



HGCA

PROJECT REPORT No. OS60

**CONTROL OF SLUG DAMAGE IN OILSEED RAPE BY SEED
TREATMENT – DEVELOPMENT AND FIELD TESTS**

MARCH 2003

Price £3.50

PROJECT REPORT No. OS60

CONTROL OF SLUG DAMAGE IN OILSEED RAPE BY SEED TREATMENT – DEVELOPMENT AND FIELD TESTS

by

L C SIMMS, C E MULLINS AND M J WILSON

Department of Plant & Soil Science, University of Aberdeen, Aberdeen, AB24 3UU

This is the final report of a twelve month research project which started in September 2001. The work was funded by a grant of £45,345 from HGCA (project no. 2510).

The Home-Grown Cereals Authority (HGCA) has provided funding for this project but has not conducted the research or written this report. While the authors have worked on the best information available to them, neither HGCA nor the authors shall in any event be liable for any loss, damage or injury howsoever suffered directly or indirectly in relation to the report or the research on which it is based.

Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended nor is any criticism implied of other alternative, but unnamed products.

CONTENTS

ABSTRACT	2
SUMMARY	3
INTRODUCTION.....	3
<i>Objectives:</i>	4
MATERIALS AND METHODS	4
<i>Laboratory bioassays</i>	4
Efficacy of seed treatments against a broad range of slug pests	4
Determination of the duration of protection given by seed treatments	4
Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.	5
<i>Semi-field experiment – mini-plots</i>	5
<i>Field experiments</i>	5
RESULTS	6
<i>Laboratory bioassays</i>	6
Efficacy of seed treatments against a broad range of slug pests	6
Determination of the duration of protection given by seed treatments	6
Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.	6
<i>Semi-field experiment – mini-plots</i>	7
<i>Field experiment</i>	7
DISCUSSION AND IMPLICATIONS.....	7
TECHNICAL DETAIL	9
INTRODUCTION.....	9
MATERIALS AND METHODS	9
<i>Laboratory bioassays</i>	9
Efficacy of seed treatments against a broad range of slug pests	10
Determination of the duration of protection given by seed treatments	10
Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.	10
<i>Semi-field experiment – mini-plots</i>	11
<i>Field experiments</i>	11
RESULTS	12
<i>Laboratory bioassays</i>	12
Efficacy of seed treatments against a broad range of slug pests	12
Determination of the duration of protection given by seed treatments	14
Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.	15
<i>Semi-field experiment – mini-plots</i>	16
Autumn mini-plot trial	16
Spring mini-plot trial	17
<i>Field experiment</i>	18
DISCUSSION AND IMPLICATIONS.....	18
ACKNOWLEDGEMENTS.....	20
REFERENCES	20

ABSTRACT

Slugs are serious pests of oilseed rape that eat rape seedlings immediately after emergence, thus reducing plant stand. Current slug control methods of broadcasting bait pellets on the soil surface often fail to give adequate protection. A previous HGCA-funded project (HGCA Project Report OS54) demonstrated that molluscicidal seed coatings are highly effective at protecting oilseed rape seedlings from slug damage under laboratory conditions. This project aimed:

- To compare efficacy of the insecticide imidacloprid as a seed treatment with that of methiocarb and metaldehyde.
- To determine efficacy of seed treatments against a broad range of slug pests.
- To determine the duration of protection given by seed treatments.
- To demonstrate efficacy of seed treatments in field and semi-field experiments.
- To develop a quantitative analytical method to monitor metaldehyde uptake by seedlings.

Laboratory experiments were done to test the efficacy of metaldehyde, methiocarb and imidacloprid seed treatments to a range of slug species. Metaldehyde and methiocarb reduced slug damage to oilseed rape seedlings caused by representative species of the Limacidae (field slugs), Arionidae (round back slugs) and Milacidae (keeled slugs), the families that comprise all UK pest species. Conversely, imidacloprid did not protect seedlings from damage by any species. In outdoor pot experiments investigating the length of protection offered by seed treatments, both metaldehyde and methiocarb protected seedlings for up to 8 weeks after planting. By this time all plants had reached at least 6 true leaves, a stage beyond which oilseed rape is capable of outgrowing all slug damage. In semi-field trials, metaldehyde and methiocarb seed treatments protected seedlings better than the controls, but not as well as slug bait pellets. However, in the field trial using standard agricultural practice, slug bait pellets protected oilseed rape seedlings from slug damage, but none of the seed treatments did. In order to determine reasons for lack of efficacy in the field we have developed a method to quantify metaldehyde residues in plant material using gas chromatography-mass spectrometry.

Both metaldehyde and methiocarb showed promise as seed treatments to control slug damage in oilseed rape from a broad range of slug species. Further investigations are required to identify the constraints to efficacy of molluscicidal seed treatments in the field. Once these have been detected, new seed dressing formulations could be produced to overcome the constraints thus increasing the efficacy of our seed treatments. It must also be noted that while seed treatments can protect seedlings from slug damage, they act as repellents and are not necessarily fatal to slugs in this type of application. It is therefore suggested that molluscicidal seed treatments may play an important role as part of an integrated pest management system, together with slug bait pellets, reducing the number of bait pellet applications and giving seedlings vital protection at their most vulnerable stages.

SUMMARY

INTRODUCTION

Slugs are serious pests of oilseed rape, causing most damage immediately after emergence when seedlings are often fatally damaged, thus reducing plant stand. Over recent years slug damage to oilseed rape has increased greatly mainly due to changes in agronomic practices such as minimal cultivation techniques and increased use of low glucosinolate varieties of oilseed rape, which are more palatable to slugs. The increase in slug damage to oilseed rape is mirrored in the increased usage of molluscicides in this crop. In 1994 30% of the area of oilseed rape grown was treated with molluscicidal baits in the UK; by 2000, this figure had almost doubled to 59%.

Slugs are usually controlled by broadcasting slug bait pellets on the soil surface, which does not always protect crops adequately, even when multiple applications are made. Since rape is only at risk during germination, emergence and early leaf stages, it may be possible to protect the crop by seed dressing, a strategy that offers both environmental and economic benefits. The timing of slug bait applications is very important. Bait pellets are often only applied when necessary, i.e. when slug damage is visible, to be cost effective. However, this is usually too late as fatal damage to seedlings may already have occurred. A protective seed dressing could protect each seedling equally at this vulnerable stage. In addition, the cost to the growers may be reduced, as a separate field pass for broadcasting baits could be avoided, also reducing the risk of wheeling damage. The amount of active ingredient may also be reduced lowering costs and environmental impact.

Previous work on seed treatments to control slug damage has focussed on protecting cereal seeds as opposed to seedlings, with the most promising chemicals being metaldehyde and methiocarb. However, slugs do not feed on oilseed rape seeds, instead causing most damage to newly emerged seedlings. Recent work in the Netherlands has demonstrated that coating wheat seedlings with molluscicides protects not only the seed, but also the emerged seedling. Based on this, HGCA-funded project 2293 at the University of Aberdeen (Project Report OS54), screened several compounds for their ability to reduce slug damage to oilseed rape. The molluscicides metaldehyde and methiocarb showed potential as seed treatments reduce slug damage to newly-emerged oilseed rape seedlings. These seed treatments if used commercially would have both economic and environmental benefits.

The current project continued this research and aimed to further test these seed treatments in laboratory, semi-field and field trials. In addition, the insecticidal seed treatment imidacloprid suggested to reduce slug damage in field trials by ADAS, during HGCA-funded project 2364 (Project Report OS57), was compared with metaldehyde and methiocarb seed treatments.

Objectives:

1. To compare efficacy of the insecticide imidacloprid as a seed treatment with that of methiocarb and metaldehyde.
2. To determine efficacy of seed treatments against a broad range of slug pests.
3. To determine the duration of protection given by seed treatments.
4. To demonstrate efficacy of seed treatments in field and semi-field experiments.
5. To develop a quantitative analytical method to monitor metaldehyde uptake by seedlings.

MATERIALS AND METHODS**Laboratory bioassays**Efficacy of seed treatments against a broad range of slug pests

Oilseed rape seeds (cv. Apex) were coated with either metaldehyde (72 g a.i./kg seed) or methiocarb (23 g a.i./kg seed) mixed with a commercial seed adhesive. Control seeds were coated with seed adhesive only. Bayer applied imidacloprid to seeds as 'Chinook' (imidacloprid and beta-cyfluthrin) at 20 ml/ kg seed and 40 ml/ kg seed. Half seed trays, containing John Innes No. 2 potting compost, were sown with 60 treated seeds. Seeds were covered with 1 cm soil, to ensure that slugs could not come into contact with the seeds, and moistened with water. Four adult slugs, either *Deroceras reticulatum* (grey field slug), *Arion distinctus* (Mabille's orange soled slug) or *Milax gagates* (jet slug), were added immediately after sowing. Each seed tray was fitted with a clear plastic propagator lid with ventilation holes. Propagator lids were then sealed at the edges. Seed trays were placed in a cold frame covered by a shading net. Slug damage and slug mortality were assessed at weekly intervals from the day of planting, for 4 weeks.

Determination of the duration of protection given by seed treatments

Oilseed rape seeds (cv. Pronto) were coated with either metaldehyde (58 g a.i./kg seed) or methiocarb (18 g a.i./kg seed) mixed with a commercial seed adhesive. Control seeds were coated with seed adhesive only. Seeds were sown in 4 inch diameter plant pots, with one seed per pot, and placed in a glasshouse. When plants had reached growth stages of either 1) both cotyledons unfolded and green, 2) first true leaf exposed, 3) second true leaf exposed and 4) third true leaf exposed, one adult *D. reticulatum* slug was added. Pots were covered with a plastic bag, with ventilation holes, to prevent slugs escaping. In all cases, crop growth stage, mean % leaf area eaten and slug mortality were recorded for five weeks from the day slugs were added.

Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.

An analytical protocol for measuring metaldehyde residues on oilseed rape seedlings was investigated. A method for extracting and purifying metaldehyde from plant material was set-up. Known concentrations of metaldehyde were determined analytically using a gas chromatograph coupled with an ion trap mass spectrometer, which can allow both identification and quantitative measurement of the chemical.

Semi-field experiment – mini-plots

The semi-field experiments were set up in mini-plots in an experimental area at IACR-Long Ashton. Each mini-plot was a plastic container 0.48 m², filled with loam soil to 20 cm depth, with drainage holes in the bottom. Each container was surrounded by a copper-mesh fence to deter slug movement between plots. Oilseed rape seeds were sown with 48 seeds per plot. Two mini-plot trials were conducted, one in autumn and one in spring. In the autumn trial oilseed rape cv. Pronto was sown with treatments of either metaldehyde dressed seeds (58 g a.i./kg seed), methiocarb dressed seeds (18 g a.i./kg seed), metaldehyde slug pellets (8 kg product/ha) and control. In the spring trial oilseed rape cv. Adder was sown with treatments of either metaldehyde dressed seeds (60 g a.i./kg seed), methiocarb dressed seeds (20 g a.i./kg seed), metaldehyde slug pellets (8 kg product/ha) and control. Seeds in the autumn trial were treated as described above, and KWS SAAT, Germany, treated seeds in the spring trial. Ten adult slugs (*D. reticulatum*) were then introduced to each plot of the four treatments described above. Plots were irrigated daily. Numbers of plants damaged by slugs were recorded weekly for 5 weeks.

Field experiments

KWS SAAT, Germany, conducted Oilseed rape field trials. Winter oilseed rape cv. Adder was sown at the rate of 3 kg/ha with treatments of either metaldehyde dressed seeds (60 g a.i./kg seed), methiocarb dressed seeds (20 g a.i./kg seed), imidacloprid dressed seeds (applied as chinook at 20 ml/kg), metaldehyde slug pellets (7 kg product/ha), methiocarb slug pellets (5 kg products/ha) and control. Plots were 6 m × 12 m. Plant numbers per m² were assessed 2, 4 and 10 weeks after sowing.

RESULTS

Laboratory bioassays

Efficacy of seed treatments against a broad range of slug pests

Deroceras reticulatum

Metaldehyde seed treatments had more surviving plants and less grazing damage than all other treatments. Methiocarb seed treatments had fewer surviving plants than the controls, but the plants had less grazing damage. Imidacloprid did not protect plants from slug damage.

Arion distinctus

Metaldehyde and methiocarb seed treatments reduced grazing damage in comparison with the controls and imidacloprid treatments, but they did not have significantly more surviving plants compared with the control. Both imidacloprid seed treatments had fewer surviving plants than the control, but there were no differences in grazing damage.

Milax gagates

Both metaldehyde and methiocarb seed treatments had more surviving plants than all other treatments. However, only metaldehyde had less grazing damage than the controls. Both doses of imidacloprid had fewer surviving plants than the controls, and the lowest dose of imidacloprid had more damage than controls.

Determination of the duration of protection given by seed treatments

Slugs added at the cotyledon stage caused significantly less grazing damage to metaldehyde plants in comparison with both control and methiocarb seed-treated plants. In addition, methiocarb seed-treated plants had more grazing damage than control plants. When slugs were added at the first true leaf stage no significant differences were found between treatments. In experiments with slugs added at the 2 true leaves and 3 true leaves growth stages, both metaldehyde and methiocarb seed-treated plants suffered significantly less grazing damage than control plants. In addition, metaldehyde seed-treated plants had less grazing damage than methiocarb seed-treated plants, when slugs were added at the 3 true leaves growth stage.

Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.

Metaldehyde was identified and qualitatively analysed using a gas chromatograph coupled with an ion trap mass spectrometer.

Semi-field experiment – mini-plots

In the autumn mini-plot trial, both metaldehyde and methiocarb seed treatments had more plants and less slug damage than control plots. However, in the spring field trial only methiocarb seed treatments had more plants and less slug damage than control. Metaldehyde slug pellets had more plants and less slug damage than all other treatments in both autumn and spring field trials.

Field experiment

None of the seed treatments tested in the field reduced slug damage to oilseed rape plants in comparison with control plots. In addition, imidacloprid seed treatments had fewer plants per m² than controls and methiocarb seed-treated plants. Plots treated with metaldehyde and methiocarb slug bait pellets had more plants than all other treatments.

DISCUSSION AND IMPLICATIONS

Both metaldehyde and methiocarb seed treatments protected oilseed rape seedlings from slug damage, in laboratory bioassays, caused by slug species from the three main slug families. When tested under semi-field conditions methiocarb seed treatments performed better than metaldehyde seed treatments, protecting oilseed rape seedlings in both autumn and spring semi-field trials. Metaldehyde seed treatments only gave limited protection in the autumn semi-field trial. However, seed treatments in the semi-field trial did not offer as much protection to seedlings as slug bait pellets. Our results for imidacloprid are less encouraging. When applied as a seed treatment imidacloprid not only failed to protect oilseed rape from slug damage, but was often found to have significantly fewer plants or more damage than control plants, in both laboratory and field trials. In the field trial using standard agricultural practice, slug bait pellets protected oilseed rape seedlings from slug damage, but none of the seed treatments did. Factors such as physical loss of seed coating caused by environmental conditions e.g. rain, microbial degradation of active ingredient, reduced uptake of active ingredient at low temperatures and loss of active ingredient from seedling through rain-wash or volatilisation may be limiting field performance of oilseed rape. We believe the analytical techniques developed in this project will enable us to carry out experiments to determine which of these factors limits field performance.

In conclusion, both metaldehyde and methiocarb show promise as seed treatments to control slug damage in oilseed rape from a broad range of slug species. Further investigations are required to identify the constraints to efficacy of molluscicidal seed treatments in the field. Once these have been established, new seed dressing formulations will be produced to overcome the constraints thus increasing the efficacy of our seed treatments. It must also be noted that while seed treatments can protect seedlings from slug damage, they act as repellents and are not necessarily fatal to slugs in this type of application. It is therefore suggested that molluscicidal seed

treatments may play an important role as part of an integrated pest management system, together with slug bait pellets, reducing the number of bait pellet applications and giving seedlings vital protection at their most vulnerable stages.

TECHNICAL DETAIL

INTRODUCTION

Over recent years slug damage to oilseed rape has increased greatly mainly due to changes in agronomic practices such as minimal cultivation and lower seed application rates, in order to lower costs of establishment, as well as incorporation of previous crop residues into the soil and cover cropping (Moens and Glen, 2002). In addition, there has been an increased use of low glucosinolate varieties of oilseed rape, which are more palatable to slugs (Glen *et al.*, 1990). The increase in slug damage to oilseed rape is mirrored in the increased usage of molluscicides in this crop. In 1994 30% of the area of oilseed rape grown was treated with molluscicidal baits in the UK, by 2000, this figure had almost doubled to 59% (M Thomas, personal communication).

Seed treatment with a molluscicidal or insecticidal active ingredient could be an alternative or added protection to oilseed rape against slug damage. The economic and environmental benefits of such a seed treatment are detailed in the summary.

This project aimed to build on a previous HGCA-funded project 2293 at the University of Aberdeen (Project Report OS54) outlined in the summary.

The specific objectives of this project are:

1. To compare efficacy of the insecticide imidacloprid as a seed treatment with that of methiocarb and metaldehyde.
2. To determine efficacy of seed treatments against a broad range of slug pests.
3. To determine the duration of protection given by seed treatments.
4. To demonstrate efficacy of seed treatments in field and semi-field experiments.
5. To develop a quantitative analytical method to monitor metaldehyde uptake by seedlings.

MATERIALS AND METHODS

All experiments were set-up as described in the summary with the addition technical information detailed below.

Laboratory bioassays

Adult *Deroceras reticulatum*, *Arion distinctus* and *Milax gagates* were field collected with traps baited with wheat. Slugs were kept at 15°C in clear plastic boxes, containing moist cotton wool until required. Slugs were fed on a mixture of 50% oat breakfast cereal (Ready Brek, Weetabix Ltd, Kettering, UK), 25% dried

milk and 25% calcium carbonate, moistened with water, and were deprived of food for 4 to 7 days before the start of experiments.

Efficacy of seed treatments against a broad range of slug pests

Oilseed rape seeds were coated with either metaldehyde or methiocarb mixed with a commercial seed adhesive, 'Sepiret' (Agrichem, Whittlesey, UK) at the rates detailed in the summary, or seed adhesive only (control). Seeds were mixed until an even distribution of the coloured adhesive was observed. The seeds were then air-dried overnight and stored in the dark until required. Bayer applied Imidacloprid to seeds as 'Chinook' as detailed in the summary. Seed trays (220 × 165 × 57 mm) were set-up as described in the summary, with John Innes No. 2 potting compost topped with 1 cm of air-dried soil (loamy sand texture), to ensure that slugs could not come into contact with the seeds, and moistened with 175 ml of tap water. Four adult slugs, either *D. reticulatum* (mean biomass = 1.8g/tray SE 0.04g), *A. distinctus* (mean biomass = 1.0g, SE 0.02g) or *M. gagates* (mean biomass = 1.8g, SE 0.02g), deprived of food 4 days before, were added immediately after sowing. Slugs were then deprived of food for a further 2 to 3 days while the seeds germinated. Each seed tray was fitted with a clear plastic propagator lid as detailed in the summary and placed in a cold frame covered by a shading net.

Slug damage and slug mortality was assessed at weekly intervals from the day of planting, for 4 weeks. Slug damage to seedlings was recorded as either fatal (loss of apical meristem) or as non-fatal grazing damage. All experiments had fully a randomised design, each with six replicates.

Determination of the duration of protection given by seed treatments

Oilseed rape seeds (cv. Pronto) were coated with either metaldehyde or methiocarb mixed with a commercial seed adhesive, 'Sepiret' (Agrichem, Whittlesey, UK) as detailed in the summary. Control seeds were coated with seed adhesive only. Seedlings were grown as described in the summary. When plants had reached growth stages of either GS1.00, GS1.01, GS1.02 or GS1.03 (Sylvester-Bradley *et al.*, 1984) one adult *D. reticulatum* slug was added. All experiments had fully a randomised design, each with ten replicate pots per treatment. In all cases, crop growth stage, mean % leaf area eaten and slug mortality were recorded at twice-weekly intervals for a period of five weeks from the day slugs were added.

Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.

An analytical protocol for measuring metaldehyde residues on oilseed rape seedlings was investigated. A method for extracting and purifying metaldehyde from plant material was set-up. This consisted of extracting metaldehyde with chloroform from oilseed rape seedlings homogenised by grinding with liquid nitrogen. Samples were purified by passing through a Whatman's No. 1 filter paper, and further cleaned-up

on a Florosil column, to give a sample that could be injected into a GC/MS. A 0.1% metaldehyde sample was determined analytically, both quantitatively and qualitatively, using a GC/MS.

Semi-field experiment – mini-plots

The semi-field experiments were set up in mini-plots in an experimental area at IACR-Long Ashton. Each mini-plot was a plastic container external dimensions 80 cm x 60 cm (0.48 m²) x 23 cm deep, internal dimensions 75 cm long x 57 cm wide (0.43 m²), with drainage holes in the bottom covered by plastic mesh to prevent slugs entering or leaving. Each plot was filled to a depth of 20 cm with unsterilised loam soil that had been stored in an air-dried condition in a polythene tunnel for several months, to ensure that all slugs originally present in the soil had died. Seeds were sown in each plot, with 48 seeds per plot in four rows, each 75 cm long and 15 cm apart, 12 seeds per row, with 6 cm between seeds in the row. Each container was surrounded by a copper-mesh fence 10 cm high to deter slug movement between plots. Each copper fence frame was sealed to the plastic container using waterproof tape. After the fences had been set in place in this way, plots were watered by gentle overhead irrigation for *ca.* 20 minutes. Mini-plots were exposed to ambient temperature, radiation and rainfall. Two mini-plot trials were conducted, one in autumn and one in spring with treatments and rates tested as detailed in the summary. Ten adult slugs (*D. reticulatum*) were then introduced to each plot of the four treatments described above. A refuge consisting of an upturned terracotta coloured plastic flowerpot saucer (approx. 7-cm diameter) was placed in the middle of each plot with slugs. In addition, no slugs were added to one plot in each block, which contained two rows of control seeds and one row of seeds with each molluscicide treatment. This additional plot provided a check on seed germination in the absence of slugs as well as a check on whether slugs were able to move between plots. Plots were irrigated daily with a sprinkler irrigation system. Bird-proof netting surrounded the whole experimental area.

The design was a randomised block with 7 replicates of each treatment. Numbers of plants damaged by slugs and slug mortality were assessed at weekly intervals from the day of planting, for 5 weeks.

Field experiments

KWS SAAT, Germany, conducted Oilseed rape field trials as described in the summary.

RESULTS

Laboratory bioassays

Efficacy of seed treatments against a broad range of slug pests

Deroceras reticulatum

An ANOVA of the number of plants, and the percentage of plants damaged revealed significant effects of both treatment ($P<0.001$) and time ($P<0.001$), but the two factors did not interact. Metaldehyde treatments had significantly more surviving plants than all other treatments (control $P<0.01$, all other treatments $P<0.001$) (Fig. 1A). Metaldehyde treatments also had significantly less grazing damage in comparison with all other treatments (methiocarb $P<0.05$, all other treatments $P<0.001$) (Fig. 1B). The controls had significantly more surviving plants compared with methiocarb and chinook 40 seed treatments ($P<0.01$) (Fig. 1A). However, methiocarb had significantly less grazing damage than control and chinook 20 treatments (control $P<0.01$, chinook 20 $P<0.05$) (Fig. 1B).

Arion distinctus

An ANOVA of the number of plants, and the percentage of plants damaged revealed significant effects of both treatment (plant numbers $P<0.01$, percentage damaged $P<0.001$) and time ($P<0.001$), but the two factors did not interact. Chinook 20 and chinook 40 seed treatments had significantly fewer surviving plants than all other treatments (control $P<0.01$, metaldehyde and methiocarb $P<0.001$) (Fig. 1C). Metaldehyde and methiocarb seed treatments had less grazing damage than all other treatments ($P<0.001$) (Fig. 1D).

Milax gagates

An ANOVA of the number of plants, and the percentage of plants damaged revealed significant effects of both treatment (plant numbers $P<0.01$, percentage damaged $P<0.001$) and time ($P<0.001$), but the two factors did not interact. Metaldehyde and methiocarb seed treatments had significantly more surviving plants than all other treatments ($P<0.001$) (Fig. 1E). However, only metaldehyde treatments had significantly less grazing damage in comparison with all other treatments ($P<0.001$). Methiocarb treatments only had significantly less grazing damage than chinook 20 ($P<0.05$) (Fig. 1F). Control seed trays were found to have more surviving plants than both chinook 20 ($P<0.001$) and chinook 40 ($P<0.05$) (Fig. 1E), but only had less grazing damage than chinook 20 ($P<0.01$) (Fig. 1F).

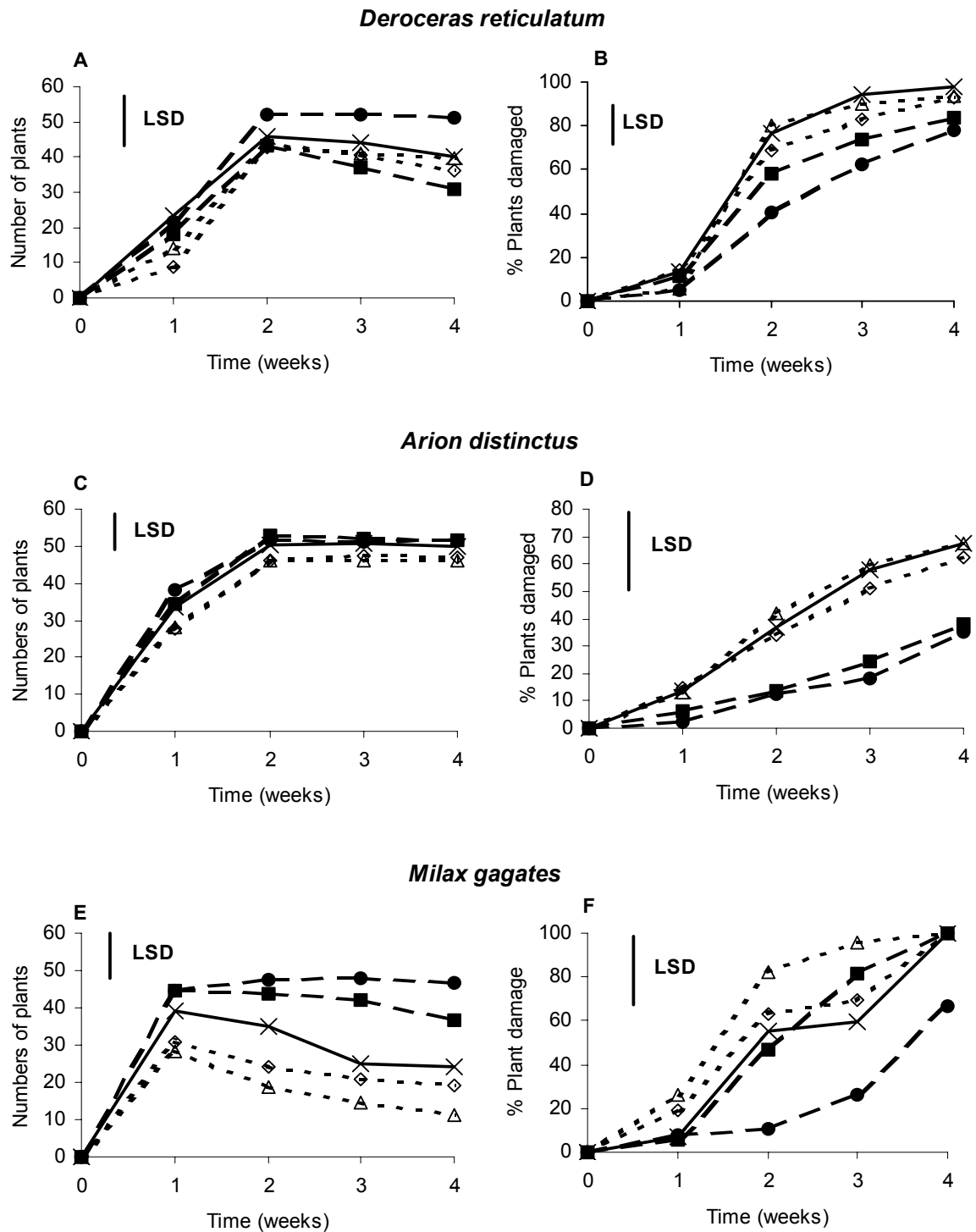


Figure 1. Comparison of three species of slugs on (A, C and E) Number of plants remaining, and (B, D and F) Percentage of plants damaged, to oilseed rape plants with seed treatments of either control (×), metaldehyde (•), methiocarb (■), chinook 20 g a.i./kg seed (Δ) and chinook 40 g a.i./kg seed (◇), in laboratory trials.

Determination of the duration of protection given by seed treatments

Growth Stage 1.00

When analysed by ANOVA the percentage of plant area damaged revealed significant effects of both treatment ($P<0.001$) and time ($P<0.001$), but the two factors did not interact. Metaldehyde treatments had significantly less grazing damage than both control ($P<0.05$) and methiocarb ($P<0.001$) treatments (Fig. 2A). Methiocarb seed treatments had significantly more grazing damage than control plants ($P<0.05$) (Fig. 2A).

Growth stage 1.01

ANOVA of the percentage of plant area damaged for treatments with slugs added at GS1.01 revealed no significant effect of treatment. Damage was found to significantly increase with time ($P<0.001$) for the mean of all treatments (Fig. 2B).

Growth Stage 1.02

When analysed by ANOVA the percentage of plant area damaged revealed significant effects of both treatment ($P<0.001$) and time ($P<0.001$), but the two factors did not interact. Both metaldehyde and methiocarb treatments had significantly less grazing damaged in comparison with control plants, when slugs were added at GS1.02 ($P<0.001$) (Fig. 2C).

Growth Stage 1.03

ANOVA of the percentage of plant area damaged revealed significant effects of both treatment ($P<0.001$) and time ($P<0.001$), but the two factors did not interact. Both metaldehyde and methiocarb treatments had significantly less grazing damaged in comparison with control plants, when slugs were added at GS1.02 ($P<0.001$) (Fig. 2D). In addition, metaldehyde seed treatments had significantly less grazing damaged than methiocarb seed treatments ($P<0.001$) (Fig. 2D).

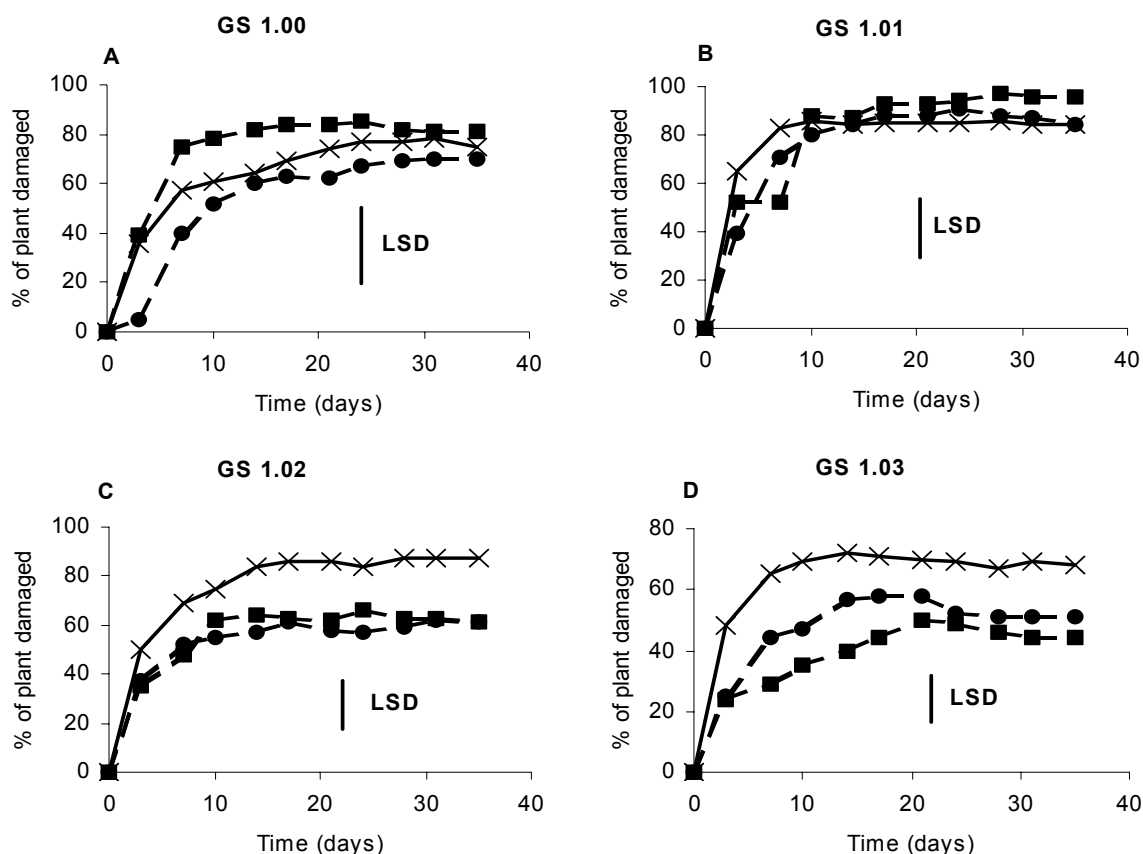


Figure 2. Damage to oilseed rape plants with seed treatments of either control (x), metaldehyde (•) or methiocarb (■), by *Deroceras reticulatum* slugs added at growth stages of either GS1.00 (A), GS1.01 (B), GS1.02 (C) or GS1.03 (D).

Development of a quantitative analytical method to determine metaldehyde uptake by seedlings.

Metaldehyde was quantitatively and qualitatively analysed using a gas chromatograph coupled with a mass spectrometer. The concentration of metaldehyde contained in a sample was determined using the abundance detected as shown in plate 1, being 0.1% in the sample shown. This was peak was confirmed as metaldehyde by the mass/charge ratio of the fragments produced by the mass spectrometer shown in the mass spectrum in plate 2. Fragment ions at m/z 45, 87, 89, 117 and 131 are characteristic of metaldehyde fragmentation by a mass spectrometer (Jones and Charlton, 1999; Fillion, 1997).

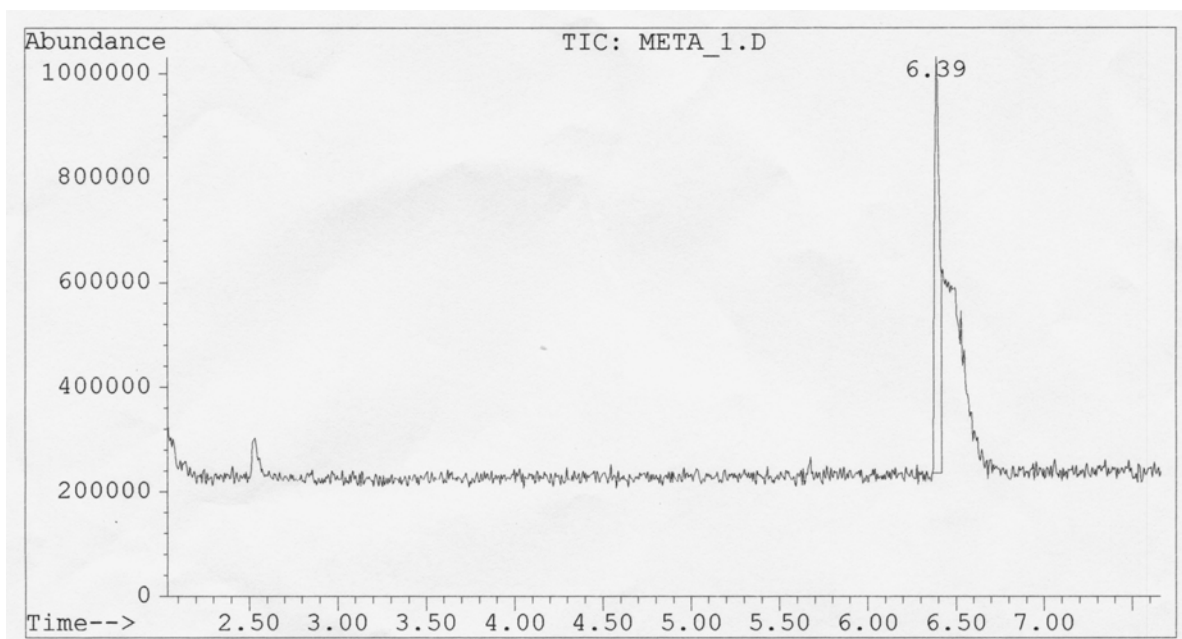


Plate 1. Chromatogram of a metaldehyde sample in chloroform, with peak of metaldehyde at 6.39 mins.

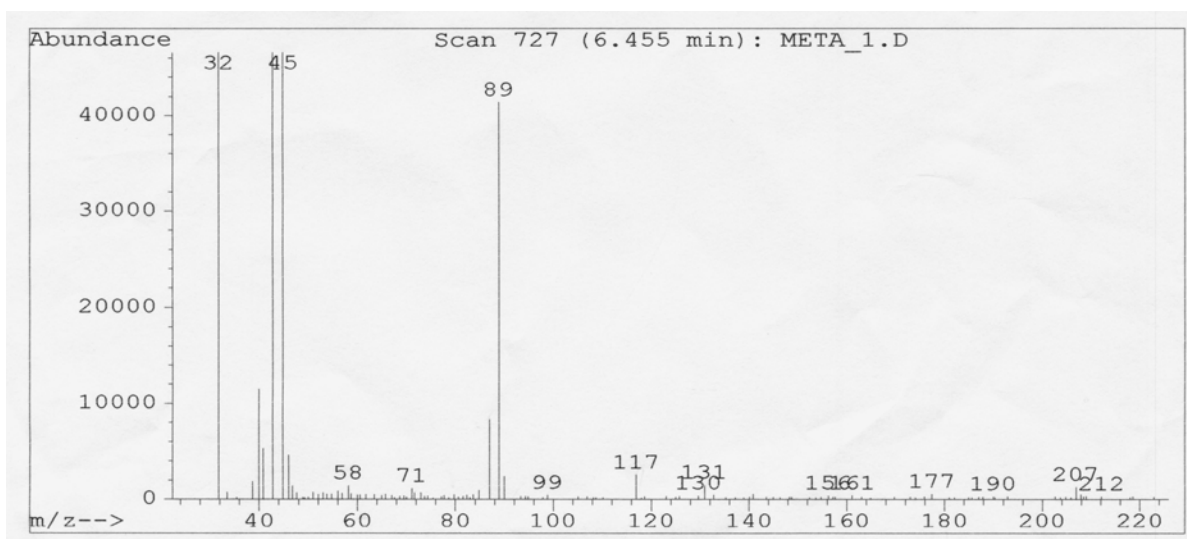


Plate 2. Mass spectrum of metaldehyde (MW = 176), analysed by GC/MS, showing characteristic fragment ions at m/z 45, 87, 89, 117, and 131.

Semi-field experiment – mini-plots

Autumn mini-plot trial

An ANOVA of the number of plants, and the percentage of plants damaged revealed significant effects of both treatment ($P < 0.001$) and time ($P < 0.001$), and an interaction between the two factors ($P < 0.001$). Metaldehyde and methiocarb seed treatments had more plants (Fig. 3A) and less damage (Fig. 3B) than control treatments

(metaldehyde $P<0.05$, methiocarb $P<0.001$). In addition, methiocarb seed treatments had more plants than metaldehyde seed treatments ($P<0.05$) (Fig. 3A). Metaldehyde slug pellet treatments had more plants (Fig. 3A) and less damage (Fig. 3B) compared with all other treatments ($P<0.001$).

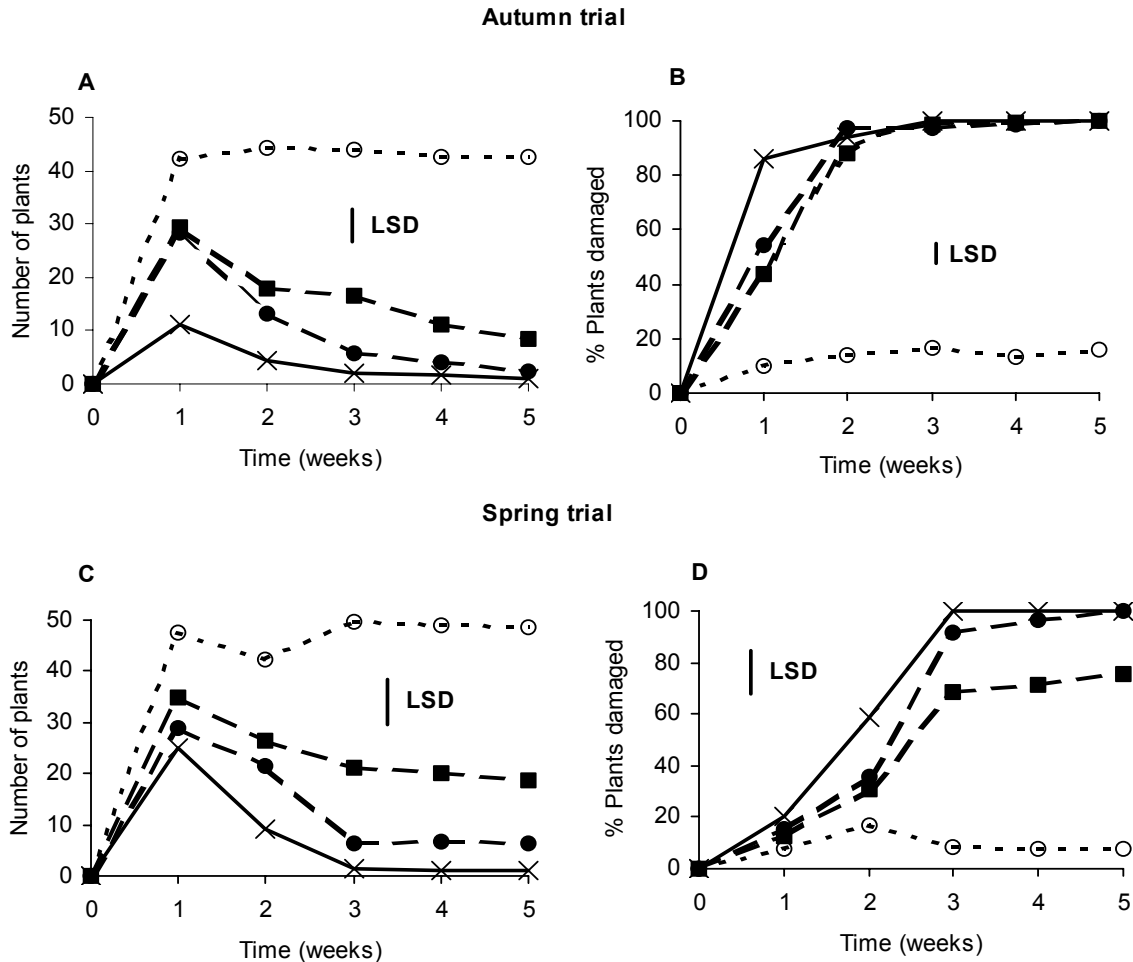


Figure 3. The effect of *Deroceras reticulatum* slugs on (A and C) Number of plants remaining, and (B and D) Percentage of plants damaged, to oilseed rape plants with seed treatments of either control (x), metaldehyde (•), methiocarb (■) or metaldehyde slug pellets (O) in mini-plot trials.

Spring mini-plot trial

Analysis of the number of plants, and the percentage of plants damaged by ANOVA revealed significant effects of both treatment ($P<0.001$) and time ($P<0.001$), and an interaction between the two factors ($P<0.001$). Methiocarb seed treatments had significantly more plants than both control treatments ($P<0.001$) and metaldehyde seed treatments ($P<0.01$) (Fig. 3C). This trend was also reflected in the percentage of plants damaged with methiocarb seed treatments having significantly more plants than both control treatments ($P<0.01$) and metaldehyde seed treatments ($P<0.05$) (Fig. 3D). Metaldehyde slug pellet treatments had more plants (Fig. 3C) and less damage (Fig. 3D) compared with all other treatments ($P<0.001$).

Field experiment

ANOVA of the number of plants revealed significant effects of both treatment ($P<0.001$) and time ($P<0.001$), but the two factors did not interact. Both methiocarb and metaldehyde slug pellets had significantly more plants than all other treatments ($P<0.001$) (Fig 4). In addition, chinook seed treatments also had fewer plants than control and methiocarb seed treatments ($P<0.001$) (Fig 4).

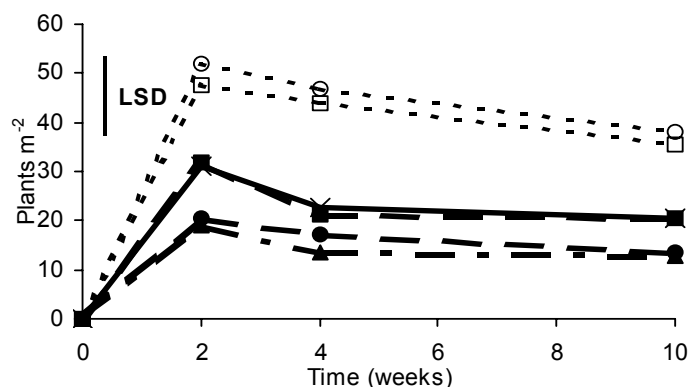


Figure 4. Number of plants remaining in a field trials with oilseed rape plants with slug control treatments of either control (x), metaldehyde seed treatment (●), methiocarb seed treatment (■), chinook seed treatment (▲), metaldehyde slug pellets (O) or methiocarb slug pellet (□).

DISCUSSION AND IMPLICATIONS

Both metaldehyde and methiocarb seed treatments protected oilseed rape seedlings from slug damage, in laboratory bioassays, by slug species from the three main slug families (Limacidae, Arionidae and Milacidae) (Fig. 1). This in agreement with Simms *et al* (2002a) who found metaldehyde and methiocarb seed treatments not only protected seedlings from slug damage by slugs from the Limacidae and Arionidae families, but these treatments performed as well as conventional bait pellets. Studies previous to this had concentrated on protecting seeds prior to germination. However, our results agree with general findings of these workers, in that both metaldehyde and methiocarb have considerable potential as seed treatments (Gould, 1962; Ester and Nijenstein, 1996a, 1996b, Ester *et al.*, 1996; Nijenstein and Ester, 1998). When tested under semi-field conditions methiocarb seed treatments performed better than metaldehyde seed treatments, protecting oilseed rape seedlings in both autumn and spring field trials (Fig. 3). Metaldehyde seed treatments only gave limited protection in the autumn filed trial (Fig. 3). This is the first report of seed treatments to control slug damage to oilseed rape under semi-field conditions. Our semi-field results are in agreement with field trials of winter wheat seed treatments of Ester *et al.* (1996) and Scott (1984), both of whom found molluscicidal seed treatments reduced slug damage. However, seed treatments in the current

semi-field trial did not offer as much protection to seedlings as slug bait pellets (Fig. 3). This is in contrast to laboratory trials conducted by Simms *et al.* (2002), where both metaldehyde and methiocarb seed treatments protected oilseed rape seedlings as well as, or even better than, slug bait pellets. In the field trial reported here, none of the seed treatments protected seedlings, while slug bait pellets reduced damage by slugs (Fig. 4). This is in contrast to the findings of Ester *et al.* (1996) and Scott (1984). Scott (1984) found wheat seeds treated with methiocarb had a significantly lower incidence of slug damage, while Ester *et al.* (1996) found both metaldehyde and methiocarb treated wheat seeds reduced slug damage. Differences in these results and the current reported findings may be due to differences in slug damage to these two different crops, or differences in seed coating formulations. As oilseed rape seeds are not attacked by slugs, the effect of the molluscicidal seed treatment may have been reduced due to field conditions, by the time the seedling has emerged. Factors such as physical loss of seed coating caused by environmental conditions e.g. rain, microbial degradation of active ingredient, reduced uptake of active ingredient at low temperatures and loss of active ingredient from seedling through rain-wash or volatilisation may be limiting field performance of oilseed rape. Also, as wheat seeds are prone to grain hollowing by slugs, the molluscicidal seed treatment may reduce slug damage at this very early crucial stage, before these factors begin to limit the performance of the seed treatment. In addition, dicotyledon seedlings, such as oilseed rape, are more susceptible to slug damage above the soil surface as their apical meristem is situated at the top of the stem. This is in contrast to monocotyledon seedlings, such as wheat, which have their apical meristem situated at the base of the stem, usually below the soil surface.

Our results for imidacloprid are less encouraging. When applied as a seed treatment imidacloprid not only failed to protect oilseed rape from slug damage, but was often found to have significantly fewer plants or more damage than control plants, in both laboratory and field trials (Fig. 1 and 4). This is in contrast to field trials reported in Simms *et al.* (2002b) and Rose & Oades (2001). Rose & Oades (2001) tested imidacloprid as a seed treatment to wheat in field trials and found slug damage to be reduced on average by 68%. Differences in these results and the current reported findings may again be due to differences in slug damage to these two different crops, discussed above. Rose & Oades (2001) found the greatest reduction in damage was to the wheat seeds resulting in an improved crop stand, but found foliage damage not to be reduced much. As oilseed rape seeds are not attacked by slugs, the effect of the imidacloprid seed treatment may have been diluted by the time the seedlings has emerged, as well as the differences in slug damage to monocotyledons and dicotyledons mentioned above. However, Simms *et al.* (2002b) reported imidacloprid seed treatments reduced slug damage to oilseed rape in the field, but this was only at a field site with low slug pressure. In field trials with a high slug pressure no significant differences of seed treatment on slug damage were found (Simms *et al.*, 2002a). It is suggested that slug pressure in the field trial reported in this current project may have been too high to show any effects of the imidacloprid seed treatments. Extremely heavy rain occurred at the beginning of the trial at the field site reported here, which would indeed encourage slug activity (data not shown). We believe the analytical

techniques developed in this project will enable us to carry out experiments to determine which of these factors limits field performance.

In conclusion, both metaldehyde and methiocarb show promise as seed treatments to control slug damage in oilseed rape from a broad range of slug species. Further investigations are required to identify the constraints to efficacy of molluscicidal seed treatments in the field. Once these have been detected, new seed dressing formulations could be produced to overcome the constraints thus increasing the efficacy of our seed treatments. It must also be noted that while seed treatments can protect seedlings from slug damage, they act as repellents and are not necessarily fatal to slugs in this type of application. It is therefore suggested that molluscicidal seed treatments may play an important role as part of an integrated pest management system, together with slug bait pellets, reducing the number of bait pellet applications and giving seedlings vital protection at their most vulnerable stages.

ACKNOWLEDGEMENTS

This work was funded by a grant from the Home-Grown Cereals Authority of the UK with support from KWS SAAT AG, Einbeck, Germany and seeds provided by Bayer Crop Science and KWS SAAT AG.

REFERENCES

- Glen, D.M., Jones, H., Fieldsend, J.K., 1990. Damage to oilseed rape seedlings by the field slug *Deroceras reticulatum* in relation to glucosinolate concentration. *Annals of Applied Biology*, **117**, 197-207.
- Gould, H.J., 1962. Tests with seed dressings to control grain hollowing of winter wheat by slugs. *Plant Pathology*, **11**, 147-152.
- Ester, A., Darwinkel, A., Floot, H.W.G., Nijenstein, J.H., 1996. Control of the field slugs (*Deroceras reticulatum*) in winter wheat using seeds treated with pesticides. *Proceedings No.66 (1996). British Crop Protection Conference Slug and Snail Pests in Agriculture*, pp165-172
- Ester, A., Nijenstein, J.H., 1995. Control of field slug *Deroceras reticulatum* (Müller) (Pulmonata: Limacidae) by pesticides applied to winter wheat seed. *Crop Protection*, **14**, 409-413.
- Ester, A., Nijenstein, J.H., 1996a. Molluscicidal seed treatment of barley, wheat and perennial ryegrass to control the field slug (*Deroceras reticulatum*). *Netherlands Journal of Agricultural Science*, **44**, 241-248
- Ester, A., and Nijenstein, J.H., 1996b. Control of field slug (*Deroceras reticulatum* (Müller)) by seed-applied pesticides in perennial rye grass assessed by laboratory tests. *Z Pflanzenk Pflanzenschutz-Journal of Plant Diseases and Protection*, **103**, 42-49.
- Fillion, J., 1997. The determination of metaldehyde in Ginseng plant material by GC/MSD. *Abstracts of Papers of the American Chemical Society*, **214**, 32.

- Jones, A., Charlton, A., 1999. Determination of Metaldehyde in Suspected Cases of Animal Poisoning Using Gas Chromatography-Ion Trap Mass Spectrometry. *Journal of Agricultural and Food Chemistry*, **47**, 4675-4677.
- Moens, R.; Glen, D., 2002. Agriolimacidae, Arionidae and Milacidae as pests in west European oilseed rape, in *Molluscs as Crop Pests*, ed. G Barker, pp 271-300. CABI Publishing: Wallingford.
- Nijënstein, J.H., Ester, A., 1998. Phytotoxicity and control of the field slug *Deroceras reticulatum* by seed applied pesticides in wheat, barley and perennial ryegrass. *Seed Science and Technology*, **26**, 501-513.
- Rose, P. W., Oades, L., 2001. Effects of imidacloprid cereal seed treatments against wireworms and slugs. *Proceedings No.76. British Crop Protection Conference, Seed Treatment – Challenges & Opportunities*, pp191-196.
- Scott, G.C., Pickett, J.A., Smith, M.C., Woodcock, C.M., Harris, P.G.W., Hammon, R.P., Koetecha, H.D., 1984. Seed treatments for controlling slugs in winter wheat. *Proceedings 1984 British Crop Protection Conference- Pests and Diseases*, pp133-138.
- Simms, L.C., Mullins, C.E., Wilson, M.J., 2002a. Seed dressings to control slug damage in oilseed rape. *Pest Management Science*, **58**, 687-694.
- Simms, L.C., Glen, D.M., Green, D.B., Wilson, M.J., 2002b. Protecting oilseed rape from slug damage using metaldehyde, methiocarb and imidacloprid seed dressings. *Proceedings 2002 British Crop Protection Conference, Pests & Diseases* In Press.
- Sylvester-Bradley, R., Makepeace, R.J., Broad, H., 1984. A code for stage development in oilseed rape (*Brassica napus* L.) *Aspects of Applied Biology, Agronomy, physiology, plant breeding and crop protection of oilseed rape*, **6**, 399-419.