient was significantly greater than one in standing cereals in Somerset ($P < 0.001$) and in Field 2 Northumberland ($P < 0.05$), but not significantly different from one in Field 1 Northumberland. Thus, slug numbers after three nights were greater than numbers after one night and, at two out of three sites, this difference increased as numbers per trap increased.

When the traps baited with chicken layers' mash were left in the field, following examination after one night (with the slugs undisturbed beneath the traps) and examined again after three nights, the result were different

from the above comparison of catches in molluscicide traps after the same intervals. In those fields of standing cereals in Somerset where slug numbers were relatively low after one night (up to about 11 slug/trap), the catches in traps baited with chicken layers' mash after three nights were similar to these after one night, with no evidence of a difference from a 1:1 relationship (Fig. 14.5b). However, in fields of standing cereals with high slug numbers (13-50 slugs per trap) after one night, numbers after three nights were consistently lower than after one night, presumably because the trap bait had become exhausted. In stubble in Cambridgeshire, where traps were put out after heavy rain on 9 September, traps were checked early in the morning of 10 September before the traps heated up in sunlight and the mean number of slugs in mash-baited traps after one night was 0.7. Dry weather followed on 10-12 September and the catch fell to a mean of 0.2 slugs per trap after three nights as the soil became drier. A significant ($P < 0.001$) regression was obtained for mean number of slugs per trap after one night compared with mean numbers after three nights' trapping (Figure 14.6) with a regression coefficient significantly less than one and regression constant significantly greater than zero. Similarly, for the two fields in Northumberland, there were significant regressions between numbers per mash-baited trap after three nights (y-axis) and one night (x-axis), with regression coefficients significantly less than one ($P < 0.001$). In both cases the regression constant was significantly greater than zero.

Table 14.2: Mean numbers of Deroceras reticulatum *and* Deroceras laeve *per trap baited with chicken layers' mash or molluscicide pellets after one and three nights in stubble. Traps were put out on 9 August. LSD is the least significant difference between numbers in traps with different baits.*

Slug species and stubble type	Interval before examination	Bait		Significance	LSD
		Mash	*Molluscicide*		
Deroceras reticulatum *Cereal & oilseed rape*	One night	7.0	5.6	NS	2.8
	Three nights	-	8.3	NS	2.5
Deroceras laeve *Oilseed rape*	One night	1.6	0.7	0.05	0.9
	Three nights	-	0.3	<0.01	0.8

For ten traps put out in cereal stubble and nine traps put out in oilseed rape stubble on 9 August in two fields in Somerset, there was no significant correlation between the numbers of *D. reticulatum* recorded overnight in traps baited with chicken layers' mash and numbers recorded after one night or three nights in traps baited with slug pellets. Analysis of variance showed no significant differences in numbers of *D. reticulatum* between mash-baited traps and pellet-baited traps after one or three nights (Table 14.2). *Deroceras laeve* was also recorded in the field with oilseed rape stubble, with significantly more of this species in mash-baited traps after one night compared to pellet-baited traps after one or three nights (Table 14.2).

In Field 1, Northumberland, small numbers of *A. subfuscus* were recorded. Numbers in mash-baited traps were not significantly correlated with numbers in traps baited with molluscicide. Analysis of variance showed significant effects of bait and the time interval before examination, with a significant interaction between these two factors ($P < 0.001$, Table 14.3). Numbers were similar after one night in the traps with both baits. After three nights, numbers in traps baited with mash had not changed significantly, but numbers had increased significantly (by a factor of 2.5) in molluscicide-baited traps.

Table 14.3: Mean numbers of Arion subfuscus *per trap baited with chicken layers' mash or molluscicide pellets after one and three nights in stubble, Northumberland. Traps were put out on 15 October.*

Interval before examination	*Bait*	
	Mash	*Molluscicide*
One night	1.0	1.1
Three nights	0.8	2.7
LSD	0.6 (0.7 for same interval before examination)	

The results presented above all relate to occasions when the soil surface remained moist for at least the first night after the traps were placed in the field. In contrast, traps were put out on 19 August 2002 in stubble in two fields where the soil surface was moist when the traps were put out, but dried overnight. Moreover, the traps were exposed to direct sun during the following days. Slug numbers (*D. reticulatum*) were relatively low and there were no significant correlations between catches in the traps with different baits. However, analysis of variance (Table 14.4) showed that catches in the traps baited with chicken layers' mash were only about half those in the traps baited with slug pellets ($P < 0.01$). Moreover, the mean numbers in traps with both baits declined from one night to three nights after the traps were put in place ($P < 0.01$), showing the importance of suitable weather during the trapping period. There was no evidence of a significant interaction between bait type and the interval before the traps were examined.

Table 14.4: Mean numbers of Deroceras reticulatum *per trap baited with chicken layers' mash or molluscicide pellets after one and three nights in cereal stubble. Traps were put out on 19 August and dry sunny weather followed.*

Interval before examination	*Bait*		*Mean*
	Mash	*Molluscicide*	
One night	2.0	4.6	3.28
Three nights	1.5	2.1	1.83
Mean	1.76	3.34	
LSD (for means)	1.1		1.0

The comparison of traps of 18 cm diameter and 25 cm diameter showed no significant differences between numbers of slugs trapped (mean numbers 2.5 and 2.0 per trap respectively) after one night.

Discussion

Refuge traps, with or without bait with have been used in several studies for monitoring slug populations (see review by South (1992). These studies have shown that traps exaggerate the proportion of surface-dwelling slugs in the population (Hunter, 1966) or larger slugs of a given species (South, 1964; Glen & Wiltshire, 1986; Paper 5, this report). The numbers recorded are also highly dependent on environmental conditions, principally soil surface moisture and temperature (Young *et al.,* 1991). Despite their disadvantages, refuge traps are convenient to use compared with other methods of estimating slug populations. This has resulted in the widespread use of traps, especially traps baited with molluscicide pellets (metaldehyde or methiocarb) for assessing the risk of slug damage in arable and horticultural crops. However, there have been a number of incidents of accidental poisoning of pets and wildlife as a result of the concentrated amounts of molluscicide pellets in traps and non-toxic baits would be preferable.

Under suitable conditions for slug activity and with numbers per trap at around the threshold level of four slugs in traps baited with molluscicide pellets, the catch of *D. reticulatum* in traps baited with chicken layers' mash after one night was similar to the catch in traps baited with molluscicide pellets after three nights. This indicates that it should be possible to use a threshold mean catch of four slugs per trap after one night, for traps baited with chicken layers' mash, to indicate a potential risk to a following crop of winter wheat. Thus, the use of traps with this bait to assess the risk of damage by *D. reticulatum* not only provides a safe alternative to the use of traps baited with molluscicide pellets, it also provides a more rapid assessment of the activity of *D. reticulatum*, the main pest species in arable crops, compared to the standard three-night recommended trapping period for traps baited with molluscicide pellets. This has obvious advantages for farmers and crop consultants during the busy period leading up to autumn drilling.

The two other slug species we encountered in sufficient numbers for analysis were either recorded in mash-baited traps in greater (*D. laeve*) or lower (*A. distinctus*) numbers compared to pellet-baited traps. For this reason, we must be a little cautious on the use of mash-baited traps where other species are present. However, the comparative efficiency of mash-baited traps for these species is likely to be a minor consideration in proportion to the major drawback of any traps, which is that they under-record the smaller slugs that make up the bulk of the population of most slug species at the time of trapping in late summer or autumn (see Paper 5, this report for further discussion).

Trapping over one night has the advantage that, since traps are always put out on an afternoon or evening when the soil and weather conditions are known to be suitable for slug activity, there is less chance that conditions will become unsuitable for slug activity during the trapping period than if traps are left in situ for three nights before they are examined. The problem of low catches in traps during drying weather is well illustrated by the results of trapping under these conditions in stubble on two occasions in August and September2002. Whilst catches in traps baited with chicken layers' mash were depressed more than traps baited with molluscicide under these conditions, slug numbers in traps with both types of bait were

considerably depressed and declined over a three-night period in August. Although slugs that had fed on pellets showed clear signs of poisoning, they were nevertheless apparently able to move out of the traps when conditions became hot and dry.

As the slugs in traps baited with non-toxic material are healthy, they can readily leave the trap after feeding. This is borne out by time-lapse video observations, which have shown that considerably more slugs enter traps baited with chicken layers' mash during the night than are present in the morning (Paper 15). However, this does not detract from the value of the traps for assessing slug activity. Under suitable weather and soil conditions, and when relatively few slugs were recorded in the traps, the numbers of slugs resting in the traps remained at about the same level from one to three nights after the traps were put out. Presumably, under these conditions the equilibrium reached between slugs entering and leaving traps during the first night was maintained for three nights. However, when larger numbers of slugs were recorded in the traps after one night, numbers dropped substantially after three nights, presumably because the bait supply became diminished and the traps became less attractive as a place to feed and rest. Thus, no advantage was gained by leaving traps baited with chicken layers' mash for more than one night. Even when numbers were around the threshold trap catch, the precision of the estimate did not improve. When numbers after one night were greater than about ten per trap, there was a risk of underestimating slug activity by leaving mash-baited traps for three nights.

Because slugs may leave trap baited with chicken layers' mash after feeding, it is not only important that they are used when weather and soil conditions are suitable for slug activity but also that they are examined when conditions are suitable for slugs to remain resting in the traps the following morning. Thus, if sunshine in the morning is likely to heat the traps above the moderate temperatures preferred by slugs, then they must be examined early in the morning because slugs will leave the trap when they heat in the sun.

Under suitable conditions for slug activity, the numbers of slugs recorded in traps baited with molluscicide increased from one night to three nights. It is likely that bait pellets in traps are slower to absorb moisture and therefore become suitable for slug feeding more slowly than the milled grain in chicken layers' mash. However, because slugs are poisoned by the molluscicide bait, the slugs gradually accumulate in these traps when conditions are suitable.

Our traps consisted of upturned plant pot saucers because we wished to compare trap baits using the same type of trap material as used by Glen *et al.* (1993). Tests showed that the small difference in trap size was unimportant. However it is worth noting that Young *et al.* (1996) recorded more slugs in traps with covers made of hardboard squares than plastic plant-pot saucers. A higher threshold catch could therefore be warranted for traps with hardboard covers, but further research is needed on this.

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Table 14.1(Alternative version): Summary of the parameters for regressions between numbers of Deroceras reticulatum *per trap baited with chicken layers' mash after one night or three nights (Mash 1 or Mash 3, respectively) and per trap baited with molluscicide pellets after one and three nights (Moll 1 or Moll 3, respectively) at different sites when conditions were suitable for tapping in 2002.*

Date(s)	Site(s) & crop	y-value	x-value	n	Prob.	Regr. Coeff.	S.E.	Regr. Const	S.E.	Prob. <0	y-value when x = 4
May-July	Somerset Cereals	Mash 1	Moll. 1	75	<0.001	1.020	0.098	5.26	1.21	<0.001	
May-July	Somerset Cereals	Mash 1	Moll. 3	75	<0.001	0.589	0.053	2.89	1.22	<0.05	5.3
May-July	Somerset Cereals	Mash 3	Mash 1	75	NS	-		-			
May-July	Somerset Cereals	Moll. 3	Moll. 1	75	<0.001	1.444	0.103	5.39	1.27	<0.001	
9 Sept	Cambs. Stubble	Mash 1	Moll. 1	-	-	-	-	-	-		-
9 Sept	Cambs. Stubble	Mash 1	Moll. 3	48?	<0.01	0.445		1.132			2.9
9 Sept	Cambs. Stubble	Mash 3	Mash 1	48?	<0.001	0.200		0.175			
9 Sept	Cambs. Stubble	Moll. 3	Moll. 1	-	-	-	-	-	-		-
15 Oct.	Northumb. Stubble 1	Mash 1	Moll. 1	50	<0.001	0.679	0.168	2.62	0.76	0.001	
15 Oct.	Northumb. Stubble 1	Mash 1	Moll. 3	50	<0.001	0.586	0.128	2.07	0.79	0.01	4.4
15 Oct.	Northumb. Stubble 1	Mash 3	Mash 1	50	0.01	0.150	0.059	1.69	0.37	<0.001	
15 Oct.	Northumb. Stubble 1	Moll. 3	Moll. 1	50	<0.001	1.091	0.093	1.16	0.42	<0.01	
29 Oct.	Northumb. Stubble 2	Mash 1	Moll. 1	50	<0.01	0.629	0.192	3.99	0.98	<0.001	
29 Oct.	Northumb. Stubble 2	Mash 1	Moll. 3	50	<0.01	0.259	0.089	2.95	1.38	<0.05	4.0
29 Oct.	Northumb. Stubble 2	Mash 3	Mash 1	50	0.01	0.316	0.118	6.149	0.94	<0.001	
29 Oct.	Northumb. Stubble 2	Moll. 3	Moll. 1	50	<0.001	1.512	0.232	7.86	1.19	<0.001	

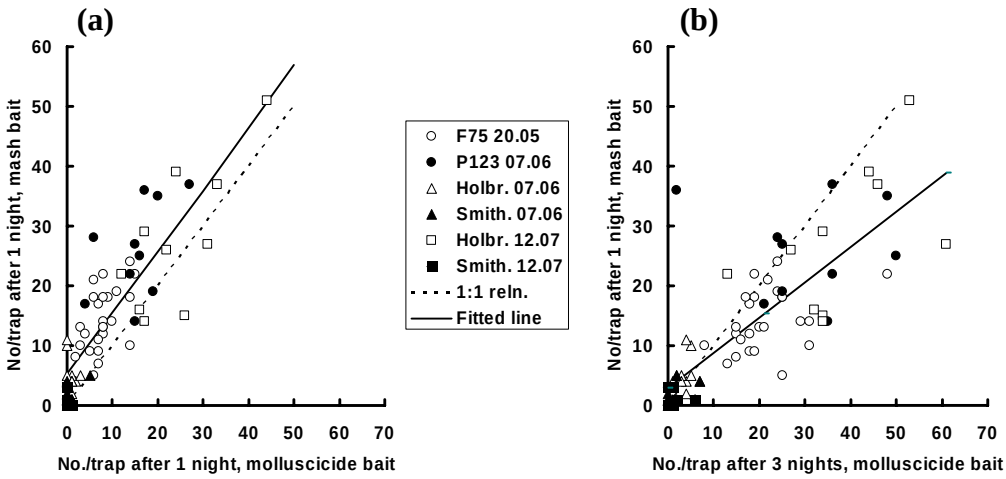

Figure 14.1: Numbers of Deroceras reticulatum *recorded after one night in traps baited with chicken layers' mash in relation to numbers recorded after (a) one night (y = 1.02x + 5.26) and (b) three nights (y = 0.59x + 2.89) in traps baited with slug pellets at four field sites on three dates in standing cereal crops in 2002.*

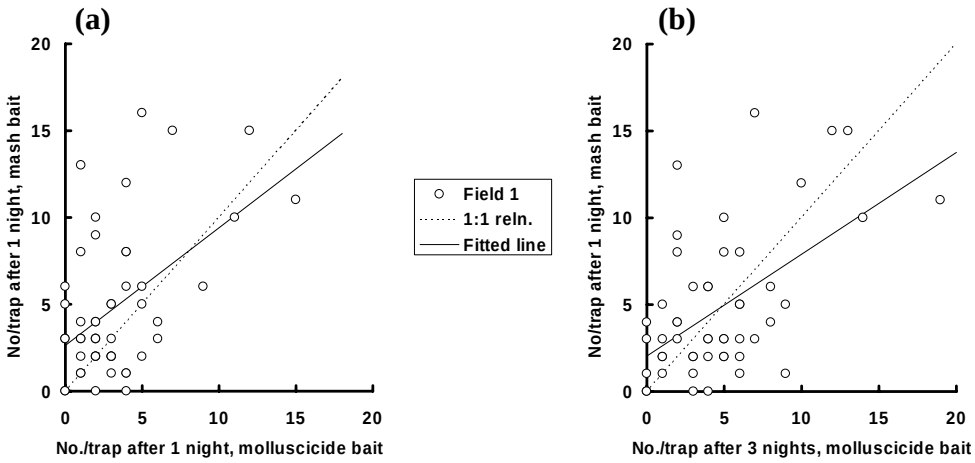

Figure 14.2: Numbers of Deroceras reticulatum *recorded after one night in traps baited with chicken layers' mash in relation to numbers recorded after (a) one night (y = 0.68x + 2.62) and (b) three nights (y = 0.59x + 2.07) in traps baited with slug pellets in stubble, Field 1, Northumberland, October 2002.*

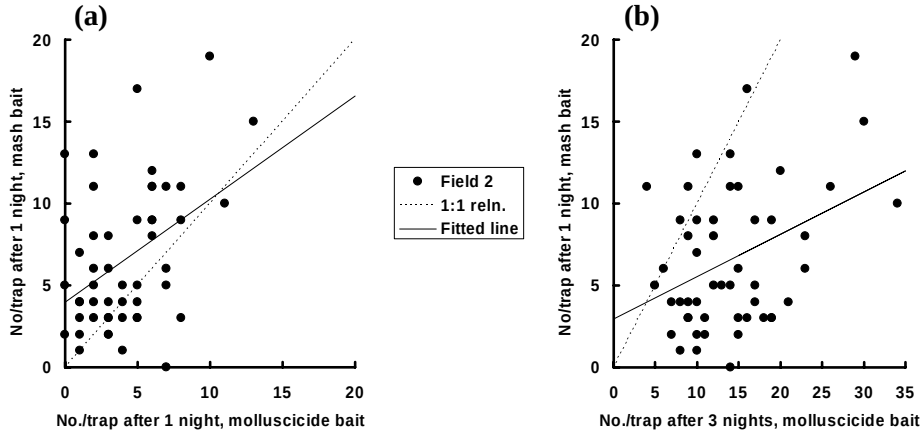

Figure 14.3: Numbers of Deroceras reticulatum *recorded after one night in traps baited with chicken layers' mash in relation to numbers recorded after (a) one night (y = 0.68x + 2.62) and (b) three nights (y = 0.59x + 2.07) in traps baited with slug pellets in stubble, Field 2, Northumberland, October 2002.*

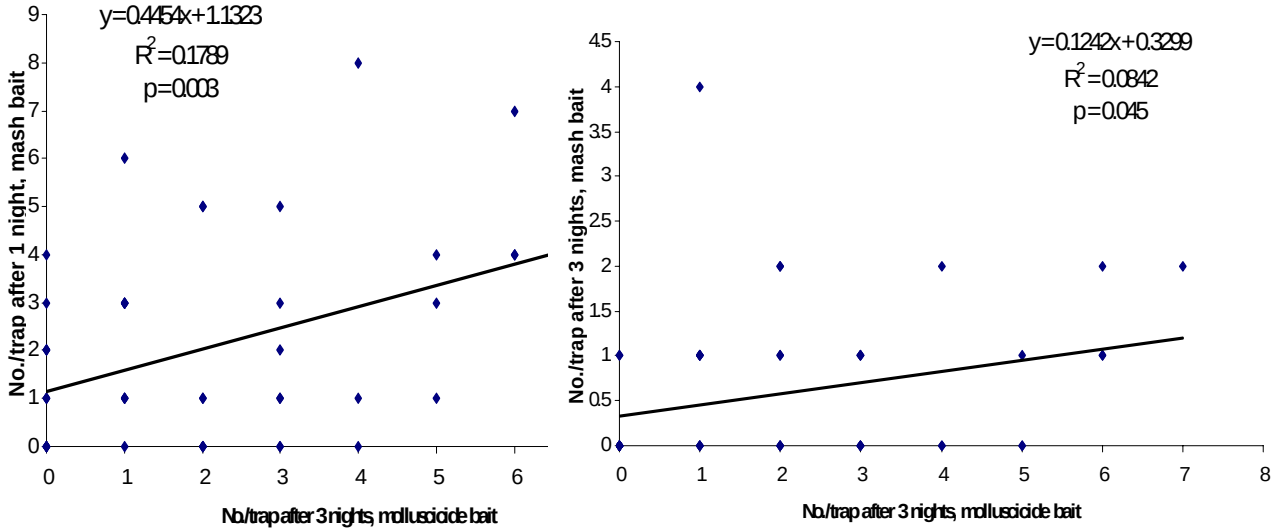

Figure 14.4. Numbers of slugs recorded in traps baited with methiocarb after three nights (x axis) in relation to numbers in mash-baited traps after one or three nights (y axis). Site: Pamplins Field South, ADAS Boxworth, Cambridgeshire.

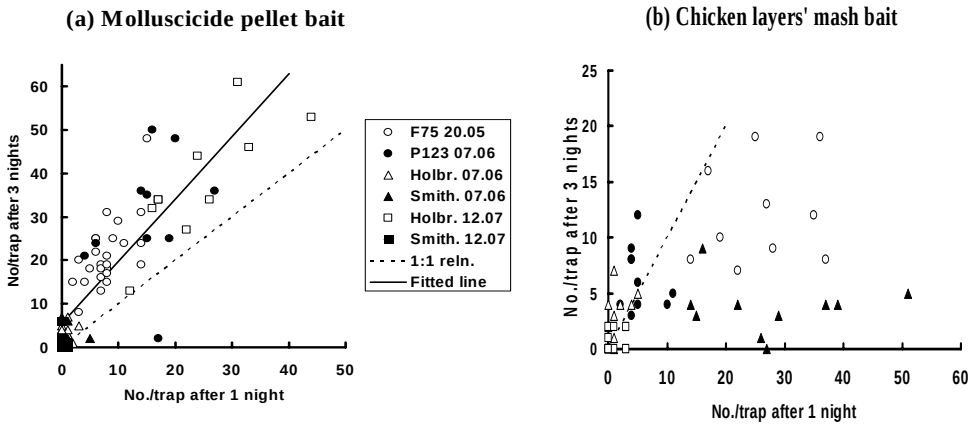

Figure 14.5: Comparison of the numbers of Deroceras reticulatum *recorded in traps after one night and three nights baited with (a) molluscicide pellets* (y = 1.44x + 5.39)*, or (b) chicken layers' mash, at three field sites on two dates in standing cereal crops. Dashed line shows 1:1 relationship between trap catches after one night and three nights.*

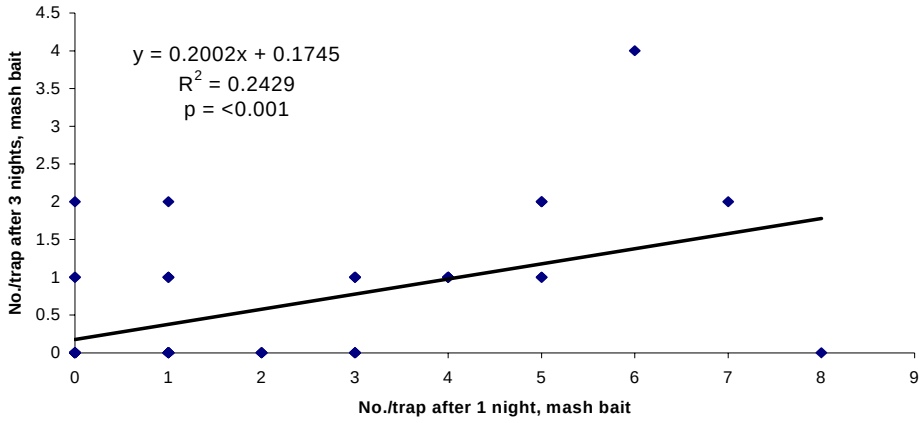

Figure 14.6. Numbers of slugs recorded in traps baited with chicken layers' mash after one night (x axis) in relation to numbers after three nights (y axis). Site: Pamplins Field South, ADAS Boxworth, Cambridgeshire.

PAPER 15 - *Objective 3*

Estimation of Surface Active Slug Populations using Refuge Traps

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Abstract

The efficacy of refuge traps and defined area traps was compared in field conditions. The most abundant species were *Deroceras reticulatum* (Müller) and *Arion subfuscus* (Draparnaud); refuge traps tended to under represent small individuals compared to defined area traps. To investigate whether size related differences in slug behaviour might explain this observation, time-lapse video techniques were used with a novel refuge trap made of infra-red transparent material to record individual movement of small (< 100 mg) and large (> 500 mg) *Deroceras reticulatum* upon encountering the trap. Approximately one third of the slugs that entered the traps at some point in the night remained there at dawn. There were no size specific differences in the number, timing or duration of trap entries. Infra-red transparent refuge traps performed as well as standard opaque saucer traps under field conditions.

Introduction

Central to any decision regarding the implementation of control measures for crop pests is an estimation of population size (Port & Port, 1986). This is not an easy task in the case of slugs due to their patchy distribution and partly subterranean habitat (Hunter, 1966) along with the dependence of their activity on factors such as soil moisture, photoperiod, humidity and temperature (Rollo, 1991). Approaches comprise both indirect and direct methods. The former include searching at night (Barnes, 1944; Barnes & Weil, 1944), inference from damage levels (Duthoit, 1961) and trapping in artificial refuges (Getz, 1959; Schrim & Byers, 1980). These sample slugs from an unknown area, but are relatively quick to complete. Direct methods, in contrast, sample slugs from a known area, but are more laborious and include soil washing (Hunter, 1968a), flooding (South, 1964) and defined area traps (DATs) (Ferguson *et al.*, 1989).

The different methods of population assessment vary in the type of estimate they provide, e.g. density, abundance or surface activity levels (Thomas, 1944; Oggier *et al.*, 1998). Their efficacy is affected by weather conditions (Hunter, 1968a) and factors such as construction material and the use of bait (Young *et al.*, 1996). In combination with the variable nature of slug activity this makes comparisons between studies

inherently difficult; even within a study variation in conditions on different assessment dates can confound results.

Despite their limitations, there are no alternatives to the current methods of population estimation. Soil sampling is considered the 'gold standard' (Hunter, 1968a; Glen & Wiltshire, 1986), but is impractical for use by farmers to assess the need for control measures due to time and labour constraints. Refuge traps, however, are transportable, cheap, easy to use and store (Schrim & Byers, 1980). Of the direct methods DATs are the most convenient and least labour intensive (Ferguson & Hanks, 1990). For these reasons these two methods are among the most frequently used and are likely to remain so for the foreseeable future. The experiments presented in this paper were carried out on this basis.

Refuge traps provide an estimate of surface activity. They consist of a shelter material which usually covers a food bait. Toxic baits, e.g. piles of molluscicide pellets, retain slugs in the traps, but are not desirable due to the hazard large amounts of pesticide under a single trap poses to wildlife and domestic animals (Voss *et al.*, 1998). In a trial of various shelter materials and non-toxic baits it was shown that hardboard squares baited with chicken layers mash was a very effective combination (Young *et al.*, 1996). Being lighter and more portable than hardboard squares, however, plastic flowerpot saucers are also commonly used (Clements & Murray, 1991).

DATs estimate population density and comprise a metal barrier sunk into the ground enclosing a known area, typically 0.1 m^2 (Ferguson *et al.*, 1989). A hardboard square or sacking is usually placed within the metal barrier to provide humid, sheltered conditions which encourage slugs to remain on the surface. The DATs are checked regularly and any slugs present are removed and counted until no further slugs are found; the area is then considered 'trapped out'.

Numerous studies have contrasted the efficacy of refuge traps with DATs (e.g. Byers *et al.*, 1989; Clements & Murray, 1991; Barratt *et al.*, 1993; Voss *et al.*, 1998). Since these assessment methods measure different aspects of the population (i.e. surface activity and density) the comparisons drawn have a relative rather than absolute meaning. It has been consistently reported that refuge traps under represent small slugs in relation to DATs and other direct methods of sampling (e.g. Glen & Wiltshire, 1986; Clements & Murray, 1991). The reasons for this are not clear; it has been suggested that large slugs may move further than small slugs and are, therefore, more likely to contact refuge traps (Glen & Wiltshire, 1986) or that small slugs are more easily overlooked (Archard *et al.*, 2004). It may also be that small and large slugs behave differently towards refuge traps when they encounter them (Howlett *et al.*, 2004, *In press*).

The experiments described in this paper were designed to investigate the behaviour of different sized slugs towards refuge traps. There were three elements to the study. Firstly, locally collected trapping data from DATs and refuge traps were compared to ascertain whether the bias reported in the literature was also

apparent in our study area. Secondly, activity studies of slugs using time-lapse video techniques were conducted to examine whether there were any size specific differences in their behavioural response to refuge traps; this involved using a novel saucer trap that was transparent to infra-red light. Thirdly the novel trap was compared to a standard saucer trap under field conditions to assess the general applicability of the behavioural study results.

Materials and Methods

Defined area traps (DATs) comprised four galvanised steel sheets arranged to form a square measuring 42 cm x 42 cm, enclosing an area of 0.18 m^2. These were sunk into the ground to a depth of 5-7 cm, such that approximately 15 cm protruded above the surface. A sheet of hardboard was placed inside each DAT to provide sheltered, moist conditions suitable for slugs. Refuge traps comprised upturned terracotta coloured plastic flowerpot saucers of 25 cm diameter. Refuge traps were baited with 2.5 g chicken layers mash. DATs were unbaited.

Small (< 100 mg) and large (> 500 mg) *D. reticulatum* used in the activity studies were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). They were maintained at 12°C in ventilated plastic containers (18 x 12 x 7 cm) filled with moist laboratory tissue for up to five days prior to use. This ensured that any slugs in the sample that were unhealthy following collection could be excluded from the experiments. During this time they were provided with Chinese cabbage and carrot *ad libitum*.

The activity studies were carried out in plastic arenas measuring 57 x 36 x 16 cm, the rims of which were painted with a Fluon® (polytetrafluoroethylene) barrier (Whitford Plastics, Cheshire, England) to prevent slugs escaping. The arenas were filled to a depth of 8-10 cm with loamy soil dug from an agricultural plot at Close House Field Station and any stones, large lumps of organic matter and soil organisms were removed prior to use. The soil surface was raked to a fine tilth. Fresh soil was used for each replicate and it was watered such that the surface appeared damp at the start of each recording.

In indoor experiments slug activity was recorded using a Panasonic AG-6040 time-lapse VHS video recorder. The camera used was a Sanyo VCB3572 IRP ½ ” high resolution 570TVL infra-red sensitive camera, fitted with a Computar 8-48 mm lens and infra red bandpass filter. The arena was illuminated at night using a Computar Uniflood LED infra red lamp (serial number CL057787). Light of this wavelength does not appear to disrupt slug activity yet permits recording to take place during darkness (Howling, 1990). Daytime lighting was provided by two 400W halogen lamps suspended 1.55 m above the arena. These switched on and off via a timer to match the prevailing sunrise and sunset times, which were updated weekly.

In outdoor experiments a Sanyo TLS-1600P/IR time-lapse VHS video recorder was used with a Baxall CD0242/IR ½ " high resolution infra-red sensitive camera. This was fitted with a Computar 4.5-10 mm lens and infra-red filter. The camera was housed in waterproof casing and mounted on a tripod. Night time illumination was provided by a waterproof infra-red LED unit and daytime lighting was not required. Power was supplied to the camera by a 12 volt car battery, otherwise mains electricity was used. In both indoor and outdoor setups the video recorder was set to record 60 times more slowly than a standard machine so that a total of 180 hours of activity could be stored on a single cassette. Recordings were played back at normal speed.

Specially designed refuge traps comprised 18 cm diameter upturned saucers manufactured from black infra-red transparent plastic (Tracksys Ltd., Nottingham, England). These were opaque to visible light, but transparent to infra-red illumination (*Fig. 15.1 (a) & (b)*). The traps were sprayed with a fine anti-mist coating (Holts anti-mist and water repellent spray, product code HMC6) to prevent condensation forming and obscuring the image during recordings. They were baited with 2.5 g chicken layers mash. Infra-red transparent refuge traps were compared with terracotta coloured standard plastic flowerpot saucers of the same diameter (18 cm). Where traps were baited, this was with 2.5 g chicken layers mash.

(a) *(b)*

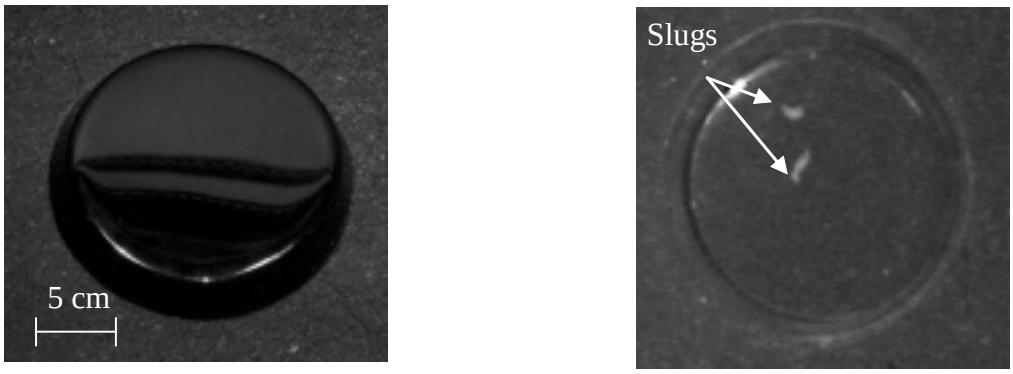


Figure 15.1: Infra-red transparent saucer (a) as viewed by the naked eye and (b) under infra-red illumination with slugs visible beneath.

Trapping data were collected from two arable fields (middle field and bottom field) at Heddon Banks Farm, Heddon-on-the-Wall, Northumberland (NZ 138 657) on ten, two-week sampling periods in 2002-03. Prior to and during trapping both fields were sown with wheat. Sixteen DATs were deployed in each field at a distance of 10 m apart in a grid arrangement. These remained in-situ for two weeks. Tall vegetation protruding above the rim of the DAT was trimmed. Any slugs that were on this vegetation were removed and replaced into the DAT. Sixteen refuge traps were deployed at the midpoint between DAT rows (i.e. a distance of 5 m between two DATs) on a single night during the two week trapping period when weather conditions were appropriate for surface activity (*Fig. 15.2)*. After this the refuge traps were removed again.

After each two week trapping period the DATs were removed and redeployed according to a rotation system (*Fig. 15.3*).

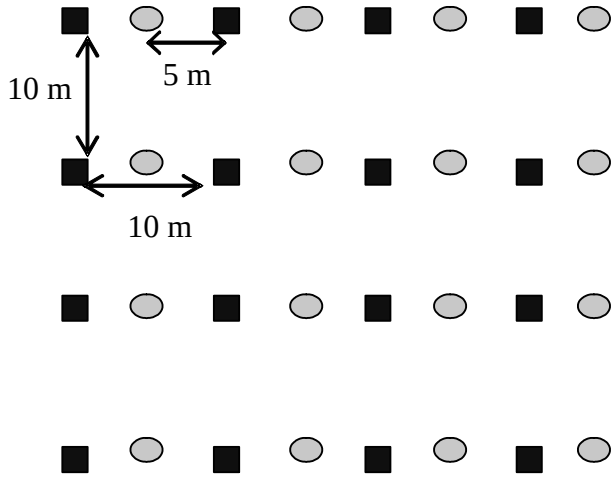


Figure 15.2: 10 m x 10 m grid arrangement of 16 DATs at Heddon Banks Farm showing the position of 16 refuge traps 5 m between DAT rows (black squares represent DATs; grey circles represent refuge traps).

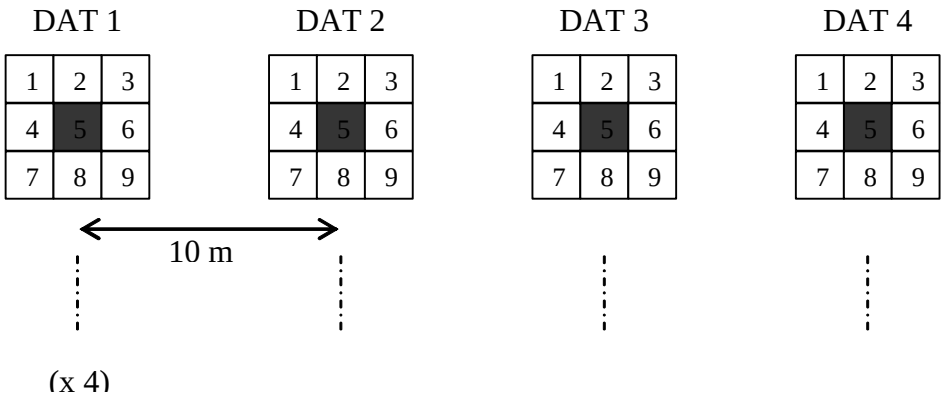


Figure 15.3: Rotation of DATs at Heddon Banks Farm.
At each DAT site within the 10 m x 10 m grid (Fig. 15.2) there is a second grid arrangement of adjacent squares numbered 1-9. A pole is placed in the centre of each grid (marked in black). The distance between poles in adjacent grids is 10 m. Each of the 16 DATs is placed at the same numerical position within its own grid (e.g. all DATs are placed at position 1 in their own grid). At the end of each two week sampling period, all DATs are moved to the next numerical position within their own grid (e.g. all DATs are moved from position 1 to position 2) etc.

Traps were checked each morning by 10.30 a.m. and slugs of all species present were removed and counted. The first 30 individuals of each species were weighed using a Mettler MT5 balance to an accuracy of 0.01

mg. DATs were checked every working day during each two week trapping period (i.e. a total of ten occasions) and the refuge traps were checked on the morning following their deployment.

In the activity studies six *D. reticulatum* were used per arena, three small (< 100 mg) and three large (> 500 mg), which is equivalent to a field density of approximately 28 slugs per m^2. These were weighed using a Mettler MT5 balance to an accuracy of 0.01 mg. To allow for atypical activity in response to a new environment slugs were placed in the arena for 24 hours prior to the commencement of recording to allow them to acclimatise (Whelan, 1982). During this time they were starved to ensure that they were motivated to forage. Following the acclimatisation period the chicken layers mash bait was applied to the arena in a central pile and was then covered with the infra-red transparent refuge trap. Recording began and continued overnight until the following morning. A total of eight replicates were recorded for both indoor and outdoor setups and different slugs were used for each. The indoor experiments were carried out at 12 ± 2°C in a controlled temperature room. The ambient temperature and soil temperature during the outdoor experiments were recorded using Tinytalk™ data loggers. Video recordings were played back and for each slug the number and timing of trap entries and exits was noted, in addition to the activity start time.

Field scale comparison of infra-red transparent and standard refuge traps was carried out in a grass plot within a walled garden at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). A total of 12 assessments were completed. Traps were deployed on mild evenings when the soil surface was moist. Two standard terracotta and two infra-red transparent refuge traps were used. These were placed 5 m apart in a grid arrangement. One of each trap type had 20 ml chicken layers mash underneath; the other had no bait (*Fig. 15.4*). The traps were left *in-situ* for a single night before being redeployed 5 m to the left of the previous site according to a rotation system (*Fig. 15.5*).

Traps were checked each morning by 10.30 a.m. and the numbers of slugs of all species present were counted. Any *D. reticulatum* were weighed on a Mettler MT5 balance to an accuracy of 0.01 mg. Traps were then wiped clean and any remaining chicken layers mash was disposed of such that there was no trace left on the soil surface.

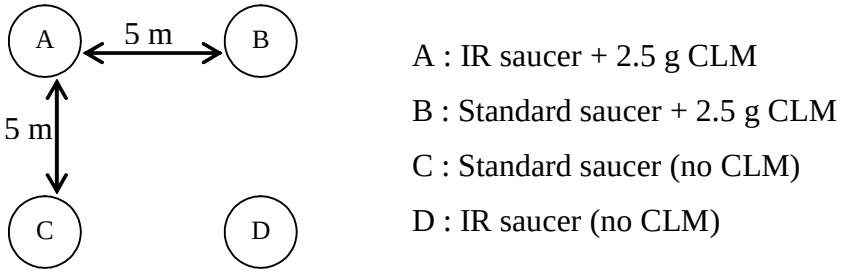


Figure 15.4: 5 m x 5 m grid arrangement of refuge traps at Close House Field Station (CLM = chicken layers mash).

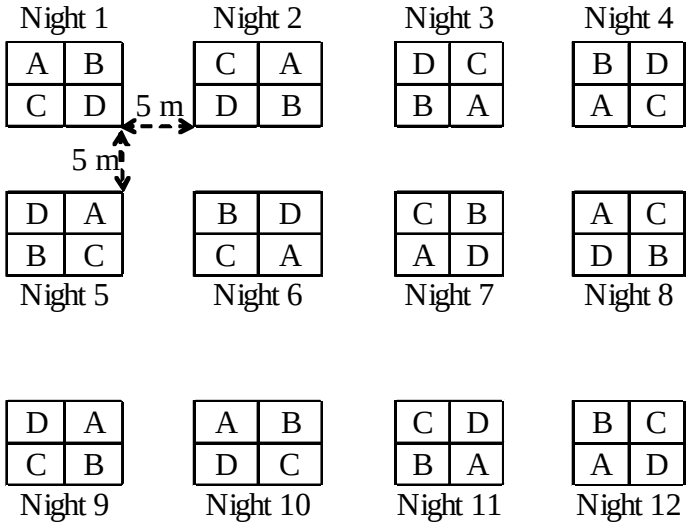


Figure 15.5: Rotation of refuge traps at Close House Field Station.

For comparisons between refuge traps and DATs the mean size of slugs in different trap types was analysed using the Wilcoxon Signed Ranks test as the data were non-parametric. For assessments of slug activity beneath refuge traps count data was analysed using Fisher's Exact test or contingency Chi square tests as appropriate. For the latter the Yates' correction was applied if necessary. For non-parametric two factor comparisons of counts the Scheirer-Ray-Hare test was used. Data concerning the timing of events were assessed with one-way analysis of variance (ANOVA) following transformation. For field scale comparisons of infra-red transparent and standard saucer refuge traps counts were analysed using the contingency chi-square test with Yates' correction. Continuous weight data were parametric; these were assessed by two-way analysis of variance (ANOVA).

Results

For the comparison of refuge traps and DATs, the months and years corresponding to each of the ten sampling occasions are shown in *Table 15.1*. There are no data for August and September due to harvesting and cultivations which prevented access to the fields. Although there were some fluctuations in relative abundance on different sampling occasions, the most abundant species summed over the total growing season were generally *D. reticulatum* and *A. subfuscus* in both trap types and fields. The single exception to this is in the middle field refuge traps where *Deroceras panormitanum* (Lessona and Pollonera) was the second most numerous species after *D. reticulatum* (*Tables 15.2 & 15.3*).

Table 15.1: Months and years corresponding to each sampling occasion.

Sample Occasion	*Month*	*Year*	*Season*
1	Oct	2002	Autumn
2	Nov	2002	
3	Dec	2002	Winter
4	Jan	2003	
5	Feb	2003	
6	Mar	2003	Spring
7	Apr-May	2003	
8	May-June	2003	Summer
9	July	2003	
10	Oct	2003	Autumn

There were more species trapped in total in the middle field for both DATs and refuge traps, although it was only *D. panormitanum* that were found in appreciable numbers in addition to *D. reticulatum* and *A. subfuscus (Fig. 15.6).* Analyses are, therefore, restricted to *D. reticulatum* and *A. subfuscus* in order to make consistent comparisons between fields. Results for both fields are summarised in *Table 15.4* for *D. reticulatum* and *Table 15.5* for *A. subfuscus.* In general refuge traps caught more slugs in early autumn and winter (sampling occasions 1-5 and 10) whereas DATs caught larger numbers in spring and summer (sampling occasions 6-9). The significance of differences tended to be greater when the total numbers caught were higher. There were no clear differences between fields for either species.

Results for slug weight for both fields are summarised in *Table 15.4* for *D. reticulatum* and *Table 15.5* for *A. subfuscus.* Note that some comparisons involve a single individual. Although the tests are valid, such results must be treated with caution. The mean weight of slugs caught by refuge traps and DATs did not differ significantly on all sampling occasions. For both species, however, it can be seen that, where there are significant differences, the refuge traps generally caught heavier slugs in autumn, winter and early spring, whereas in summer these were found in the DATs. For the growing season as a whole (i.e. all sampling occasions combined) the only instance where a significant difference in mean slug weight between trap types persisted was in the bottom field for *A. subfuscus* where the DATS caught heavier slugs.

Table 8.2: Species abundance in DAT traps in middle and bottom field on each of ten sampling occasions at Heddon Banks Farm (2002-03).

Field	Sampling Occasion	Arion circumscriptus	Arion distinctus	Arion subfuscus	Deroceras panormitanum	Deroceras reticulatum
Bottom	1	-	-	-	-	-
	2	-	-	2	-	7
	3	-	-	1	-	3
	4	-	-	-	-	4
	5	-	-	-	-	3
	6	-	-	2	-	13
	7	-	-	11	-	30
	8	-	-	8	-	94
	9	-	1	26	-	103
	10	-	-	1	-	-
	Total	0	1	51	0	257
Middle	1	-	-	3	6	23
	2	-	-	3	12	50
	3	-	2	4	1	22
	4	1	-	11	9	30
	5	-	-	7	4	19
	6	-	1	12	4	24
	7	-	1	34	8	69
	8	-	-	115	9	52
	9	-	1	178	6	19
	10	-	-	-	-	-
	Total	1	5	367	59	308

Dates for each sampling occasion are shown in Table 8.1.

Table 8.3: Species abundance in refuge traps in middle and bottom field on each of ten sampling occasions at Heddon Banks Farm (2002-03).

Field	Sampling Occasion	Arion subfuscus	Deroceras panormitanum	Deroceras reticulatum
Bottom	1	-	-	3
	2	-	-	1
	3	9	-	20
	4	4	-	12
	5	8	-	20
	6	1	-	10
	7	6	-	23
	8	1	-	2
	9	3	-	8
	10	-	-	-
	Total	*32*	*0*	*99*
Middle	1	1	8	24
	2	1	-	14
	3	17	53	114
	4	21	19	55
	5	26	17	66
	6	2	4	20
	7	6	2	26
	8	1	1	3
	9	5	2	1
	10	1	1	3
	Total	*81*	*107*	*326*

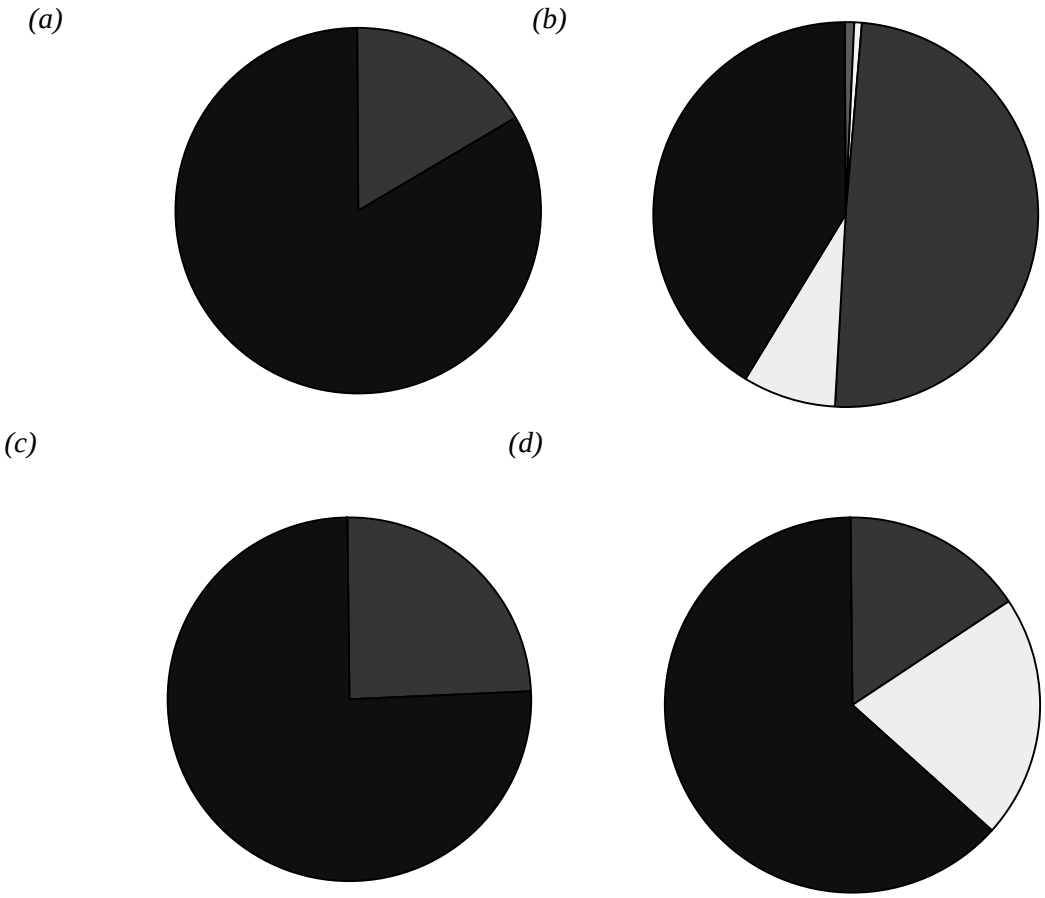


Figure 15.6: Slug species abundance at Heddon Banks Farm, Northumberland 2002-03 (a) DAT bottom field (b) DAT middle field (c) refuge trap bottom field (d) refuge trap middle field (blue segment = Deroceras reticulatum; *yellow segment* = Deroceras panormitanum*; red segment* = Arion subfuscus*; pale blue segment* = Arion distinctus*; green segment* = Arion ater*).*

Table 8.4: Results of Wilcoxon Signed Ranks Test to compare mean weights of Deroceras reticulatum *between DATs and refuge traps in middle and bottom fields, Heddon Banks Farm, Northumberland (2002-03). Data refer only to those trap pairs where at least one slug was caught on a given sampling occasion († indicates that this is a single individual).*

Field	*Sampling occasion*	*Number of trap pairs*	*Number of slugs*		G^2	*P-value*	*Mean weight ± S.E. (mg)*		*Z*	*P-value*
			Refuge trap	*DAT*			*Refuge trap*	*DAT*		
Middle	1	14	24	23	0.09	*n.s*	153.00 ± 21.94	85.36 ± 11.69	-1.977	*< 0.05*
	2	16	14	50	19.14	*< 0.001*	142.70 ± 38.14	162.33 ± 9.88	-0.414	*n.s.*
	3	12	114	22	63.60	*< 0.001*	111.51 ± 14.24	157.23 ± 27.12	-0.784	*n.s.*
	4	13	55	30	7.95	*< 0.01*	151.43 ± 20.30	263.00 ± 25.55	-1.572	*n.s.*
	5	12	66	19	27.11	*< 0.001*	217.14 ± 22.65	186.50 ± 28.79	-0.628	*n.s.*
	6	13	20	24	0.20	*n.s.*	415.26 ± 44.47	177.32 ± 33.76	-2.411	*< 0.05*
	7	16	26	69	18.57	*< 0.001*	419.35 ± 92.16	173.76 ± 14.49	-2.741	*< 0.01*
	8	15	3	52	41.89	*< 0.001*	79.69 ± 98.54	179.47 ± 18.66	-2.613	*< 0.01*
	9	10	1	19	14.45	*< 0.001*	32.17†	271.20 ± 50.34	-2.803	*< 0.01*
	10	3	3	0	5.33	*< 0.05*	235.82 ± 160.72	-	-	-
	TOTAL	*124*	*326*	*308*	*0.57*	*n.s.*	*199.10 ± 14.02*	*176.01 ± 10.03*	*-0.347*	*n.s.*
Bottom	1	2	3	0	5.33	*< 0.05*	441.11 ± 56.66	-	-	-
	2	6	1	7	3.13	*n.s.*	124.63†	277.78 ± 67.17	-1.153	*n.s.*
	3	10	20	3	14.09	*< 0.001*	316.39 ± 35.76	10.03 ± 12.65	-2.803	*< 0.01*
	4	9	12	4	5.06	*< 0.05*	129.15 ± 31.97	90.47 ± 71.52	-0.533	*n.s.*
	5	9	20	3	14.09	*< 0.001*	376.10 ± 47.65	55.47 ± 65.87	-2.429	*< 0.05*
	6	10	10	13	0.17	*n.s.*	321.52 ± 83.79	185.32 ± 61.44	-1.172	*n.s.*
	7	16	23	30	0.68	*n.s.*	266.10 ± 36.26	404.81 ± 27.33	-2.223	*< 0.05*
	8	15	2	94	86.26	*< 0.001*	49.50 ± 93.51	321.71 ± 20.83	-2.953	*< 0.01*
	9	12	8	103	79.60	*< 0.001*	167.07 ± 87.64	172.76 ± 10.18	-0.235	*n.s.*
	10	0	-	-	-	-	-	-	-	-
	TOTAL	*89*	*99*	*257*	*69.24*	*< 0.001*	*219.79 ± 22.26*	*201.44 ± 12.57*	*-0.305*	*n.s.*

Table 8.5: Results of Wilcoxon Signed Ranks Test to compare mean weights of Arion subfuscus *between DATs and refuge traps in middle and bottom fields, Heddon Banks Farm, Northumberland (2002-03). Data refer only to those trap pairs where at least one slug was caught on a given sampling occasion († indicates that this is a single individual).*

			Number of slugs				*Mean weight ± S.E. (mg)*			
Field	*Sampling occasion*	*Number of trap pairs*	*Refuge trap*	*DAT*	χ^2	*P-value*	*Refuge trap*	*DAT*	*Z*	*P-value*
Middle	1	3	1	3	0.25	*n.s*	47.41†	284.78 ± 164.42	-1.069	*n.s.*
	2	4	1	3	0.25	*n.s*	85.36†	55.09 ± 28.23	-0.365	*n.s.*
	3	12	17	4	9.33	*< 0.01*	225.26 ± 63.27	11.61 ± 12.40	-2.667	*< 0.01*
	4	14	21	11	3.78	*n.s*	140.96 ± 31.73	109.80 ± 55.83	-1.161	*n.s.*
	5	14	26	7	12.12	*< 0.001*	309.35 ± 56.61	36.34 ± 34.02	-3.296	*< 0.01*
	6	7	2	12	5.79	*< 0.05*	154.01 ± 241.00	118.62 ± 21.86	-0.507	*n.s.*
	7	14	6	34	18.23	*< 0.001*	274.37 ± 196.46	312.91 ± 35.48	-0.596	*n.s.*
	8	14	1	115	110.08	*< 0.001*	30.70†	219.01 ± 13.80	-2.794	*< 0.01*
	9	12	5	178	161.66	*< 0.001*	315.90 ± 188.02	175.96 ± 11.58	-0.706	*n.s.*
	10	0	-	-	-	-	-	-	-	-
	TOTAL	*95*	*81*	*367*	*181.31*	*< 0.001*	*296.62 ± 32.96*	*143.67 ± 9.06*	*-0.635*	*n.s.*
Bottom	1	0	-	-	-	-	-	-	-	-
	2	2	0	2	0.50	*n.s*	-	549.81 ± 388.77	-1.342	*n.s.*
	3	6	9	1	8.10	*< 0.01*	554.91 ± 149.44	12.01†	-1.992	*< 0.05*
	4	3	4	0	6.25	*< 0.05*	177.42 ± 68.49	-	-	-
	5	6	8	0	10.13	*< 0.01*	528.82 ± 160.46	-	-	-
	6	2	1	2	0.00	*n.s*	102.45†	423.94 ± 423.93	-0.447	*n.s.*
	7	11	6	11	0.94	*n.s*	364.38 ± 178.49	884.49 ± 164.47	-1.689	*n.s.*
	8	6	1	8	4.00	*< 0.05*	177.25†	1247.51 ± 147.85	-1.992	*< 0.05*
	9	12	3	26	16.69	*< 0.001*	341.40 ± 364.97	1327.26 ± 260.30	-3.059	*< 0.01*
	10	0	-	-	-	-	-	-	-	-
	TOTAL	*49*	*32*	*51*	*3.90*	*< 0.05*	*334.86 ± 82.83*	*718.45 ± 91.74*	*-2.711*	*< 0.01*

When studying the slug activity beneath refuge traps a pilot study showed that the anti-mist spray used on infra-red transparent saucers to prevent condensation had no differential effect on the activity of slugs compared to an unsprayed saucer. There were no block effects between replicates in either indoor or outdoor experiments and data were therefore pooled for each setup. In all analyses the results of the indoor experiments were confirmed by outdoor experiments.

The mean time elapsed between the onset of activity and the first occasion slugs entered the trap was shorter for large than small slugs in indoor experiments and the converse in outdoor experiments (*Table 15.6*). There was, however, considerable variation in entry times and these differences were not significant in either case. (ANOVA: Indoors: $F_{1, 41} = 0.450$, *n.s.;* outdoors: $F_{1, 30} = 0.058$, *n.s.*)

Table 15.6: Mean time (± S.E.) between onset of activity and first trap entry for small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *in indoor and outdoor experiments.*

Experimental Setting	*Slug size*	*N*	*Mean time (mins) to first trap entry (± S.E.)*
Indoor	Small	19	107.84 ± 38.37
	Large	24	75.24 ± 20.48
Outdoor	Small	16	50.36 ± 26.42
	Large	16	129.31 ± 91.85

The number of small and large slugs entering the trap on at least one occasion did not differ significantly; for both size classes the majority of slugs entered the trap at least once during the night (Fishers Exact test: indoors: N = 47, P = 0.234, *n.s.*; outdoors: N = 41, P = 1.000, *n.s.*) (*Fig. 15.7*).

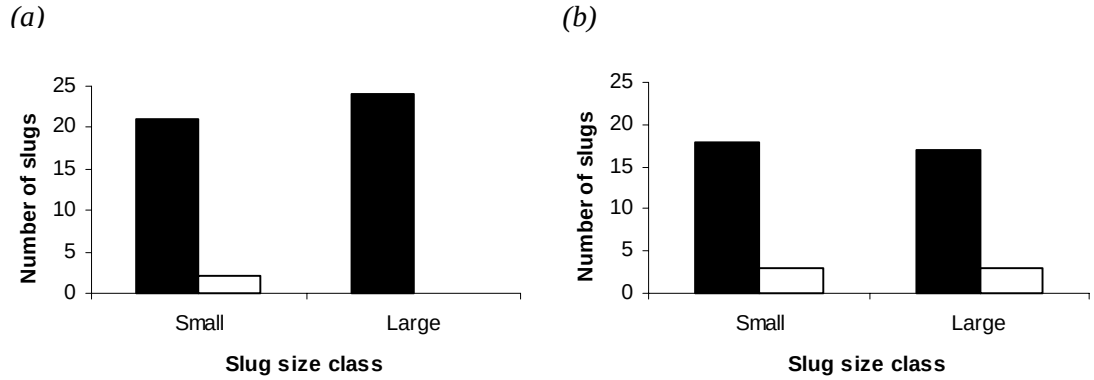


Figure 15.7: Number of small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *entering the refuge trap at least once during the night (a) indoors and (b) outdoors (black bars = slugs entering trap; white bars = slugs not entering trap).*

There was no significant difference in the mean time slugs of different sizes spent under the trap on their first entry (ANOVA: indoors: $F_{1, 39} = 1.021$, *n.s.*; outdoors: $F_{1, 31} = 1.423$, *n.s.*) (*Table 15.7*). This remained the

case when data were subdivided into slugs that entered the trap only once and those that went on later to re-enter the trap. Slugs remained under the trap for 3-5 hours on average on the first entry.

Table 15.7: Mean time (± S.E.) spent under refuge trap on the first entry for small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *in indoor and outdoor experiments.*

Experimental Setting	*Slug size*	*N*	*Mean time (mins) under trap on first entry (± S.E.)*
Indoors	Small	19	238.71 ± 40.09
	Large	22	306.28 ± 51.58
Outdoors	Small	16	199.86 ± 38.29
	Large	17	262.23 ± 35.72

Counts of slugs entering the trap more than once were resolved into two categories; those re-entering once and those re-entering two or more times; any further categories resulted in numbers too small for analysis. There were no significant differences according to size with similar numbers in each category (Chi-squared: indoors: N = 34, df = 1, $G^2_c = 1.000$, *n.s.*; outdoors: N = 24, df = 1, $G^2_c = 1.000$, *n.s.*) (*Fig. 15.8*). There were no significant differences between small and large slugs in the mean time elapsed between initially leaving the trap and the first re-entry (ANOVA: indoors: $F_{1, 32} = 0.271$, *n.s.*; outdoors: $F_{1, 22} = 3.452$, *n.s.*). Similarly, the elapsed time between leaving the trap after the first re-entry and re-entering a second time did not differ according to slug size (ANOVA: indoors: $F_{1, 12} = 0.417$, *n.s.*; outdoors: $F_{1, 10} = 0.099$, *n.s.*). The mean elapsed time between re-entries are summarised in *Table 15.8*. The large standard errors reflect the considerable variation between individuals.

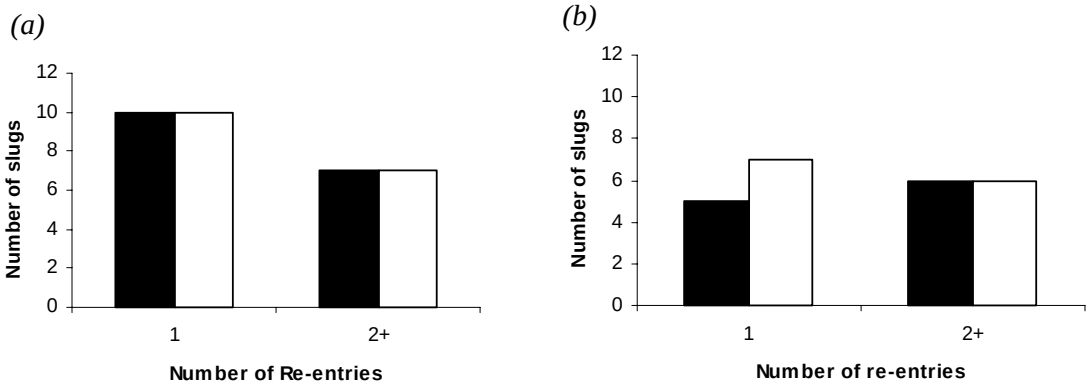


Figure 15.8: Number of small (< 100 mg) and large (> 500mg) Deroceras reticulatum *re-entering refuge once or two or more times during the night (a) indoors and (b) outdoors (black bars = small slugs; white bars = large slugs).*

Table 15.8: Mean elapsed time (± S.E.) between first and second trap re-entries for small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *in indoor and outdoor experiments.*

Experimental setting	*Re-entry*	*Slug size*	*N*	*Mean time (mins) elapsed between refuge trap re-entries (± S.E.)*
Indoor	1	Small	17	85.14 ± 24.10
		Large	17	130.19 ± 65.30
	2	Small	7	50.78 ± 30.04
		Large	7	53.75 ± 24.88
Outdoor	1	Small	11	20.32 ± 6.28
		Large	13	65.53 ± 20.49
	2	Small	6	92.66 ± 58.42
		Large	6	30.59 ± 8.79

More slugs of both sizes entered the trap during the night than were present at dawn, with a reduction in numbers of approximately two thirds (*Fig. 15.9*). This was significant at $P < 0.05$ in both indoor and outdoor experiments (*Table 15.9*). In indoor experiments slightly more large slugs entered the trap than small slugs whereas this was reversed in outdoor experiments. In both conditions, however, the reduction in numbers observed at dawn did not differ significantly according to size.

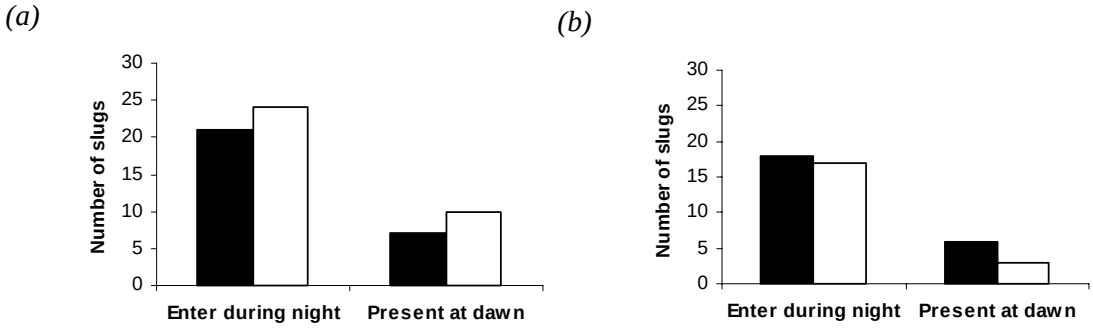


Figure 15.9: Number of small (< 100mg) and large (> 500 mg) Deroceras reticulatum *entering refuge traps during the night and number of each size present at dawn (a) indoors and (b) outdoors (black bars = small slugs; white bars = large slugs).*

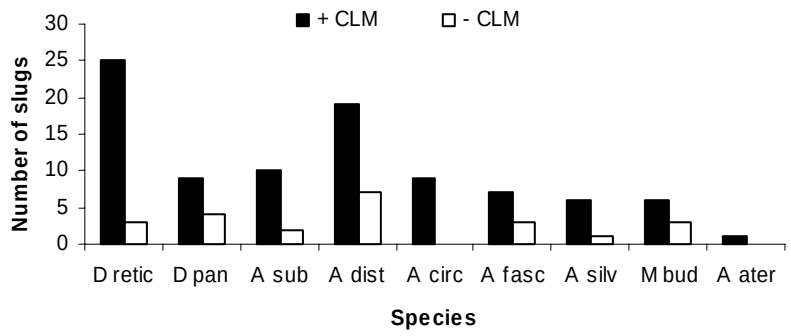
Table 15.9: Results of Scheirer-Ray-Hare test to compare numbers of small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *that enter refuge traps with the number present at dawn.*

Experimental Setting	*Factor/Interaction*	*SS/MS $_{total}$*	*df*	*P-value*
Indoors	Slug size	0.06	1	*n.s.*
	Number entering trap and present at dawn	4.54	1	*< 0.05*
	Interaction	0.19	1	*n.s.*
Outdoors	Slug size	0.02	1	*n.s.*
	Number entering trap and present at dawn	5.04	1	*< 0.05*
	Interaction	0.00	1	*n.s.*

Between dawn and midday it was observed that most slugs remained under the refuge traps in both indoor and outdoor experiments; one small slug and no large slugs left during this period in indoor experiments and a single individual from each size class left in outdoor experiments. Slug size did not, therefore, significantly affect such activity (Fishers Exact test: indoors: N = 17, P = 0.412, *n.s.*; outdoors, N = 9, P = 1.000, *n.s.*). The three single individuals that left the traps did so 1-2 hours after sunrise.

In the field scale comparison of infra-red transparent and standard refuge traps there were no block effects between assessment nights and, therefore, data were pooled. *Fig. 15.10* shows the counts of different species caught in standard and infra-red transparent refuge traps. Baited traps caught markedly more slugs than unbaited traps. *Arion silvaticus* (Lohmander) was caught exclusively in standard traps, however, patterns are otherwise similar between trap types and analyses are restricted to the two most abundant species, *D. reticulatum* and *A. distinctus.*

(a)



(b)

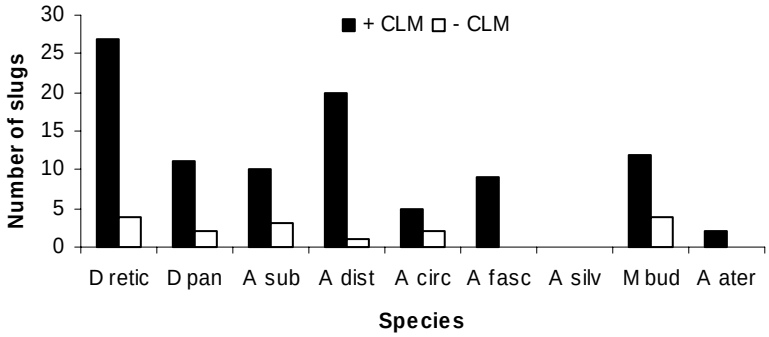


Figure 15.10: Total counts of slug species caught by (a) standard refuge traps and (b) infra-red transparent refuge traps in a grass plot at Close House Field Station over 12 nights (CLM = chicken layers mash bait; D retic = Deroceras reticulatum*; D pan* = Deroceras panormitanum*; A sub* = Arion subfuscus*; A dist* = Arion distinctus*; A circ* = Arion circumscriptus*; A fasc* = Arion fasciatus*; A silv* = Arion silvaticus*; M bud* = Milax budapestensis*; A ater* = Arion ater*).*

For both *D. reticulatum* and *A. distinctus* the infra-red transparent traps caught comparable numbers of slugs to the standard refuge traps according to whether or not they were baited (Chi-squared: *D. reticulatum*: N = 59, df = 1, $G_c^2 = 0.000$, *n.s.*; *A. distinctus*: N = 47, df = 1, $G_c^2 = 2.623$, *n.s.*). The trap type and presence of bait did not affect the mean weight of *D. reticulatum* caught (*Tables 15.10* and *15.11*).

Table 15.10: Results of two-way analysis of variance (ANOVA) to compare mean weight of Deroceras reticulatum *under infra-red transparent and standard refuge traps, baited and unbaited.*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Trap type	1	68830.71	68830.71	1.90	*n.s*
Bait	1	72891.73	72891.73	2.01	*n.s*
Interaction	1	16077.54	16077.54	0.44	*n.s*
Error	55	1990932.84	36198.78		

Table 15.11: Mean weights (mg) (± S.E.) of Deroceras reticulatum *under infra-red transparent and standard refuge traps, baited and unbaited.*

Trap type	*Bait*	*N*	*Mean weight (mg) (± S.E.)*
Standard	+	25	353.46 ± 44.66
	-	3	411.64 ± 108.95
Infra-red transparent	+	27	408.54 ± 30.30
	-	4	502.00 ± 89.75

Discussion

In both refuge traps and DATs and both fields the two most abundant species caught were *D. reticulatum* and *A. subfuscus*. The presence of large numbers of *D. reticulatum* is not surprising since it is one of the most serious and widespread slug pests of cereal crops in the UK (Schley & Bees, 2003). *A. subfuscus*, in contrast, is not generally regarded as a dominant pest of cereals. This may, however, simply reflect regional differences between the study locations and, indeed, earlier work at Heddon Banks Farm also found *A. subfuscus* in noticeable numbers (Young, 1990). This species may, therefore, be an important member of the slug fauna in this particular area.

Although more species in total were caught in the middle field than the bottom field, the numbers of individuals constituting each of these additional species groups was small. It is unlikely that crop related differences in slug distribution would account for this disparity as the crop prior to and during trapping was the same in both fields. Physical differences between the two sites would seem a more probable explanation; the soil in the bottom field is much heavier than the middle field. It is also wetter and prone to pools of standing water as it lies on the flood plain of the River Tyne which may restrict the species it can support.

For both *D. reticulatum* and *A. subfuscus* there was considerable variation in the numbers and mean weights of slugs caught throughout the study period which may explain why the differences between trap types were not significant on some occasions. When there were differences, however, the trends were similar for *D. reticulatum* and *A. subfuscus*. Relative to DATs, refuge traps tended to catch fewer slugs in early spring and summer, but more in autumn and winter, confirming the results of Byers *et al.* (1989) and Barratt *et al.* (1993). The lower catches in spring may be due to the cool temperatures in north east England at this time of year which prevent slugs becoming more active until later in the season. It is not clear why they performed poorly in summer, but this was exceptionally warm and dry in 2003 which may have driven slugs below the ground to avoid desiccation. These results may, therefore, be atypical. In autumn the often wet and mild conditions encourage much surface activity, increasing the likelihood that slugs will encounter a refuge trap. Neither trap performs particularly well in winter (Hunter, 1968a), but DATs are particularly affected by freezing conditions (Barratt *et al.*, 1993) which may explain the apparent improved catch of refuge traps in this season.

Regardless of the total numbers caught, refuge traps had a lower proportion of small slugs in all seasons relative to DATs, except in summer. These patterns may reflect differences in activity related to the breeding cycle (Glen & Wiltshire, 1986). In spring there is a preponderance of newly hatched slugs which, it is suggested, spend a large proportion of time below ground (Archard *et al.*, 2004) and would not be detected by refuge traps. The summer population is composed principally of juveniles; the sizes of slugs are, therefore, more evenly spread masking differences due to extremes. By autumn the population is maturing and there are many surface active adults, which would encounter the refuge traps while searching for mates and laying eggs. In winter the population is comprised mainly of eggs and juveniles over-wintering below ground.

The techniques used in the slug activity experiments allowed the behaviour of slugs beneath refuge traps to be studied for the first time. The species used was *D. reticulatum*, a particularly surface active species (South, 1965). It was hypothesised that behavioural differences between small and large slugs on encountering the traps might partly explain why they tend to be biased towards large slugs. These studies, however, found no evidence of size-related differences in the response of small and large *D. reticulatum* for any of the behaviours assessed. This was the case for both indoor and outdoor experiments.

The timings of the initial trap entry varied considerably between individuals in agreement with Grimm & Schaumberger (2002), but the mean value corresponded with that reported by Hommay (1998) in his study of activity and sheltering behaviour in this species. The bait used was highly palatable to slugs (Young, 1990). Although olfaction is not thought to play a role in locating pellets or single seeds over distances greater than 3-4 cm (Bailey *et al.*, 1989; Howling, 1991), it may have been of greater significance with the larger quantities of food used in the current study (2.5 g chicken layers mash). Furthermore, its presence in

the traps may have influenced the time spent underneath once slugs had entered, particularly as slugs had been starved for 24 hours prior to recordings which has been shown to increase the first meal length (Bailey, 1989).

Most slugs were observed to enter the trap at least once with many re-entering multiple times. This result may be exaggerated due to restricted movement along the margins of the arena; this is an unavoidable limitation of video assessments of activity. Although studies have indicated that the arena size does not affect the distance moved (Bailey, 1989; Howling, 1990) it might affect the direction of movement; this could be important when there is an object in the centre of the arena, the response to which is the variable of interest.

The number of slugs in the traps at dawn was approximately one third of the total active population. Experiments would need to be repeated under a wider range of conditions and with different species to gauge how reliable this figure is, but such data could help to refine damage risk estimates. Caution must be exercised in extrapolating laboratory results of behaviour studies to field situations since the former are controlled so as to be repeatable whereas in their natural environment animals are subjected to a great many more variables which may modify their behaviour (Bailey, 1989). For this reason the experiments presented here were performed indoors and outdoors. The necessity of constraining slugs in a fixed arena meant, however, that only semi-field conditions could be achieved; slugs were exposed to naturally fluctuating temperatures and light intensity, but the influence of the arena edges on directional movement and their buffering effects against air currents could not be eliminated. The concordance of indoor and outdoor results indicates that the activity of slugs in the laboratory setup realistically represented behaviour in the field under the conditions tested, but further work is needed to confirm this, for example repeating the experiments in larger arenas and at different times of the year.

The time of year when experiments were conducted (early April 2004) may have particularly influenced the results concerning numbers of slugs leaving the traps between dawn and midday. It was expected that outdoors, as the air temperature rose following sunrise, conditions under the trap would become increasingly inhospitable for slugs causing them to leave (Judge, 1972; Schrim & Byers, 1980). Although the weather was mild (mean minimum temperature 11.6°C; range 8-21°C) with some sunny days, Tinytalk™ data loggers showed that there was only a 1°C difference between the mean soil and air temperature. Under the conditions of this study, therefore, any heating effect was minimal, but it seems unlikely that this would be the case in the summer time.

The weight difference between small (< 100 mg) and large (> 500 mg) slugs in these experiments was quite wide. This was necessary in order to distinguish between them on the monochrome recordings; natural contraction and elongation of slugs during locomotion alters their appearance on screen and the weights chosen for the two size categories allowed them to be discriminated despite this.

Food deprivation has been shown to increase the locomotor activity of *D. reticulatum* (Airey, 1987). A short period of starvation prior to studies of foraging and feeding is common practice in order to improve the chances that slugs will be responsive during experiments and was also the case in the current work. Since slugs are known to be capable of tolerating such conditions for several weeks (Lovett & Black, 1920 in Arias & Crowell, 1963), a short starvation period of 24 hours is unlikely to have unduly altered basic physiology over and above simply increasing motivation to feed and there is no evidence to suggest that small and large slugs are differentially affected over such short time intervals.

In summary, the under representation of small slugs by refuge traps relative to DATs does not appear to be due to differences in their behavioural response compared to large slugs upon encountering the trap; they spent similar amounts of time under the traps, entered them similar numbers of times and left before dawn in comparable numbers. The suggestion that small slugs may be overlooked by observers (Archard *et al.*, 2004) may be feasible if farmers are hurriedly checking their own traps, but is unlikely to be the case for research reports which are conducted by biologists with dedicated time for the task and considerable experience (D. Glen, *pers. comm.)*. A failure of small slugs to reach the traps under natural conditions would seem to be a more likely explanation.

The field scale comparison of infra-red transparent and standard refuge traps was done primarily to ensure that the infra-red transparent traps performed as well as standard traps in the field and this was found to be the case. The infra-red traps are a reliable substitute for opaque plastic saucers and a useful research tool for slug behavioural studies. The two most abundant species were *D. reticulatum* and *Arion distinctus* (Mabille), both of which are prevalent in the walled garden at Close House. Baited traps caught significantly larger numbers of slugs than unbaited traps. As mentioned previously, olfaction is not believed to play a role in attraction to food over a distance (Bailey *et al.*, 1989; Howling, 1991). It may be that trap entry was a random process, but once underneath the baited saucers the slugs remained to feed and rest whereas they left unbaited traps again. The presence of these slugs under the baited traps would also have provided mating opportunities to attract and retain others. That the mean weight of *D. reticulatum* did not vary between baited and unbaited traps suggests that the added effect of bait is on total numbers and is not size-related.

In conclusion, it was found that:

1. Refuge traps under represented small *D. reticulatum* and *A. subfuscus* relative to DATs in all seasons except summer.
2. Refuge traps caught fewer *D. reticulatum* and *A. subfuscus* relative to DATs in spring and summer, but caught more in autumn and winter.
3. Behavioural differences towards refuge traps did not differ significantly between small and large *D. reticulatum.*

4. Infra-red transparent refuge traps performed as well as standard opaque refuge traps in field conditions.
5. Many factors influence the efficacy of different methods of population estimation and this must be borne in mind when interpreting the results of comparative studies. It may help if statements regarding traps are qualified with the weather conditions, habitat and the time of year measurements were made.

It would be interesting to see what effect alternative food has on slug behaviour towards refuge traps. Although seedlings would have reduced the clarity of video recordings, it would be possible to use ungerminated seeds in the arena as these would not obscure the view of slugs. It would also be useful to extend the work to different species; it may be that those that are generally less surface active than *D. reticulatum* would behave differently. it would be helpful to formally assess the effect of the arena edges on directional movement in order to interpret the results of the video experiments more fully.

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PAPER 16 – *Objective 4*

System of Risk Assessment and Integrated Control for Winter Wheat

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Abstract

A systematic approach for integrated control of slug damage in winter wheat is described. Firstly, damage risk is assessed, based on the slug catch in traps baited with chicken layers' mash, left out in the field overnight before cultivation, together with other factors known to influence the ability of slugs to inflict severe damage. Damage risk is minimised by the use of cultural control measures. However, if the risk is high, slug pellets should be applied at around the time of drilling, before significant damage has occurred. All crops should be monitored closely from emergence until tillering, then at intervals throughout the winter and slug pellets broadcast if the crop is slow to emerge or to grow through the early vulnerable stages and does not appear to be outgrowing slug damage.

Introduction

Winter wheat is highly vulnerable to slug attack during establishment (Glen & Moens, 2002). Wheat seeds are especially at risk and severe damage can result in complete crop failure or such severe loss of stand that the crop will not recover. This paper describes a system for integrated control of slug damage in winter wheat that has been developed in the UK by the partners in a Sustainable Arable LINK Project.

Risk assessment and cultural control

Risk assessment and cultural control measures are very closely interlinked.

There are four main elements, as described below:-

5. Trap to assess slug activity during period before cultivation and possibly after drilling.
6. Use trap catches together with other information to assess the risk of slug damage
7. Reduce the risk of slug attack by cultivations and adjustment of drilling depth
8. Monitor crops throughout the early susceptible growth stages

1. Trapping to assess slug activity

Slug activity on the soil surface is dependent on moist and mild conditions. Traps act as refuges for slugs active on the soil surface in the surrounding area. Traps predominantly record slugs >100 mg, whereas smaller slugs are under-recorded compared to their densities in soil, even though behavioural studies have shown no differences in the rates of entry and leaving traps (Howlett *et al.*, 2005). If conditions are suitable for surface activity, the catch will give an indication of the number of slugs >100 mg in the area. Placing bait beneath traps increases the numbers of slugs trapped.

Although traps baited with slug pellets have been used routinely for monitoring slug activity (e.g. Gratwick, 1992), we do not now recommend slug pellets as trap bait because recent research shows that chicken layers' mash is a safe and effective alternative (Young *et al.* 1996; Glen *et al.*, 2003). Traps with this bait need only be left out for one night to record slug numbers similar to traps baited with slug pellets left out for three nights (Glen *et al.*, 2003). Traps may consist of inverted plant saucers, mats (for example, as described by Hommay & Briard, 1988) or pieces of hardboard etc. Traps should be of about 25 cm diameter or width. A heap of around 20 ml (two heaped teaspoonfuls) of chicken layers' mash is placed under each trap.

Trapping should be done during the period before cultivation. After cultivation, trapping may under-estimate the true slug population as surface activity is reduced. However, trapping between drilling and emergence is valuable if wet weather persists, because under these conditions increasing slug populations may pose a threat to emerging wheat. It is essential to take advantage of suitable weather for trapping. Traps are put in place only when the soil surface is moist and temperatures are favourable for slug activity (minimum night temperature greater than 5^{o}C, maximum daytime temperature less than 25 ^{o}C).

Nine traps should be laid out in a 'W' pattern in each field (13 traps if the field is larger than 20 ha). If certain areas of the field are known to suffer from slug damage (e.g. areas of heavy clay or silt soil), traps should be concentrated in these areas. Traps are left overnight and examined the following morning. Slugs will remain in traps while the sky remains overcast, but will leave traps if they heat up when exposed to sunshine. In sunny weather, traps should be examined as early as possible, before direct exposure to sunshine.

For winter wheat, a catch of an average of four or more slugs per trap will justify slug pellet treatment, provided that favourable conditions for slug activity (and control) continue and provided that other risk factors (described below) are positive.

Trapping will provide a useful guide to levels of slug activity, when carried out under favourable conditions while following the guidelines given above. Monitoring should be considered well in advance of drilling to maximise flexibility in subsequent operations.

2. Use trap catches together with other information to assess the risk of slug damage

When trap catch exceeds the threshold, slug pellet treatment is advised when one or more of the following criteria are met:

- the field is drilled during a period of generally wet weather
- wet weather delays sowing in a prepared seedbed
- the seedbed tilth is coarse and cloddy, and further consolidation is not possible following sowing
- wet weather continues after drilling and further trapping shows evidence of high slug activity on the seedbed
- the crop is slow to emerge or to grow through the early vulnerable stages and symptoms of slug damage are seen.

3. Reduce the risk of slug attack by cultivations and adjustment of drilling depth

Damage to seeds and seedlings of winter wheat before emergence directly affects yield, but is the hardest to predict as it may not be linked to surface activity. The best approach to prevent early damage is sowing at sufficient depth (3 cm) in a fine, consolidated seedbed to deny access to the seeds by slugs and provide conditions for rapid germination. In cloddy seedbeds, seeds should be sown a little deeper than normal (4-5 cm).

The more cultivations and the more intensive the cultivation method, the greater the likelihood that slug numbers will be reduced, especially if the weather is dry. However, whatever method of cultivations are used, it is important that the seedbed is fine and firm to protect seeds and young seedlings. Thus, although reduced tillage methods can allow more slugs to survive compared with ploughing, they can have advantages for slug control if the farmer is able to produce finer seedbeds compared with ploughing. Moreover, surviving slugs are not buried to some depth by reduced tillage, as happens after ploughing, so slugs are more likely to be surface-active following reduced tillage and therefore vulnerable to slug pellets. Reduced tillage methods also retain seedbed moisture for germination under dry conditions, which helps the crop to grow rapidly through the early vulnerable stages.

4. Monitor crops throughout the early susceptible growth stages

Crops should be examined regularly for slug damage. Slug trapping is not normally necessary at this stage, but trapping should be done if there is any doubt about whether the damage is caused by slugs. Cereal crops are most susceptible to damage from sowing to first tillering (GS 21). After this growth stage is reached, further damage is unlikely to result in additional loss of plants. However it is important to continue to monitor crops throughout the winter and be ready to treat if there is evidence of fresh damage to young leaves and plants show signs of being set back by slug damage.

Control using slug pellets

Timing of slug pellet treatment

An application of slug pellets at the recommended rate will generally depress the slug population and feeding activity for several weeks following treatment. The population will recover more rapidly if conditions are especially favourable (mild and wet), or more slowly if the intervening period is dry. The proportion of the population killed will depend on surface activity in the week following application. Heavy splashy rain soon after treatment could result in reduced efficacy. If there is heavy rain within three days of application, treated fields should be examined to check whether pellets are still visible. The highest probability of an economic response to treatment occurs during the first month of crop growth, so that an application immediately following drilling and rolling is most likely to produce a useful effect.

Application shortly before drilling may be effective if the conditions at the time are suitable for surface activity and if the soil can be left undisturbed for three days after treatment. However, because of the importance of timely sowing in good conditions for control of slug damage, it is not worth delaying sowing to allow a treatment to be applied (Gratwick, 1992).

In the dry autumns of 2002 and 2003, pellets applied to stubble up to 6 weeks before drilling winter wheat were as effective as pellets applied after drilling. In contrast, in the wet autumn of 2004 pellets applied to stubble had lost their efficacy by the time that wheat was at risk; pellets applied after drilling were significantly more effective. Thus, the earlier before sowing that treatments are applied the more likely it is that slug populations will have recovered by the time the crop is exposed to damage.

When risk assessment shows that an application of pellets is not justified around the time of drilling, a pre-emergence treatment may be justified if wet weather has continued since drilling and traps placed on the seedbed show high slug activity.

Treatments are often applied after crop emergence in response to fresh leaf shredding damage. They will normally only be worthwhile in the period before the crop has reached the less susceptible 3-4 true-leaf/tillering growth stage and, as always, if the conditions following treatment are suitable for surface

activity. However treatment after tillering may be justified if there is evidence of fresh damage to young leaves and plants show signs of being set back by slug damage.

Slug pellet application

Pellets may be applied broadcast to the soil surface or admixed with seed and applied at drilling. Mixing should be carried out immediately before use, calculating the quantity needed to ensure that there is negligible surplus of the mixture. Storage of grain/slug pellet mixtures is not good practice and should be avoided.

Broadcasting is the method of application that will give the most consistent slug control, especially when combined with the preparation of fine, firm seedbeds that protect seeds and young seedlings from attack before emergence.

Pellet admixtures with wheat seeds may be effective when winter wheat is direct-drilled or drilled into open cloddy seedbeds. However when seeds are sown, as recommended, into fine seedbeds, admixed pellets will be ineffective because, like the seeds, they will be unavailable to slugs. Slugs will survive to attack the emerging seedlings.

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PAPER 17 – *Objective 4*

Risk Assessment and Integrated Control for Oilseed Rape

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Abstract

Observations of slug activity in standing wheat crops and on cereal stubbles were made at 29 sites in summer and autumn 2002 (3 sites), 2003 (14 sites) and 2004 (12 sites). At ten of these sites, trapping was also undertaken in standing cereal crops. At six sites, trapping was possible only in standing cereal crops. Damage assessments were made in following crops of winter oilseed rape at the two-leaf stage (GS 1,02). Second damage assessments were made at the four-leaf stage at five sites in the Midlands and six sites in Somerset. Slug trap catches were substantially higher in standing cereals than in stubble, but the trap catch in standing cereals was not significantly related to subsequent damage to oilseed rape. Significant regressions were obtained between mean the percentage of oilseed rape plants damaged by slugs at an average two-leaf stage (GS 1,02) and mean number of slugs per trap on cereal stubbles using combined data for 2002-2004. These data provided the most useful predictions of crop damage, provided that stubble trapping was undertaken when the soil surface remained moist during wetter weather conditions favourable for slug activity. Regression analysis for data for 21 sites where the conditions were suitable for trapping indicated that a mean of one slug per trap on a cereal stubble would result, on average, in 5% seedling plants damaged by slugs at the two-leaf stage in a subsequent crop of oilseed rape (P=0.015). However, a pragmatic estimate of the upper limit of the relationship indicated that more than 10% of plants could be damaged following a catch of one slug per trap. This level of damage at the two-leaf stage is potentially serious because studies at six sites showed that it could result in more than 30% plant losS by the four-leaf stage. We have therefore suggested, as a guideline, that a catch of 1 or more slugs/trap in cereal stubble indicates possible risk if other risk conditions are met, as described in HGCA Topic Sheet No. 85. Because there is only a short period between harvesting cereals (especially wheat) and drilling oilseed rape, trapping may not be possible at this time and we therefore suggest that it may be worthwhile trapping in standing cereals, up to 10 days before harvest. If this is done, a catch of 4 or more slugs/trap in standing cereals indicates a potential risk, if other conditions are met.

Introduction

Oilseed rape seeds are not damaged by slugs, but the seedlings are attacked and they are most vulnerable to slug damage in the early stages of germination (Moens & Glen, 2002). For effective control, it would therefore be valuable to be able to assess the risk of slug damage before drilling and, if necessary, apply molluscicides as soon as possible after drilling, before seedlings start to germinate. Moens and Glen (2002) suggest that modern cultivars of oilseed rape, with their low concentrations of glucosinolates, are more susceptible than winter wheat to slug damage. However, there is little information on the relationship between slug population densities in soil and the severity of damage to oilseed rape at establishment. In this study, we have made a preliminary appraisal of whether it is feasible to evaluate damage risk to oilseed rape by using slug traps to estimate slug activity in standing cereal crops and cereal stubble, just prior to oilseed rape establishment.

Methods

Within each study field from 2002 to 2004, nine or ten saucer traps, 25 cm in diameter, were baited with chicken layers mash (i) within standing crops of winter wheat in late July-early August and (ii) on cereal stubbles during August, prior to drilling oilseed rape in late August or early September. Suitably moist periods were selected for monitoring (the soil surface was visibly moist when traps were put in place) and traps were left in position for 1-3 nights during suitable rainfall events. (For further details of trapping methods, see Paper 14, this report).

Where trapping had indicated slug activity in the pre-drilling period, follow-up assessments of damage incidence were made in subsequent oilseed rape crops. For six oilseed rape crops in the western Midlands, plant populations were assessed at the two leaf stage. Percentages of plants with evidence of slug grazing were determined from ten 30x30 cm quadrats per plot. Untreated strips were left in two oilseed rape crops which were treated post-drilling with slug pellets at Billingsley, Shropshire (sites 5 and 6). At these sites, plant counts and damage assessments were made on untreated and treated field areas. Second damage assessments were determined on 26 November from counts made on 25 randomly-selected plants within treated and untreated areas.

For crops in Somerset, plant numbers and slug damage on nine or ten untreated subplots in each study field were compared with the same number of subplots treated with slug pellets (Metarex, 5% metaldehyde, De Sangosse UK) (i) as soon as possible after drilling and (ii) at about the one- to two-leaf stage. If analysis of variance showed significant differences in plant numbers or the percentage of plants with slug damage between treated and untreated plots, the percentage reduction in plant stand and the percentage of damage to plants were calculated on untreated subplots.

Results and discussion

Summer & autumn 2002

At the two sites where slugs were trapped in both standing cereals and cereals stubble, trap catches were substantially higher in the standing cereals than the stubble (Table 17.1). The highest level of damage, with a 46% reduction in plant numbers, was recorded on untreated subplots at the site where trap catches in standing cereals were highest (28.9 slugs/trap). The trap catch in cereal stubble at this site was substantially lower (3.9 slugs/trap). Bright sunny conditions in the morning when traps were examined in the stubble could have contributed to this reduction in numbers. No significant reduction in plant numbers was recorded at the other two sites, but 10 and 48% of plants were damaged by slugs at these sites.

Table 17.1. Summary of mean number of slugs per refuge trap in standing winter wheat crops or on wheat stubbles and plant population and damage assessments made in subsequent winter oilseed rape crops in autumn 2003.

Site	*Trapping period*	*Traps sited in:*	*Mean no. slugs per trap*	*Oilseed rape drilled:*	*% Decrease in plant no.*	*% Plants damaged by slugs*	*Assessment dates (growth stage in brackets)*
1. Field 75, North Somerset	24.06-10.07.02	W. wheat Crop	10.0	30.08.02	0	48.4%	10.09-23.09 (1,00-1,04)
	12.08.02	W. wheat stubble	0.5				
2. Holbrook, Somerset	12.07.02	W. wheat Crop	28.9	04.09.02	46	81%	20.09-07.10 (1,01-1,04)
	19.08.02	W. wheat stubble	3.9				
3. Cowley's, Somerset	9.08.02	W. wheat stubble	7.5	11.09.02	0	9.6%	20.09-09.10 (1,00-1,04)

Summer and autumn 2003

A high incidence of slug activity was recorded in standing winter wheat crops at three sites in Shropshire in traps established in wet conditions on 31 July and recovered in moist conditions between 0845 and 1030 on 1 August. Means of 14.0, 17.1 and 28.9 slugs per trap were recorded after one night at sites 7-9 respectively (Table 17.2). The trap catch was also high (33/trap) in standing wheat in Glebe Field, Somerset (site 17). Sites 8 and 9 were selected for more detailed studies on treated and untreated areas of crop. Slug activity on stubbles was low in dry conditions in August, even at sites showing an earlier high incidence of slug activity in standing winter wheat crops (sites 4-6 described previously). At the two sites in Somerset, trapping was not done in stubble due to the dry conditions.

A low or zero incidence of slug damage in subsequent oilseed rape crops was recorded at sites in Staffordshire (sites 4-6); Shropshire (site 7); Norfolk, North Yorkshire, Herefordshire, Cambridgeshire (sites

10-15) and Somerset (sites 13 & 14). At sites 8 and 9, where more detailed assessments were made, incidence of damage in the following oilseed rape crops in September was initially slight although increases occurred during moister conditions from late October. Data summaries for all sites are presented in Table 17.2.

Table 17.2. Summary of sites showing mean number of slugs per refuge trap in standing winter wheat crops or on wheat stubbles and plant population and damage assessments made in subsequent winter oilseed rape crops in autumn 2003.

Site	*Trapping period*	*Traps sited in:*	*Mean no. slugs per trap*	*Oilseed rape drilled:*	*Plants /m², or % reduction*	*Percentage plants damaged by slugs*	*Assessment date (growth stage in brackets)*
4. Oakley, Staffs.	11-12.08.03	W. wheat stubble	0	17.08.03	78.8	1.4%	23.09.03 (1,03-1,04)
5. Shutt Green A, Staffs	11-12.08.03	W. wheat stubble	0.3	17.08.03	62.7	0%	23.09.03 (1,03-1,04)
6. Shutt Green B, Staffs	11-12.08.03	W. wheat stubble	0.1	17.08.03	71.5	0%	23.09.03 (1,03-1,04)
7. Much Wenlock, Shropshire	31.7-1.8.03	W. wheat crop	14.0	01.09.03	110.0	0%	23.09.03 (1,02)
	11-12.08.03	W. wheat stubble	0				
8. Billingsley A, Shropshire	31.7-1.8.03	W. wheat crop	17.1	01.09.03	69.3 unt 72.6 trt	1.6% unt 0% treated	23.09.03
(Cottage Field)	11-12.08.03	W. wheat stubble	0			44.0% unt 36.0% trt	26.11.03
9. Billingsley B, Shropshire	31.7-1.8.03	W. wheat crop	28.9	01.09.03	93.5 unt 104.5 trt	1.2% unt 0% treated	23.09.03
(Snow Hill)	11-12.08.03	W. wheat stubble	0			52.0% unt 8.0% trt	26.11.03
10-12. Terrington, Norfolk (3 sites)	11-12.08.03	W. wheat stubbles	0.03, 0, 0	From 30.09.03	Not assessed	0%	
13. Duggleby, North Yorkshire	11-12.08.03	W. wheat stubble	0		Not assessed	0%	
14. Rosemaund, Herefordshire	11-12.08.03	W. wheat stubble	0		Not assessed	0%	
15. Boxworth, Cambridgeshire	11-12.08.03	W. wheat crop	0		Not assessed	0%	
16. Pad. 1,2&3, Somerset		W. wheat crop	3.2	26.08.03	0% reduction	2.5%	
		W. wheat stubble	-				
17. Glebe Field, Somerset	18.7-1.08.03	W. wheat crop	33.1	25.08.03	0% reduction	4.6%	
	30.08.03	W. wheat stubble	-				

Unt = molluscicide-untreated area; trt = molluscicide-treated field area

Two winter oilseed rape crops, in which strips remained untreated with slug pellets, were drilled on 1 September and established rapidly (sites 5 and 6). A high incidence of slug activity had been recorded in standing winter wheat crops at these sites on 1 August. Mean number of plants/m² ranged from 69.3/m² on untreated areas at site 5 to 104.5/m² on areas of crop treated with slug pellets pre-crop emergence at site 6. Damage assessments made at sites 8-9 on 23 September showed a zero or low incidence of slug-damaged plants and no evidence of slug activity on the soil surface. Similar results were also obtained at site 7 at which a high incidence of slug activity had also been recorded in the preceding, standing winter wheat crop.

Table17.3: Slug activity and damage assessments in oilseed rape at site 8. Winter oilseed rape cv. Recital, Cottage field, Billingsley, Shropshire – untreated strip compared with treated field.

Trapping period or assessment date	*Untreated area*	*Treated area of osr crop*
31.07-01.08 (standing winter wheat crop)	Mean 17.1 slugs per trap	-
11-12.08.03 (winter wheat stubble)	Mean 0 slugs per trap	-
11-12.09.03 (osr crop)	Mean 0 slugs per trap	Mean 0 slugs per trap
11-26.11.03 (osr crop)	Mean 0.1 slugs per trap	Mean 0 slugs per trap
Plant number/m² 23.09.03 GS 1,0 (cotyledon) -1,01	69.3	72.6
Percentage plants damaged by slugs 23.09.03	1.6%	0%
Percentage plants damaged by slugs 26.11.03 GS 1,06	44.0%	36.0%
Mean damage severity score 26.11.03	0.56	0.64

Table 17.4. Slug activity and damage assessments in oilseed rape at site 9. Winter oilseed rape cv. Recital, Snow Hill field, Billingsley, Shropshire – untreated strip compared with treated field.

Trapping period or assessment date	*Untreated area*	*Treated area of osr crop*
31.07-01.08 (standing winter wheat crop)	Mean 28.9 slugs per trap	-
11-12.08.03 (winter wheat stubble)	Mean 0 slugs per trap	-
11-12.09.03 (osr crop)	Mean 0 slugs per trap	Mean 0 slugs per trap
11-26.11.03 (osr crop)	Mean 0.4 slugs per trap	Mean 0.1 slugs per trap
Plant number/m² 23.09.03	93.5	104.5
Percentage plants damaged by slugs 23.09.03 GS 1,01-1,02	1.2%	0%
Percentage plants damaged by slugs 26.11.03 GS 1,06	52.0%	8.0%
Mean damage severity score 26.11.03	1.0	0.12

As soils moistened in late October 2003, slug activity started to increase in oilseed rape crops with 44% and 52% of plants with slug damage at GS 1,6 on 23 November in untreated strips at sites 8 and 9 respectively (Tables 17.3 and 17.4). A lower incidence of crop damage (mean 8.0% damaged plants) was recorded in areas treated with molluscicide at site 9.

During the warm and drier than average conditions in August 2003, slug activity was low on winter wheat stubbles, including sites where a previous high incidence of slug activity had been recorded in a standing winter wheat crops (sites 7, 8 and 9 in Shropshire). Overall, only a low incidence of damage to oilseed rape crops was recorded at early emergence stages at three sites.

Summer and autumn 2004

Monitoring was undertaken at 12 sites in harvest year 2005. Data are summarised in Table 17.5. Trapping was undertaken in standing winter wheat crops at six sites. The highest incidence of slug activity was recorded in Somerset (site 29), with a mean of 20.0 slugs/trap in standing wheat, and at sites 21 and 22 in Shropshire with 4.8 and 9.2 per trap respectively.

Table 17.5: Sites monitored in summer and autumn 2004. Note that the percentage reduction in plant numbers is given for Site 24

Site	*County*	*Location*	*Trapping on cereal stubble or standing wheat crop*	*Mean no. slugs per trap*	*Mean max. no. slugs per trap*	*Mean % slug damaged osr plants*	*Mean max. % slug dam. plants*
18	Staffs	Oaken	Stubble ww	0.6	0.6	0	0
19	Staffs	Brewood	Stubble ww	0.7	0.7	10.0	10.0
20	Staffs	Codsall	Stubble ww	2.7	2.7	12.0	12.0
21	Shropshire	Billingsley (Railway Fd)	st crop only ww	3.9	4.8	25.0	100
22	Shropshire	Billingsley (18 acres Fd)	st crop & stubble ww	0.3	9.2	26.0	84.2
23	Shropshire	Much Wenlock	Stubble ww	3.6	3.6	34.0	100
24	Norfolk	Terrington A	st crop only ww	0.3	0.3	24.6	24.6
25	Norfolk	Terrington B	st crop only ww	0.3	0.3	6.0	6.0
26	Norfolk	Terrington C	st crop only ww	1.1	1.1	8.0	8.0
27	N. Yorkshire	Duggleby (Crow wood)	Stubble wb	2.7	2.7	13.3	13.3
28	N. Yorkshire	Duggleby (Kirbys)	Stubble wb	5.0	5.0	5.3	5.3
29.	Somerset	Higher Clapton	St crop ww Stubble ww	20.0 5.7	39.5 5.7	!36.2% Reduction	91.7

wb = winter barley; ww=winter wheat

Trapping on stubbles was undertaken at eight sites. At site 29, Somerset, trapping on winter wheat stubble gave a mean of 5.7 slug/traps. At ADAS High Mowthorpe, North Yorkshire, trapping on winter barley stubble was done in wet conditions on 17-18 August, and resulted in a mean total of 5.0 slugs per trap after one night. A lower incidence of slug activity was recorded on cereal stubbles at the remaining sites. Trapping in the standing wheat crop and on the stubble was undertaken at site 22 in Shropshire, which showed a low incidence of activity on the stubble; mean 0.3 per trap in the period 3-4 September 2004 when conditions were dry and relatively unsuitable for trapping. However, with a very short period between the delayed winter wheat harvest and drilling the crop of oilseed rape crop, this was the only opportunity to trap at the site which had previously indicated a mean of 9.2 slugs per trap in the standing wheat crop on 23-24 August.

Assessments of slug damage at the two leaf stage (GS 1,2) were undertaken at 12 sites in winter oilseed rape crops following winter cereals. At three sites in Shropshire (sites 21-23), second assessments of plant damage were made at the four-leaf stage (GS 1,4). The highest incidence of damage was recorded at site 29, in Somerset, where there was a reduction in plant numbers and 92% of plants were damaged by the four-leaf stage, and at site 23 where 100% of plants were damaged by slugs.

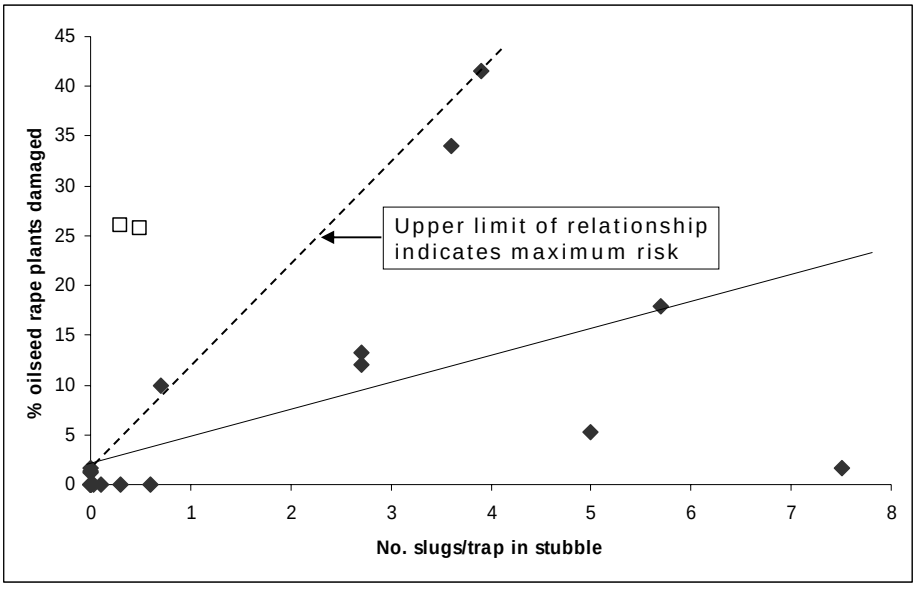

Figure 17.1: Relationship between the number of slugs per trap in cereal stubble and the subsequent percentage of oilseed rape plants damaged by slugs at GS1,02 (two-true-leaves), 2002-2004. The open squares represent two sites where conditions were sub-optimal, with the soil surface dry or drying. Solid diamonds represent sites where weather conditions were suitable for trapping (the soil surface remained moist). The solid line shows the regression (y = 2.59x + 2.51), whilst the dashed line shows a pragmatic estimate of the upper limit of damage risk.

Relationship between slug trap catch and damage to oilseed rape

The most useful damage predictor was the highly significant regression obtained between the percentage of oilseed rape plants damaged at GS 1,02 (two-true leaves) and the mean number of slugs per trap on cereal stubble (Fig. 17.1) using combined data for all three years.

Trapping at one site had, by necessity, to be conducted in dry conditions (site 22 in autumn 2004) as there was only a very short available period between wheat harvest and ploughing for the following crop of oilseed rape. Similarly, trapping at site 1 in 2002 was conducted under drying conditions: although the soil surface was moist when the traps were put out, it was recorded as drying when the traps were examined. If data for these two site are removed from the analysis, as shown in Fig. 17.1, this gives a significant relationship between trap catch in stubble and damage to the oilseed rape crop at GS 1,02 (P = 0.015, y = 2.59x + 2.51). The regression line for the 21 sites where the conditions were suitable for trapping indicates that a mean of one slug per trap on cereal stubble would result, on average, in 5% seedling plants being damaged by slugs at the two-leaf stage in a subsequent crop of oilseed rape. However, for the purpose of damage forecasting, the regression line describing the average relationship between these two factors does not adequately describe damage risk because, as shown in Fig, 17.1, in some cases the level of damage was considerably higher than expected from this relationship. The upper limit of the relationship between trap catch in stubble and slug damage at the two-true-leaf stage is much more important for damage forecasting; a pragmatic estimate of this upper limit is shown by the dashed line in Fig. 17.1.

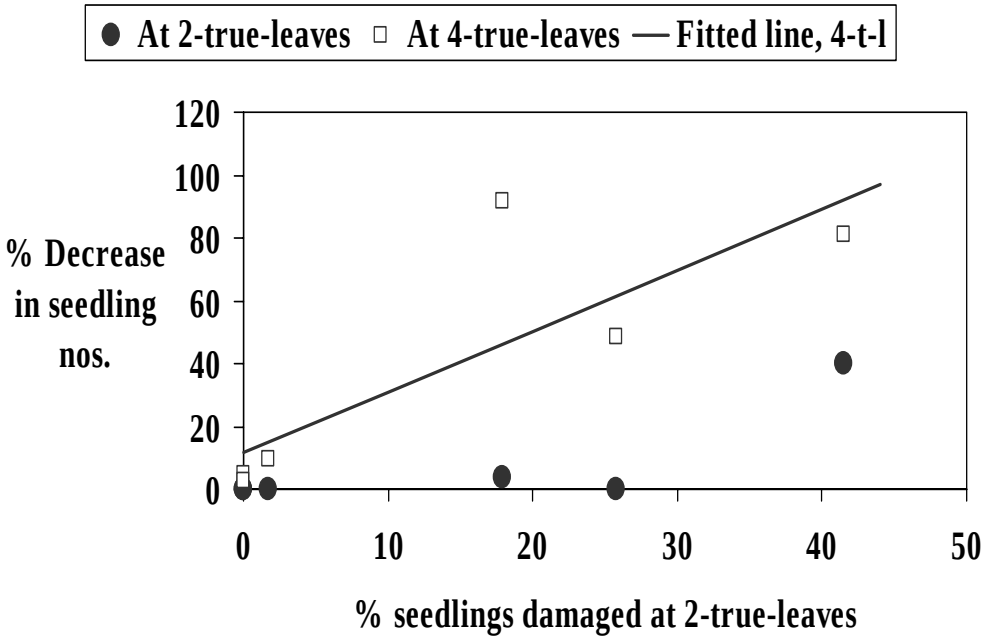

Figure 17.2: Relationship between the percentage of oilseed rape plants damaged by slugs at (two-true-leaves or at four-true-leaves and the percentage decrease in plant numbers at six sites where this was measured, 2002-2004.

The importance of slug damage to oilseed rape plants at the two-leaf stage can be gauged by the way that this damage is related to plant losses, not only at this stage, but up to the four-true-leaf stage (by which time the plants are generally considered to be able to withstand and outgrow any further damage). These relationships are shown in Fig. 17.2, for the six sites in Somerset where both parameters were measured. The relationship was significant ($P < 0.05$) for the percentage decrease in plant numbers at the four-leaf stage and the regression line indicates that 10% plant damage at the two-leaf stage could lead to a loss of about 30% of plant stand at the four-leaf stage. Not surprisingly, there was a greater reduction in seedling numbers by the four-leaf-stage than at two leaves; the relationship between percentage damage and plant loss at the two-leaf stage indicates no plant loss even when about 25% of plants were damaged. This could possibly suggest that farmers would not need to treat after drilling until the anticipated damage at the two-leaf stage exceeded 25%. However, the results presented here are only of a preliminary nature and we believe that we should be cautious about expected damage. On this basis we suggest as a guideline that, if the trap catch in cereal stubble reaches or exceeds an average of one slug per trap, farmers should apply slug pellets as soon as possible after drilling and rolling oilseed rape, in order to protect against the risk of slug damage.

Trapping data obtained within standing crops of winter wheat provided a poor predictor of damage. Regressions for the tested variates were not significant, in contrast to trapping data for cereal stubbles. Nevertheless, we know from previous studies that slug activity, trap catches and crop damage are very weather dependent. Therefore, it may not be possible to select suitably wet conditions for slug trapping in the short period between harvesting cereals (especially winter wheat) and cultivating for oilseed rape. In such conditions, it is possible that slug numbers in traps on stubble will be low but a following crop may still suffer significant slug damage. Our data provided evidence of this in autumn 2002 (site 1 with a mean of 0.5 slugs per trap on wheat stubble but 26% oilseed rape plants damaged by slugs at GS 1,02)and 2004 (site 22 with a mean of 0.3 slugs per trap on wheat stubble but 26% oilseed rape plants damaged by slugs at GS 1,02). Conversely, in autumn 2003, trapping in standing wheat crops had shown a high incidence of slug activity at three sites in Shropshire and one site in Somerset but subsequent activity on the wheat stubbles was slight with little damage in the following oilseed rape crops at the two leaf stage.

Because weather conditions during the short period between harvesting cereals (especially wheat) and drilling winter oilseed rape, may be unsuitable for slug trapping, it may therefore be worthwhile to trap in standing cereals up to 10 days before harvest, particularly if farmers plan to broadcast seeds into standing cereals or stubble (eg Autocast). In this study it is clear that, where trapping was done in both standing cereals and stubble, trap catches were at least three to four times higher in standing cereals than stubble. It is likely that traps are more attractive to slugs in standing cereasl than on stubble because (1) there is little alternative food for slugs in standing cereals, whereas food is abundant in the form of spilt grain etc on stubble and (2) the soil-surface microclimate is more suitable for slugs in standing cereals than on stubble. We suggest, as a guideline, that an average catch of 4 of more slugs/trap in standing cereals may justify treatment with slug pellets soon after drilling oilseed rape.

We must emphasise that predictive data were available in this study for only 29 sites. By contrast, a large data set comprising 93 sites, throughout the UK, was obtained in previous collaborative studies on damage risk winter wheat (Glen *et al.*, 1993). Finally, the question of how much damage an oilseed rape crop can sustain before yield loss occurs needs to be explored. This may relate both to plant population and losses due to slugs on individual plants. Seedbed conditions and rates of crop emergence may also need to be considered in any future studies.

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