Project Report No. 379

November 2005

Price: £3.75

Efficacy of a metaldehyde-based seed treatment for slug control in winter wheat and winter oilseed rape

by

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This is the final report of a thirty-four month project which started in December 2003. The work was funded by a contract of £96,664 from the HGCA (Project No. 2719) and an in-kind contribution from Luxan UK (£22,000), making a total of £118,664.

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ABSTRACT

Slugs are major pests of oilseed rape and wheat with most serious damage occurring just after sowing and seedling emergence. Current chemical control measures (bait pellets) often do not give adequate protection to plants, and pose an environmental hazard. Two HGCA-funded projects at the University of Aberdeen have demonstrated that metaldehyde seed coatings are highly effective at protecting oilseed rape seedlings from slug damage under laboratory conditions. However, in the second project, preliminary field experiments showed that protection was less than in the laboratory and was short-lived. If the factors responsible for limiting efficacy of molluscicidal seed treatments in the field can be identified, it may be possible to use modern seed-treatment chemistry to overcome these constraints. Factors are likely to include loss of seed coat caused by the irrigation of plots, volatilisation of active ingredients, reduced uptake at low temperatures and/or microbial breakdown of active ingredients.

We tested 5 environmental factors likely to cause this reduction in efficacy using an analytical method not previously published that analyses metaldehyde directly without prior depolymerisation. Metaldehyde residues in harvested shoots were analysed by GC-FID and showed metaldehyde seed treatments were broken down by soil micro-organisms and washed off seeds by rainfall. Neither wind, nor simulated rain on seedlings significantly reduced metaldehyde residues.

New seed treatment formulations developed by our industrial partner, Luxan (UK), were tested in 3 mini-plot trials on oilseed rape and wheat. In 2 of the mini-plot trials the industry-produced metaldehyde seed treatment protected seeds and seedlings, as well as bait pellets. When plots containing plants with the metaldehyde seed dressing received a half dose of bait pellets 5 days after sowing, protection from slugs was greater than that offered to control plants with a full dose of pellets applied at the time of sowing. In the third mini-plot trial, only treatments with slug bait pellets successfully reduced slug damage. It is likely the reduction in protection offered by the metaldehyde seed dressing was due to 2 unusually high rainfall events the 2nd and 3rd day after sowing. Precipitation for this month was 3 times the average for the time of year when the trial was conducted.

Previous HGCA-funded projects focussed on seed treatments to oilseed rape. The work reported here was extended to include wheat, which is severely damaged by slugs in the UK. Metaldehyde seed-dressings significantly reduced slug damage to wheat seedlings at all doses tested. However, no significant differences were found between doses tested.

SUMMARY

Introduction

Slugs are important pests of oilseed rape and wheat, damaging seeds and seedlings, thus reducing plant stand. Current slug control methods using bait pellets are often inadequate, due to unavailability of pellets to slugs during wet weather and failure of slugs to ingest a lethal dose. In addition, slug bait pellets lying on the soil surface are a potential threat to wildlife.

One alternative to bait pellets is seed treatment, which is used widely in the agricultural and horticultural industries to control insects and fungi. A molluscicidal seed treatment has many advantages over conventional bait pellets:

- amount of active ingredient per unit area reduced,
- active ingredient better targeted,
- avoids a separate field-pass for pesticide application,
- active ingredient less available to wildlife, if seeds are drilled,

This project aimed to build on two previous projects funded by the HGCA (Simms *et al.,* 2002; 2003). Our initial project, which screened several compounds under laboratory conditions, clearly demonstrated that metaldehyde and methiocarb seed treatments reduced slug damage to newly emerged oilseed rape seedlings. Optimum doses of metaldehyde and methiocarb seed treatments protected oilseed rape seedlings as well as or better than convention slug bait pellets. The second project testing these seed treatments in semi-field and field trials showed that protection was less than in the laboratory and short-lived. Possible explanations for the difference between laboratory and field experiments include:

- 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,
- loss of active ingredient from seedling through rain-wash or volatilisation.

If the factors responsible for limiting efficacy of molluscicidal seed treatments in the field can be identified, it may be possible to use modern seed-treatment chemistry to overcome these constraints. We developed a new method to analyse low concentrations of metaldehyde in plant material and used this method to identify environmental factors limiting metaldehyde uptake by seedlings. The results were used by our industrial partner to develop new seed treatment formulations, which were tested in semi-field trials.

The previous HGCA-funded projects focussed on seed treatments to oilseed rape. The reported project extended seed treatments to wheat, which is severely damaged by slugs in the UK. This would increase the potential market for any seed treatment product developed.

Methods

Optimum dose of metaldehyde to protect wheat seedlings from slugs

Wheat seeds (cv. Savannah) were treated with 3 doses of metaldehyde (4.6, 6.6 and 8.6 g a.i./kg seed). Treatments were either metaldehyde mixed with a commercial seed adhesive or our industrial partner's (Luxan UK) initial metaldehyde formulation mixed with seed adhesive. Wheat seeds treated with the seed adhesive only were used as a control. Seeds were sown in half seed trays containing John Innes No. 2 compost and surrounded by a copper mesh fence to deter slug movement. Four adult *Deroceras reticulatum* (Grey field slugs) were added to each tray. Plants were assessed weekly for slug damage.

Identification of factors limiting field performance

Oilseed rape (cv. Pronto) and wheat plants (cv. Savannah) treated with metaldehyde mixed with a commercial seed adhesive at the rates of 58 g a.i. /kg seed for oilseed rape and 6.6 g a.i./kg seed for wheat to give the same amount metaldehyde per seed. Seeds were sown in half seed trays (except where stated) and subjected to the following environmental conditions. All harvested plants were then analysed for metaldehyde by a method developed at Aberdeen University.

<u>Effect of soil micro-organisms on degradation of metaldehyde seed treatment</u>

Seeds were sown in seed trays containing sterilised soil and covered with a further 2cm of sterilised soil. Soil was watered every two days until the end of the experiment with either sterilised water or water inoculated with naturally abundant soil micro-organisms. Plants were harvested after 14 days.

<u>Effect of rain on metaldehyde seed treatment</u>

John Innes No. 2 compost was brought to field capacity with tap water; then seeds were sown on the surface. 'Rain' treatments were watered again with a watering can at the time of sowing and again 24 hours later. Plants were harvested 7 days after sowing.

<u>Effect of rain on seedlings</u>

Seeds were sown in half seed trays containing John Innes No. 2 compost, covered with a further 2cm of compost, moistened and covered with a propagator lid and left for 3 days to allow seeds to germinate.

Propagator lids were then removed and each tray was watered either onto the emerged cotyledons or onto the soil adjacent using a wash bottle every day for 5 days. Plants were harvested 7 days after sowing.

Effect of wind on volatilisation of metaldehyde from seedlings

Seeds were sown in seed trays containing John Innes No. 2 compost and covered with a further 2cm of John Innes No. 2 compost, moistened to field capacity and covered with a propagator lid. 'Wind' treatments had a continuous flow of air provided by an aquarium pump attached with tubing to a hole at one end of the propagator lid with another hole at the opposite end to allow air to escape. All treatments had small holes in the top of the propagator lids to allow for gaseous exchange. All seed trays were placed on fleece matting kept wet. Plants were harvested 14days after sowing.

Effect of temperature on metaldehyde uptake

Seeds were sown on 2 cm of agar in clear plastic boxes. Boxes were fitted with a clear plastic lid and placed in growth cabinets at either 10°C or 20°C with 12 hour light and 12 hour dark. Plants were grown for 20 days in 10°C and 10 days in 20°C before harvesting.

Mini-plot trials

The experiments were set up in mini-plots in an experimental area at Aberdeen University. Each mini-plot was a plastic container, 0.5 m^2 area, filled with sandy loam soil. Plots were painted with fluon and surmounted by copper-mesh fences 10 cm high to deter slug movement between plots. Oilseed rape or wheat seeds were sown with 48 seeds per plot. Three mini-plot trials were conducted, two in spring and one in autumn. In all trials seeds were treated with a new metaldehyde formulation developed by our industrial partner mixed with a commercial seed adhesive. Control seeds were treated with seed adhesive only. Treatments consisted of (1) metaldehyde-dressed seeds (58 g a.i./kg seed for oilseed rape cv. Pronto or 6.6 g a.i./kg seed for wheat cv. Savannah), (2) metaldehyde dressed seeds (doses as above) plus half dose metaldehyde slug pellets applied 5 days after sowing (4 kg metarex green/ha) (3) control plus metaldehyde slug pellets applied at the time of sowing (8 kg metarex green/ha) and (4) untreated control. Five adult slugs (*D. reticulatum*) were then introduced to each plot. Plants were assessed for any signs of slug grazing weekly for a period of four weeks.

Key results

- All doses of metaldehyde seed treatment reduced slug damage to wheat plants in comparison to control plants. However, no differences were found between the doses tested (Figure 1).

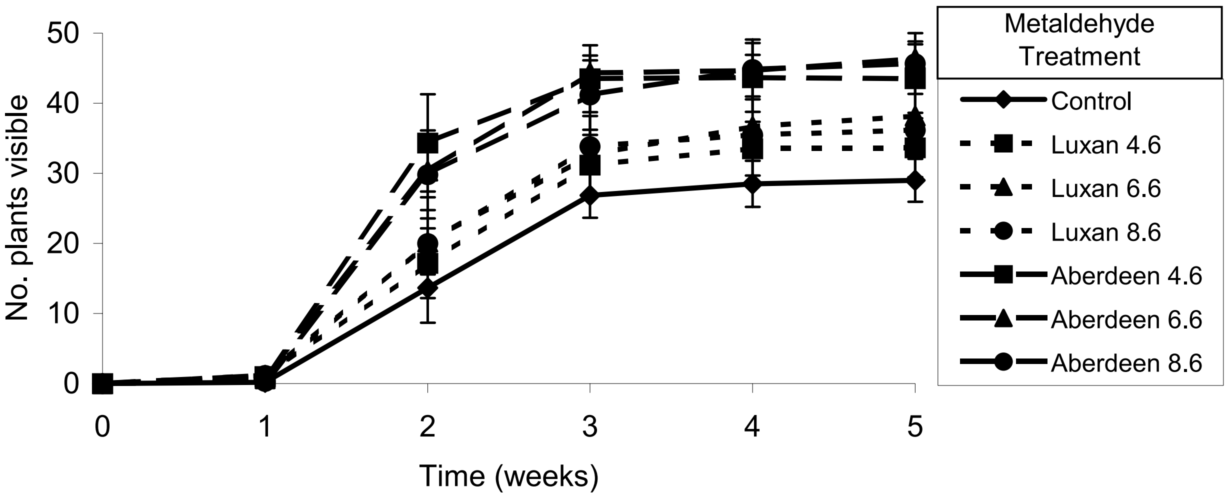

Figure 1. Number of wheat seedlings, with metaldehyde seed treatments either formulated by Luxan UK (Luxan) or Aberdeen University (Aberdeen), in seed trays containing 4 *Deroceras reticulatum* (grey field slug).

- Both oilseed rape and wheat plants grown in soil inoculated with soil micro-organisms had less metaldehyde in comparison to those grown in sterilised soil. Rain on the seeds at the time of sowing reduced metaldehyde in oilseed rape seedlings but not in wheat seedlings (Figure 2b). However, rain on the seedlings after germination had no effect on either oilseed rape or wheat (Figure 2c). 'Wind' also had no effect on the amount of metaldehyde in both oilseed rape and wheat seedlings (Figure 2d). There were no metaldehyde peaks present in any control plant samples. Oilseed rape plants grown at 20°C for10 days were found to have more metaldehyde than those grown at 10°C for 20 days (Figure 2e). However, no significant differences were found between wheat plants grown in different temperatures.

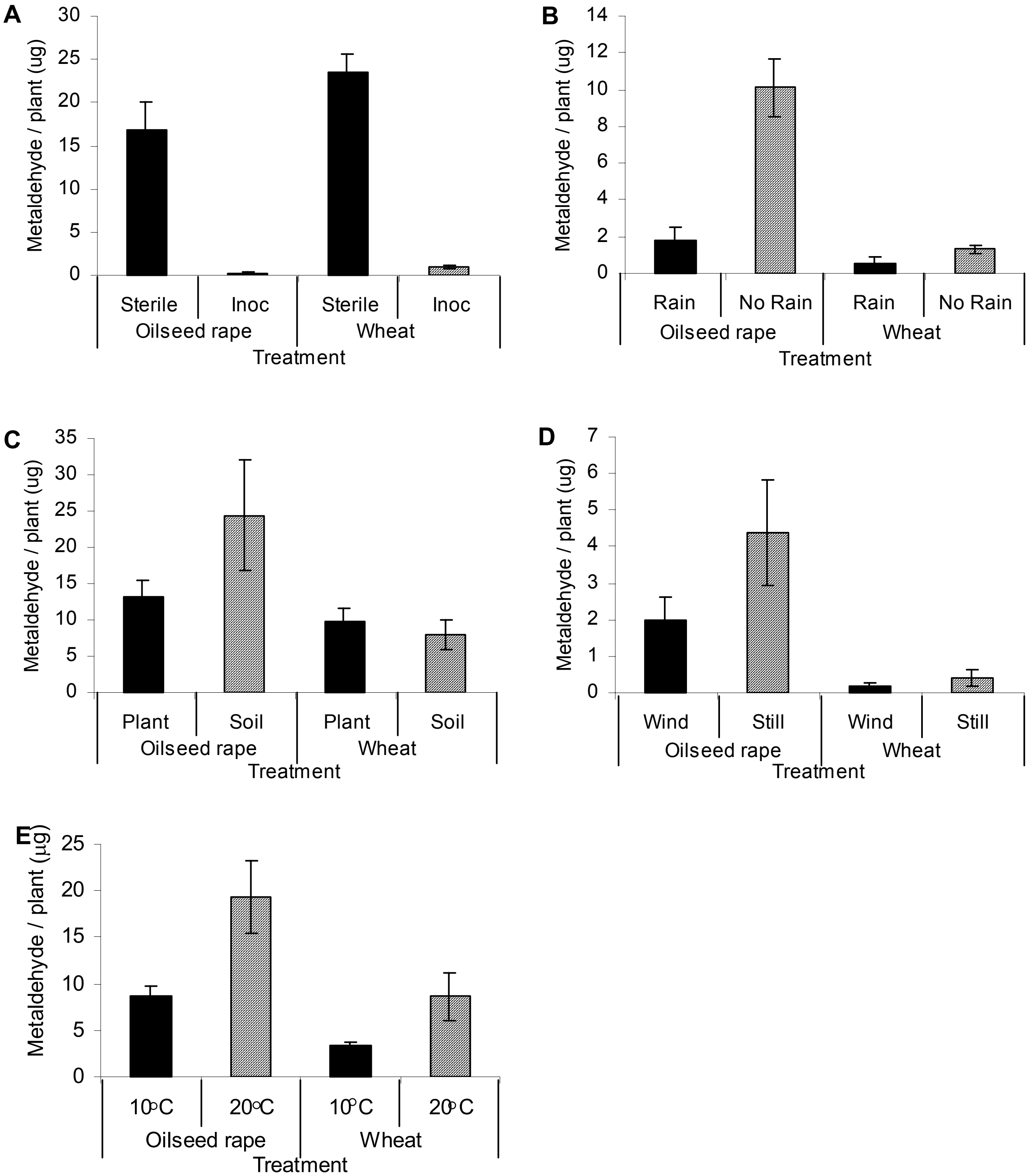

Figure 2. Amount of metaldehyde per plant extracted from oilseed rape and wheat seedlings (a) grown in sterile soil or sterile soil inoculated with soil micro-organisms, (b) with and without simulated rain on seeds, (c) with and without simulated rain on seedlings, (d) with and without simulated wind, (e) grown in 2 different temperatures.

- In two of the mini-plot trials conducted the new industry-produced metaldehyde seed treatment protected oilseed rape and wheat plants as well as conventional bait pellets applied at the time of sowing at the recommended dose. In addition, metaldehyde seed dressings together with a half dose of bait pellets applied 5 days after sowing performed better than the full dose of bait pellets alone (Fig 3). In the third mini-plot trial only treatments with bait pellets (control seeds with a full dose of pellets and metaldehyde seed dressing with a half dose of bait pellets) offered any protection to plants from slugs. Oilseed rape and wheat plants in these treatments had significantly less damage and more plants than controls (with the exception of the number of wheat plants in metaldehyde seed dressing with a half dose of pellets).

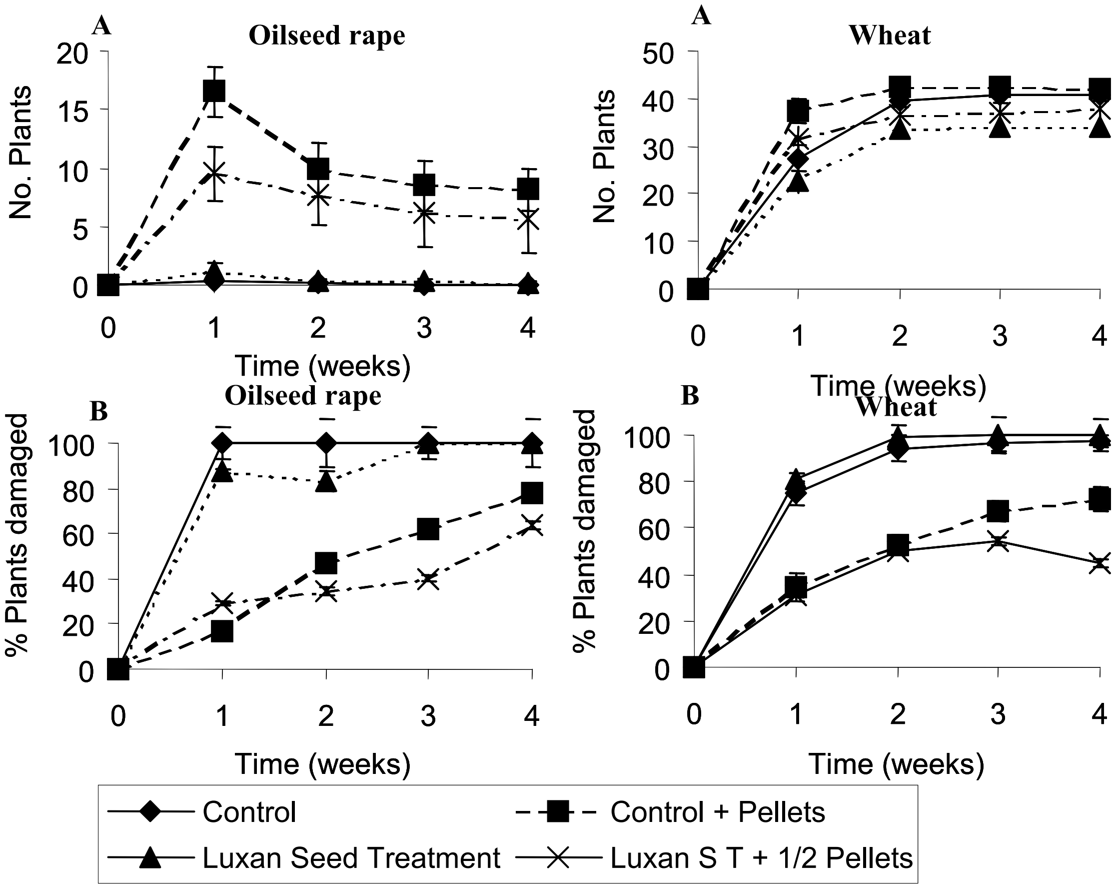

Figure 3. (A) Number of oilseed rape and wheat plants visible and (B) percentage of plants damaged by slugs, in a mini-plot trial done in Aberdeen, Scotland during autumn 2004.

Conclusions

Metaldehyde seed treatments successfully reduced slug damage to wheat in our laboratory trials. No extra protection appears to be offered by a higher dose of metaldehyde within the doses tested here. However, differences do appear to be present between the 2 different formulations. Plants with seeds treated with metaldehyde only (mixed with a seed adhesive) suffered less damage than those treated with initial industry-produced metaldehyde seed treatment formulation.

The sensitivity of the method for metaldehyde analysis in plant material was excellent, detecting very small residues of metaldehyde in small plant samples. As anticipated, the presence of soil micro-organisms had the greatest effect on metaldehyde uptake. Both wheat and oilseed rape plants grown in sterile soil had significantly more metaldehyde than those grown in soil inoculated with naturally abundant soil micro-organisms. Metaldehyde is a broken down very easily by soil micro-organisms in the soil. Provided the active ingredient can be protected on the seed, this rapid breakdown in soil makes metaldehyde an ideal choice for a seed treatment in comparison to other molluscicides, as any a.i. that leaches from the seed will have a limited effect on the environment. Rain on the seeds immediately after sowing reduced the uptake of metaldehyde in both plants, but results were only significant for oilseed rape. This is likely to be due to the difference in surface texture of the two different seed types. The rough surface texture of a wheat seed will allow the seed treatment to form a stronger bond with the seed in comparison with the shiny, smooth surface of an oilseed rape seed. Metaldehyde uptake was not significantly affected by either wind or rain on the seedling. This suggests that the metaldehyde is taken up by the root, transported and stored within the plant. If metaldehyde simply adhered to the cotyledons as they emerged we would expect these factors, and in particular rainfall, to have had a greater effect. It appears that temperature has a slight effect on the uptake of metaldehyde by oilseed rape reducing the amount of metaldehyde per plant at low temperatures. However, the use for this information is limited as it is unlikely that any amendments could be made to commercial seed formulations to overcome this. In addition, differences in physiology between plants grown at different temperatures make interpretation of the results difficult.

The new industry-produced seed treatment formulation increased the efficacy of metaldehyde seed treatments in semi-field conditions in both spring trials in 2004 and 2005. In these trials, all seeds treated with industry-produced metaldehyde formulations had significantly more plants and less slug damage than control plants. In some cases the industry-produced metaldehyde seed treatment alone, performed as well as bait pellets. In addition, when plots with the industry-produced metaldehyde seed treatment received a half dose of bait pellets, plant numbers were more and slug damage less than the full dose of bait pellets applied at time of plants. In the trial done in the autumn of 2004 industry-produced metaldehyde seed treatment only successfully reduced damage to plants in conjunction with bait pellets. Immediately after the sowing of this trial 2 extremely heavy rain events were experienced in Aberdeen. In addition, the rainfall for the month was approximately 3 fold that of an average year. It is suggested that the heavy and prolonged nature of the precipitation during the beginning of this trial had washed a sufficient amount of the metaldehyde seed treatment off the seeds before the seeds germinated, and had an opportunity to take up the treatment, to leave the plants vulnerable to damage by the introduced slug population.

Implications

Modern seed-treatment chemistry has been able to overcome the loss of metaldehyde from the seed due to microbial breakdown and rainfall to produce a seed treatment with greater efficacy in the field. If commercialised the benefits of such a product would be 2-fold. Economically, the cost to farmers could be reduced by avoiding the need for a separate field pass for bait pellet application and reduce the loss of crops by protecting seeds and seedlings at their most vulnerable stage. This is particularly true for oilseed rape that is drilled at the most busy time on the farm. Environmentally, better targeting of the molluscicide allows the amount of active ingredient applied per unit area to be reduced.

TECHNICAL DETAIL

INTRODUCTION

Slugs are a major pest of oilseed rape and wheat in the UK and many parts of North West Europe (Moens & Glen, 2002). The most serious damage, resulting in plant death, occurs just after sowing for wheat when slugs hollow out grains, and just after emergence for oilseed rape when the apical meristem is often destroyed. This can result in loss of plant stand, and is particularly serious for oilseed rape because the short period of time in which this crop can be established means that re-drilling is usually impractical. Current control methods rely on baited pellets containing either metaldehyde, carbamate or iron phosphate active ingredients (Henderson & Triebskorn, 2002). Slug control with pellets is often inadequate, due to unavailability of pellets to slugs during wet weather and failure of slugs to ingest a lethal dose. In addition, slug bait pellets lying on the soil surface are a potential threat to wildlife. Since the only economic damage done by slugs is to seeds or very young newly emerged seedlings, one possible approach to controlling this pest is the use of repellent seed treatments. This would offer the potential benefits of reducing the amount of active ingredient applied to the environment, reduce the threat to non-target organisms and negate the need for a separate tractor pass for pellet application. Seed treatments with metaldehyde and methiocarb have been shown to be effective at controlling slug damage to wheat under field conditions (Ester et al., 1996). We have also previously shown that these compounds can protect oilseed rape from slug damage as effectively as bait pellets under laboratory conditions in two previous short-term projects funded by the HGCA (Simms et al., 2002b; 2003). However, in our semi-field (mini plot) experiments with oilseed rape, although metaldehyde and methiocarb seed treatments significantly reduced slug damage compared with control plants, this effect was short lived, particularly with metaldehyde (Simms et al., In Press). In addition, protection offered by seed-treatments was significantly less than that offered by metaldehyde bait pellets. If the factors responsible for limiting efficacy of molluscicidal seed treatments in the field can be identified, it may be possible to use modern seed-treatment chemistry to overcome these constraints. Factors are likely to include loss of active ingredient from leaves or seed coat due to rain or irrigation of plots, reduced uptake of active ingredient at low temperatures, volatilisation of active ingredients and/or microbial breakdown of the active ingredients. The last possibility seems particularly likely because metaldehyde because activity was particularly short-lived and it is known to depolymerise into acetaldehyde rapidly in the soil.

Here we describe experiments designed to investigate the 5 most likely environmental factors, mentioned above, reducing metaldehyde in oilseed rape and wheat seedlings using GC-FID. Previously published methods on analysis of metaldehyde in plant material involve removing naturally occurring acetaldehyde prior to depolymerisation of metaldehyde and derivatisation of the resulting acetaldehyde (Brown *et.al*., 1996; Giang and Smith, 1956; Selim and Seiber, 1973). Other methods published were also unsuitable due to concerns with either: sensitivity, as the method relies on colour development and measurement photometrically (Kimura and Miller, 1964); safety, as the method involves boiling concentrated sulfuric acid in a closed system at high temperature (Griffiths, 1984); or lack of 'clean-up' steps necessary for the complex mix of compounds found in plant material (Jones and Charlton, 1999). However, due to the low concentrations of metaldehyde expected in harvested seedlings, we decided to use a method analysing the parent compound to avoid background interference of acetaldehyde and derivatives, and to reduce the number of steps involved and hence the loss of metaldehyde. The method, not previously published, involved an acetonitrile extraction, various clean-up steps, careful evaporation and analysis by gas chromatography (GC) with a flame ionisation detector (FID). Due to the increased movement of water and increased microbial diversity and biomass present in semi-field trials compared to laboratory trials we suspected these factors to be those most likely to be significantly affixing metaldehyde uptake.

The results of metaldehyde uptake experiments were used by our industrial partners to design a new seed treatment formulation to improve the efficacy in the field. This new formulation was tested in a series of 3 semi-field experiments for efficacy against the most widespread slug pest species, *Deroceras reticulatum*.

In addition, previous HGCA-funded projects have until now focussed on seed treatments to oilseed rape. The work reported here was extended to include wheat, which is severely damaged by slugs in the UK. This will increase the potential market for any seed treatment product developed.

Objectives:

1. Confirm optimum dose of metaldehyde to protect wheat seeds.
2. Identify the factors that have limited field efficacy of seed treatments.
3. Develop new seed coating formulations containing adjuvants, barrier layers and/or slow release compounds to overcome the above limitations.
4. Investigate the efficacy of new seed treatment formulations under controlled semi-field conditions.
5. Investigate the efficacy of new formulations under field conditions.

MATERIALS AND METHODS

Optimum dose of metaldehyde to protect wheat seeds

Winter wheat (cv. Savannah) seeds were coated in 20 – 50 g lots with either metaldehyde, or a metaldehyde seed formulation created by our industrial partner mixed, with a commercial seed adhesive, Sepiret (Agrichem, Whittlesey, UK). Control seeds had seed adhesive coating only, at the same application rate as treated seeds. 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.

Doses tested were based on the most effective doses found by Simms *et al.* (2002) for oilseed rape of 58g a.i./kg seed. This rate was then adapted to wheat according to the thousand-grain weight, 6.6g a.i./kg seed, to give the same mass of active ingredient per seed. Two further doses of 8.6g a.i./kg seed and 4.6g a.i./kg seed were also tested.

Sixty treated seeds were sown in seed trays (220 × 165 × 57 mm) surrounded by a copper mesh fence (10cm high) to prevent slugs escaping and placed in a greenhouse. Seed trays were continuously watered with a drip irrigation system. Seedlings showing any signs of slug grazing were assessed at weekly intervals from the day of planting, for 4 weeks. All experiments had a fully a randomised design, each with six replicates.

Identification of factors limiting field efficacy of seed treatments

Plant material and treatments

Oilseed rape (cv. Pronto) and winter wheat (cv. Savannah) seeds were coated in 20 – 50 g lots with metaldehyde mixed with a commercial seed adhesive, Sepiret (Agrichem, Whittlesey, UK). Control seeds had seed adhesive coating only, at the same application rate as treated seeds. 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.

Doses tested were based on the most effective doses found by Simms *et al.* (2002) for oilseed rape of 58g a.i./kg seed. This rate was then adapted to wheat according to the thousand-grain weight, 6.6g a.i./kg seed, to give the same mass of active ingredient per seed.

In all experiments 100 treated seeds were sown in seed trays (220 × 165 × 57 mm) placed in a greenhouse, and had fully a randomised design, each with six replicates.

<u>Effect of soil micro-organisms on degradation of metaldehyde seed treatment</u>

Soil (sandy loam texture) was sterilised by autoclaving to 121°C, in small batches, allowed to cool, mixed and autoclaved a further two times. All seeds were sown in seed trays containing sterilised soil and covered with a further 2cm of sterilised soil. Soil was moistened with 200ml of either autoclaved sterile tap water or inoculated water 3 days before planting, the day of planting and every two days until the end of the experiment. The water was inoculated with soil micro-organisms by mixing the same non-sterilised soil with an equal volume of tap water, mixing vigorously, allowing to settle for 5 minutes before pouring off the water fraction. Plants were harvested after 14 days, weighed and stored at -20°C.

<u>Effect of rain on metaldehyde seed treatment</u>

Seed trays were filled with John Innes No. 2 compost and brought to field capacity with tap water. Seeds were sown on the surface of the compost. 'Rain' treatments were further watered with approximately 200ml of tap water with a watering can at the time of sowing and again 24 hours later. Plants were harvested 7 days after sowing, weighed and stored at -20°C.

Effect of rain on seedlings

Seeds were sown in seed trays containing John Innes No. 2 compost and covered with a further 2cm of John Innes No. 2 compost, moistened with 175 ml of tap water, covered with a propagator lid and left for 3 days to allow seeds to germinate. Propagator lids were then removed and each tray was watered with 100ml of tap water either onto the emerged cotyledons or onto the soil adjacent using a wash bottle every day for 5 days. Plants were harvested 7days after sowing, weighed and stored at -20°C.

Effect of wind on volatilisation of metaldehyde from seedlings

Seeds were sown in seed trays containing John Innes No. 2 compost and covered with a further 2cm of John Innes No. 2 compost, moistened to field capacity with tap water and covered with a propagator lid. 'Wind' treatments had a continuous flow of air provided by a aquarium pump (1 pump per 4 seed trays) attached with tubing to a 1cm-diameter hole at one end of the propagator lid with another 1-cm hole at the opposite end to allow air to escape. All treatments had 20 x 1mm holes in the top of the propagator lids to allow for gaseous exchange. All seed trays were placed on fleece matting kept wet with tap water. Plants were harvested 14days after sowing, weighed and stored at -20°C.

Effect of temperature on metaldehyde uptake

Seeds were sown on 2 cm of agar (technical no.3) in clear plastic boxes (cm x cm) sterilised with ethanol. Boxes were fitted with a clear plastic lid and placed in phytotrons at either 10°C or 20°C (constant temperature inside enclosed box) with 12 hour light and 12 hour dark. Plants were grown for 20 days in 10°C and 10 days in 20°C before harvesting.

Metaldehyde extraction and analysis

Extraction

Plant samples were homogenised in a pestle and mortar with liquid nitrogen. A sub-sample of 2.5g was placed into a Wheaton vial and 10ml of acetonitrile was added to each vial and placed in an orbital shaker for 5 minutes. An oilseed rape and wheat control sample were each spiked with 0.001g metaldehyde to determine the recovery of metaldehyde.1g NaCl was added to each vial and placed in an orbital shaker for a further 5 minutes. Samples were passed through a Whatman's No. 1 filter paper, followed by an additional 10ml acetonitrile. The water phase at the bottom of each vial was then removed and discarded.

Clean-up

Three solid phase extraction (SPE) tubes were connected in series and pre-conditioned with 6ml acetonitrile per tube, and the eluant discarded. A further 6ml approximately acetonitrile was added to all columns to produce no air gaps between columns. A C_{18} SPE column (Supelco, 6ml, 1g) was connected to the top of an Envicarb SPE column (Supelco, 6ml, 500mg) which was in turn connected to an aminopropyl (NH_2) SPE column (Supelco, 6ml, 1g). 1g Na_2SO_4 was added to the Envicarb column. A 10ml reservoir was connected to the top of the C_{18} column. The 20ml sample was added and eluted using a vacuum manifold. 5ml acetonitrile was then added to elute the sample fraction left in the C_{18} column. The C_{18} column was discarded and 5ml of 3:1 acetonitrile-toluene was added to the remaining columns to elute the extract. The samples were then evaporated to just under 1ml under nitrogen in a water bath at 40°C and brought up to 1ml with toluene. Metaldehyde standards of between 1 to 200mg/l in toluene were prepared for calibration. All samples were spiked with 10µl of the internal standard hexachlorobenzene (10g/l in toluene).

GC analysis

The metaldehyde samples were analysed using GC-FID (Thermoquest GC 8000). Samples (1 µg) were injected onto a capillary column (Phenomonex ZB1, 30 m x 0.32 mm i.d). The temperature programme started at 90°C for 1.5 min, and increased to 280°C at 15°C/min to 280°C and then held at this temperature for 10 minutes. The injector temperature was 250°C and the detector temperature was 310°C. Under these conditions, metaldehyde and hexachlorobenzene retention times were 5.4 and 11.8 min respectively. The quantities of metaldehyde per plant were calculated using the peak area under the metaldehyde peak and quantified using the metaldehyde standard calibration curve.

Mini-plot trials

The experiments were set up in mini-plots in an experimental area at Aberdeen University. Each mini-plot was a plastic container (80 cm x 60 cm (0.48 m^2) x 23 cm deep, filled with sandy loam soil to 20 cm depth, with drainage holes in the bottom covered by plastic mesh to prevent slugs entering or leaving. Plots were painted with fluon and surmounted by copper-mesh fences 10 cm high to deter slug movement between plots. Oilseed rape or wheat seeds were sown with 48 seeds per plot. Three mini-plot trials were conducted, two in spring and one in autumn. In all trials seeds were treated with a new industry-produced metaldehyde formulation mixed with Sepiret blue seed adhesive. Control seeds were treated with seed adhesive only. Treatments consisted of (1) metaldehyde-dressed seeds (58 g a.i./kg seed for oilseed rape cv. Pronto or 6.6 g a.i./kg seed for wheat cv. Savannah), (2) metaldehyde dressed seeds (doses as above) plus half dose metaldehyde slug pellets applied 5 days after sowing (4 kg metarex green/ha) (3) control plus metaldehyde slug pellets applied at the time of sowing (8 kg metarex green/ha) and (4) untreated control. There were six replicate plots of each treatment arranged in randomised blocks. Five adult slugs (*D. reticulatum*) were then introduced to each plot. Plots were irrigated as required. Plants were assessed for any signs of slug grazing weekly for a period of four weeks.

Statistical analysis

Statistical analyses were performed with Genstat 5 (Numerical Algorithms Group, Oxford). All data were subject to analysis of variance (ANOVA) and when ANOVA revealed significant treatment effects or interactions, individual means were compared using the LSD test.

RESULTS

Optimum dose of metaldehyde to protect wheat seeds

All doses of metaldehyde seed treatments had a significantly greater number of plants in comparison to the controls (Figure 1). Metaldehyde seed treatments formulated by Aberdeen University had a significantly greater number of seedlings in comparison to all industry-produced seed treatments. No significant differences were found in number of plants between different doses within the same formulation.

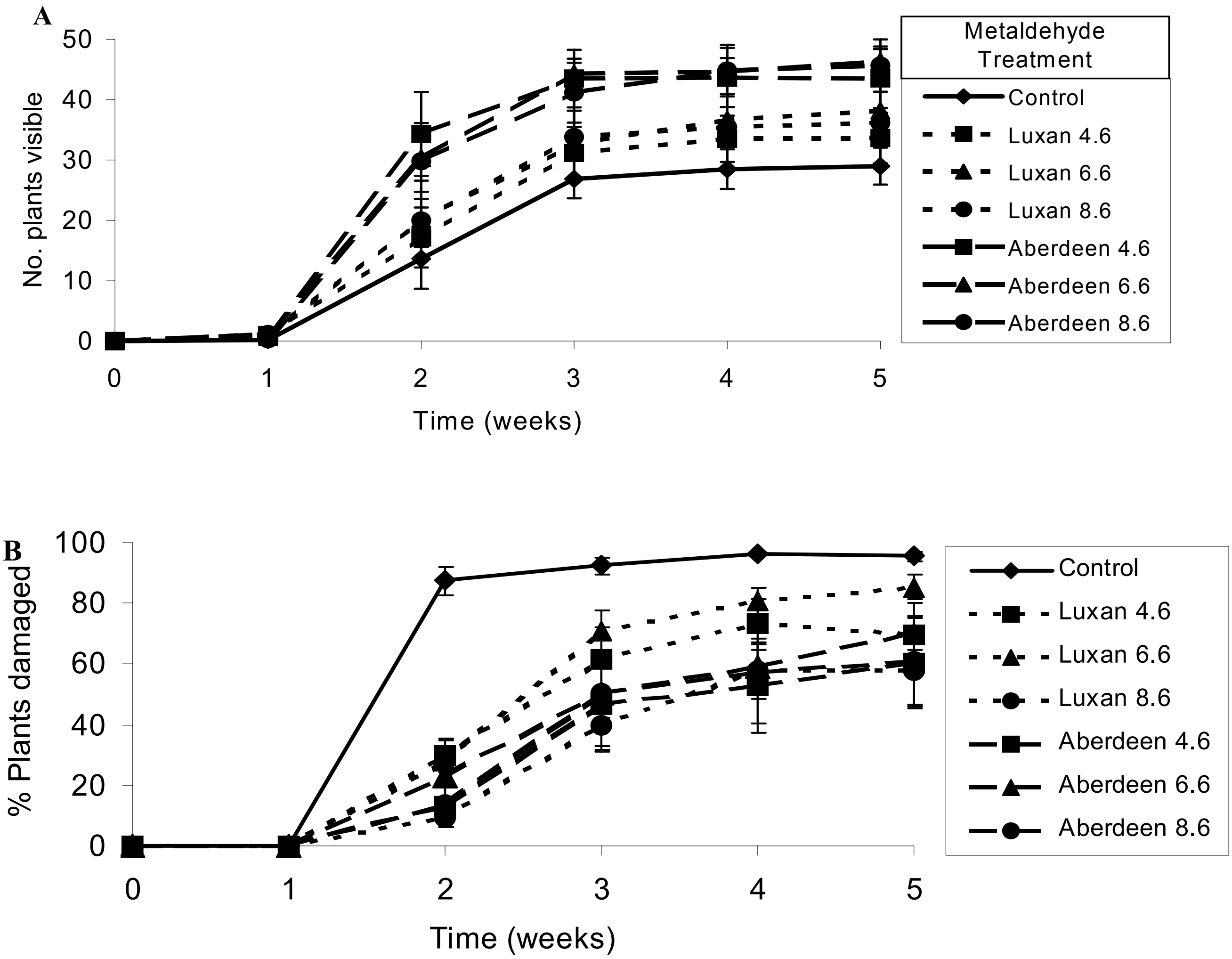

Figure 1. Damage to wheat seedlings, with metaldehyde seed treatments either formulated by industry (Luxan) or Aberdeen University (Aberdeen), in seed trays containing 4 *Deroceras reticulatum* (grey field slug). (A) Number of plants visible, and (B) Percentage of plants damaged.

Identification of factors limiting field efficacy of seed treatments

Metaldehyde analysis

Metaldehyde calibration was carried out for each individual experiment to confirm both the retention time of metaldehyde and to calibrate peak area to metaldehyde concentration. An internal standard, hexachlorobenzene was added to every sample to check for any deviations in retention time. Retention times for metaldehyde and hexachlorobenzene were found to be 5.36 and 11.77 minutes respectively in every sample (Figure 2 a-d). In addition, there was no interference found between the metaldehyde and hexachlorobezene peaks with other plant compound peaks. Metaldehyde was detected by GC-FID down to a concentration of 1mg/l (Figure 3).

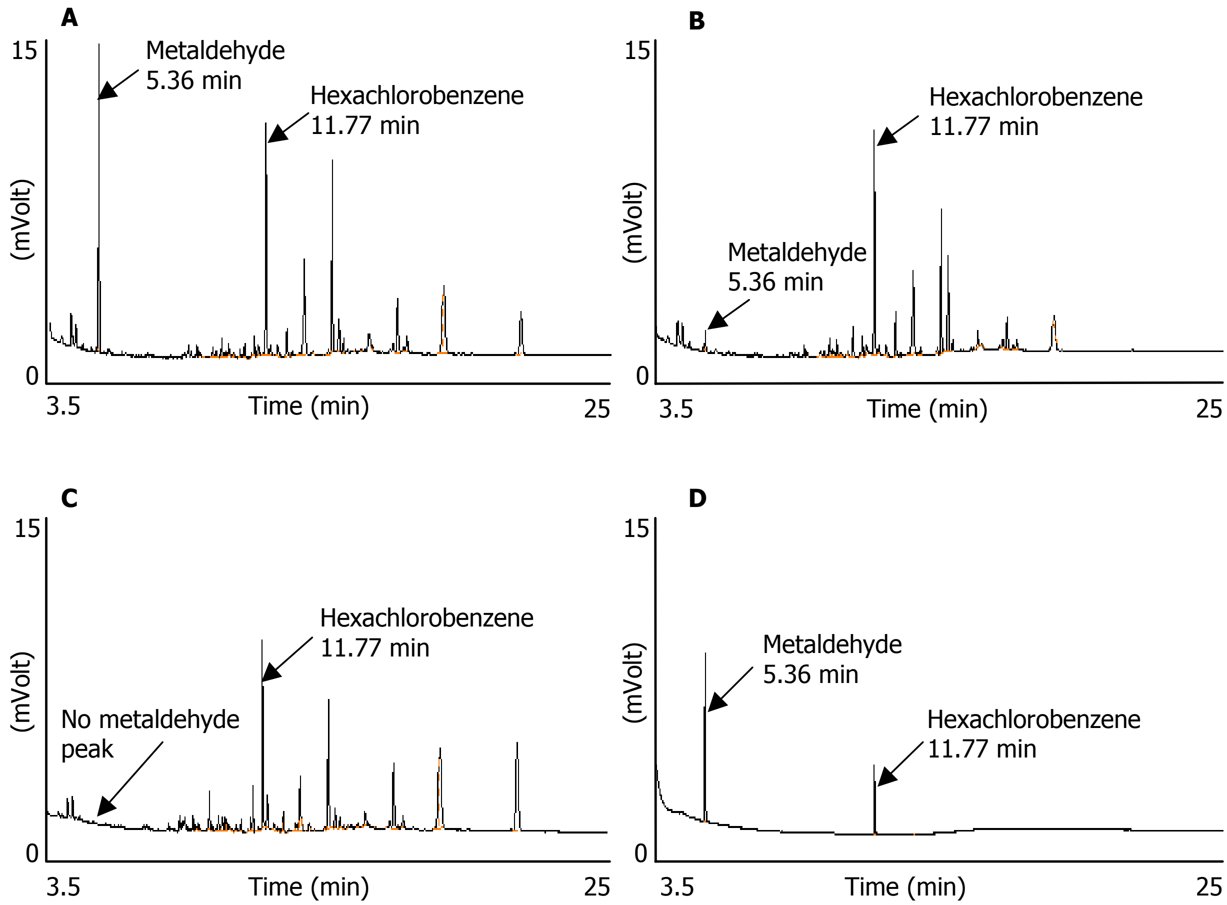

Figure 2. Typical chromatograms of shoot extracts from oilseed rape with (A) metaldehyde seed treatment grown in sterilised soil, (B) metaldehyde seed treatment grown in sterilised soil re-inoculated with soil micro-organisms, (C) control seed treatment grown in sterilised soil re-inoculated with soil micro-organisms, and (D) metaldehyde control (200mg/l) with internal standard hexachlorobenzene.

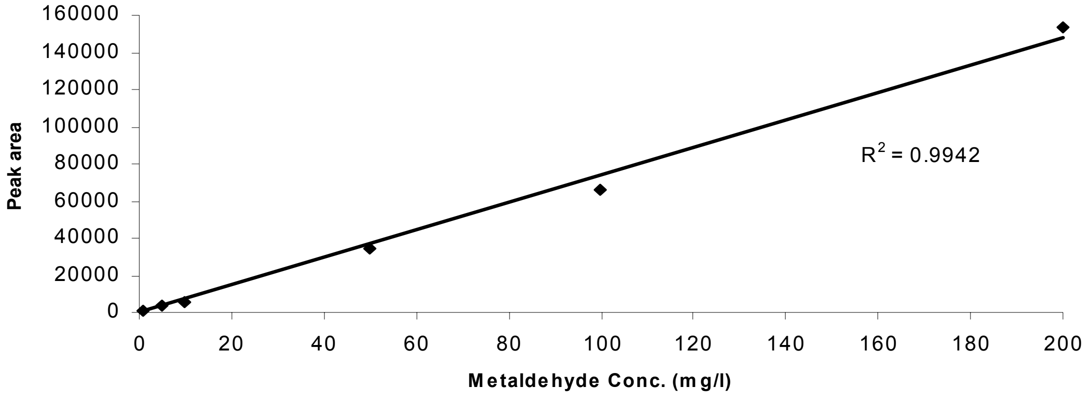

Figure 3. Calibration curve of metaldehyde in toluene analysed by GC-FID.

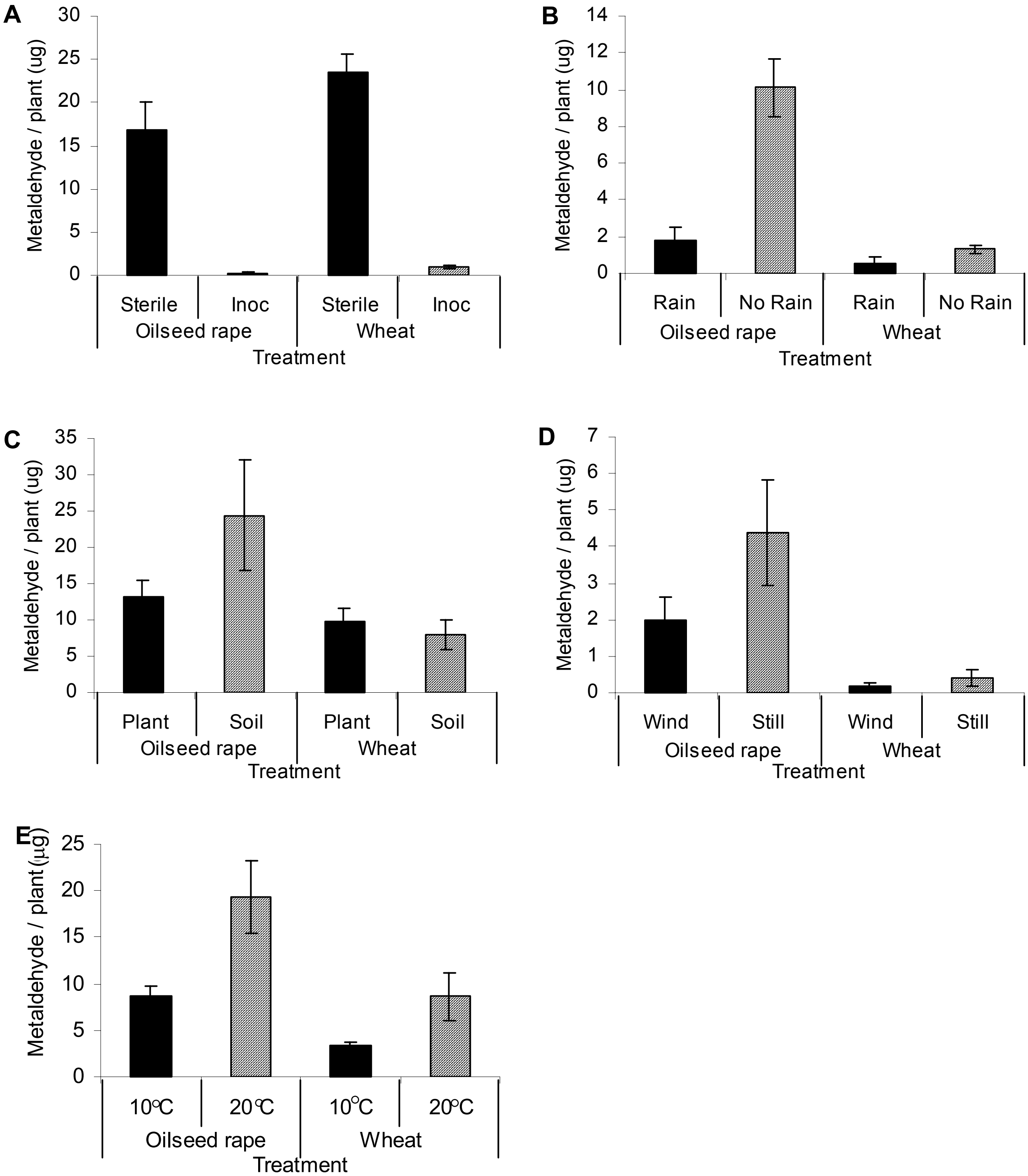

Figure 4. Amount of metaldehyde per plant extracted from oilseed rape and wheat seedlings (a) grown in sterile soil or sterile soil inoculated with soil micro-organisms, (b) with and without simulated rain on seeds, (c) with and without simulated rain on seedlings, (d) with and without simulated wind, (e) grown at 2 different temperatures.

Effect of environmental factors on metaldehyde uptake

- Both oilseed rape and wheat seeds had significantly more ($P< 0.001$) metaldehyde when grown in sterile soil in comparison to those grown in soil inoculated with soil micro-organisms (Figure 4a). Rain on the seeds at the time of sowing significantly reduced metaldehyde in oilseed rape seedlings ($P< 0.001$) but not in wheat seedlings ($P=0.074$) (Figure 4b). However, rain on the seedlings after germination had no effect on either oilseed rape or wheat (Figure 4c). 'Wind' also had no effect on the amount of metaldehyde in both oilseed rape and wheat seedlings (Figure 4d). There were no metaldehyde peaks present in any plant control samples with seed adhesive seed treatment only (Figure 4c). Oilseed rape plants grown at 20°C for 10 days were found to have more metaldehyde than those grown in 10°C for 20 days ($P< 0.05$) (Figure 4e). However, no significant differences were found between wheat plants grown in different temperatures ($P< 0.066$).

Mini-plot trials

In the spring 2004 mini-plot trial, all slug control treatments had significantly more oilseed rape and wheat plants and fewer plants damaged by slugs than the controls (Figure 5). In oilseed rape plots, industrial metaldehyde seed treatment plus a half dose of slug pellets had significantly more plants and fewer plants respectively damaged than all other treatments. Although oilseed rape plants with bait pellet protection did not have more plants than the other slug control treatments they did have less slug damage than metaldehyde seed treatment alone. In wheat plots no significant differences in plant numbers were found between any of the slug control treatments. However, differences were found in the percentage of plants damaged. Slug bait pellet treatments had fewer plants damaged in comparison with metaldehyde seed treatments and metaldehyde seed treatments together with a half dose of bait pellets had fewer plants damaged than all other treatments.

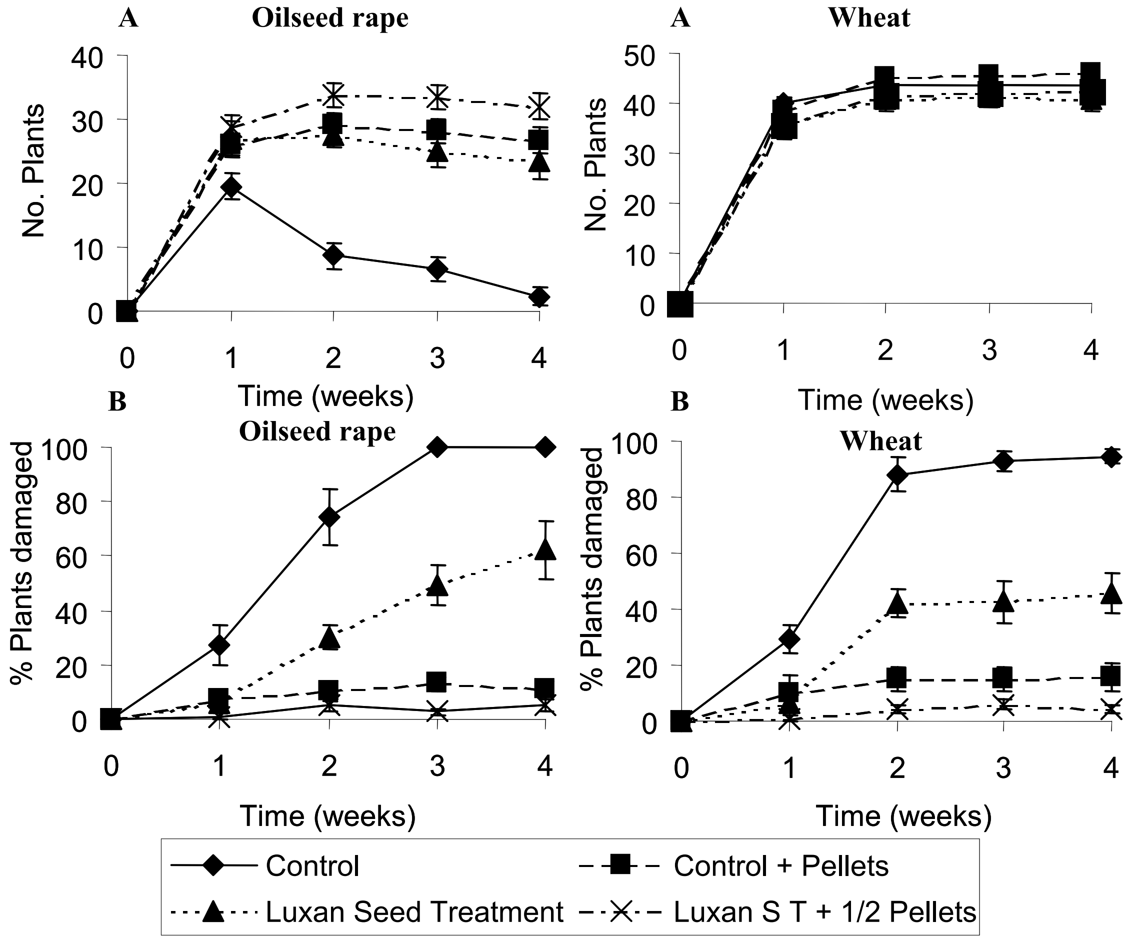

Figure 5. (A) Number of oilseed rape and wheat plants visible and (B) percentage of plants damaged by slugs, in a mini-plot trial done in Aberdeen, Scotland during spring 2004.

In the second mini-plot trial done during the autumn of 2004 only treatments containing pellets protected plants from slug damage (Figure 6). Both oilseed rape and wheat control seeds with a full dose of bait pellets and industrial metaldehyde treated seeds plus a half dose of pellets had significantly fewer plants damaged by slugs than control and metaldehyde treated seeds without pellets added. The same trend is true for the number of oilseed rape plants visible with both control plots with a full dose of pellets and metaldehyde seed treatment plots having more plants than the other two treatments. However, for all oilseed rape treatments plant numbers were very low with less that 35% establishment. In wheat plots only control treatments with the full dose of bait pellets showed signs of protection from slug damage in terms of plant numbers with significantly more plants visible than all other treatments.

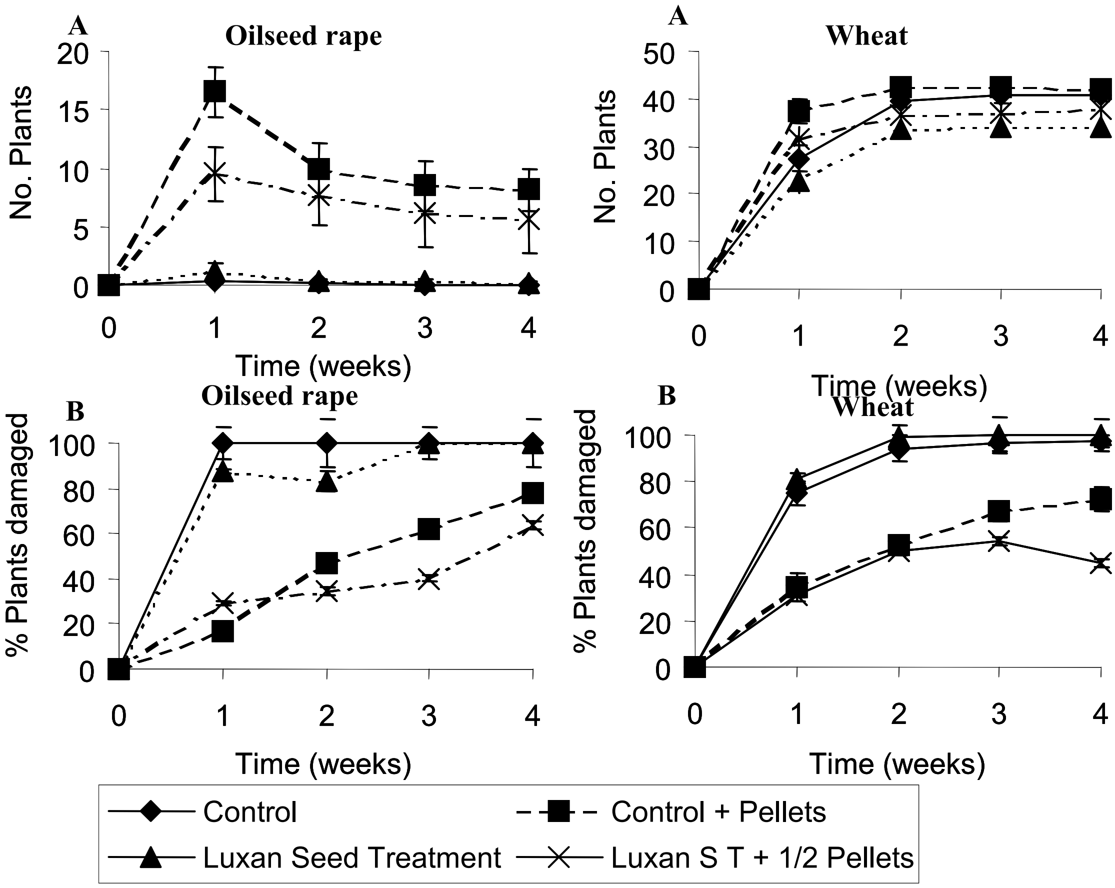

Figure 6. (A) Number of oilseed rape and wheat plants visible and (B) percentage of plants damaged by slugs, in a mini-plot trial done in Aberdeen, Scotland during autumn 2004.

In the trial done in the spring of 2005 all slug control treatments in oilseed rape plots had significantly more plants and significantly less damage (Figure 7). In addition, control oilseed rape plants with bait pellets had significantly more plants than all other treatments. However, no difference in slug damage was found between this treatment and both metaldehyde seed treatments. In contrast, only wheat plants with metaldehyde seed treatments with a half dose of bait pellets had significantly more plants and less damage in comparison to all other treatments. Control plants with bait pellets had fewer plants damaged than metaldehyde seed treatment alone and control plots, but no difference were between numbers of plants in these treatments.

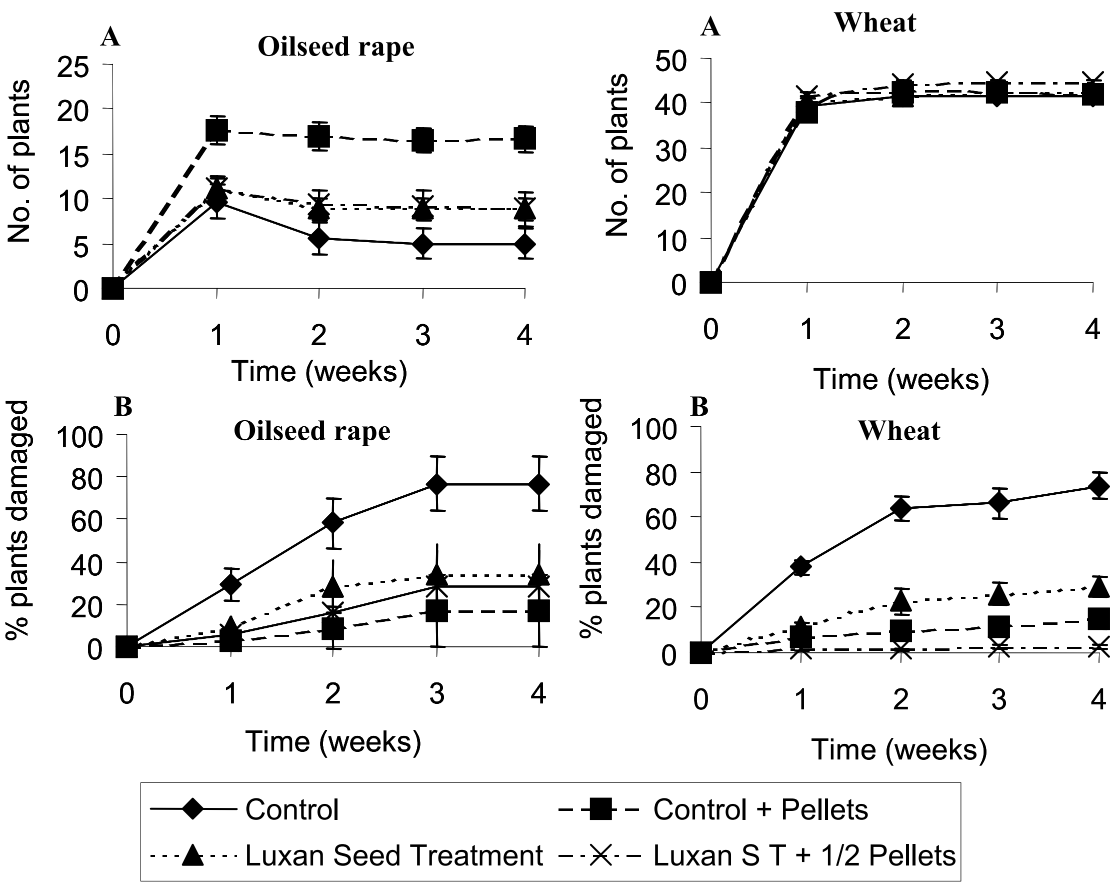

Figure 7. (A) Number of oilseed rape and wheat plants visible and (B) percentage of plants damaged by slugs, in a mini-plot trial done in Aberdeen, Scotland during spring 2005.

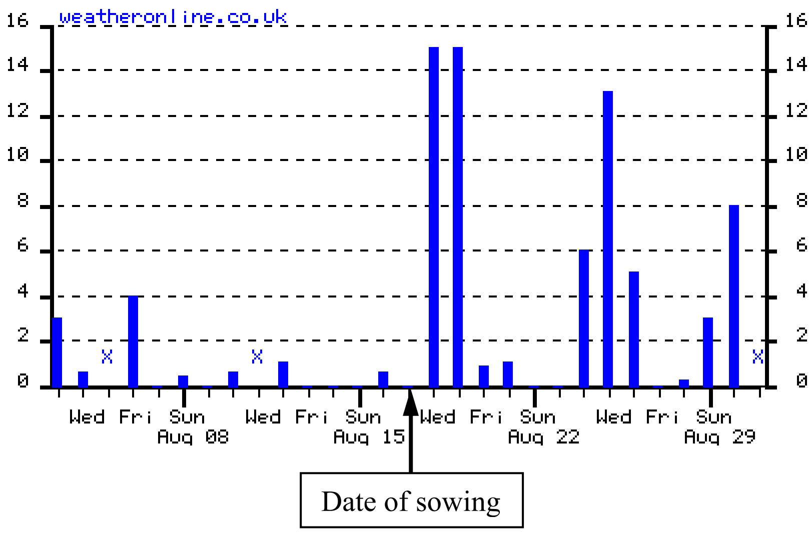

Figure 8. Rainfall (mm) during August 2004 in Aberdeen, Scotland.

DISCUSSION

Metaldehyde seed treatments have been shown to reduce slug damage to wheat. No extra protection was offered by a higher dose of metaldehyde within the doses tested here. However, there were differences between the 2 formulations. There was less damage to plants with seeds treated with metaldehyde only (mixed with a seed adhesive) than with initial industrial metaldehyde seed treatment formulation.

The sensitivity of the method for metaldehyde analysis in plant material was excellent, detecting very small residues of metaldehyde in small plant samples. Calibration of metaldehyde by GC-FID was linear on each occasion, with retention times being very consistent throughout in plant, calibration and blank samples. The 3 step clean-up process allowed most plant compounds to be removed, allowing very clean metaldehyde and hexachlorobenzene peaks. The calibration of metaldehyde provided confidence in the results that metaldehyde was being detected with no interference detected in any control plant samples. This method involves fewer steps than previously reported methods (Giang and Smith, 1956; Selim and Seiber, 1973; Iwata et al., 1982), so reducing the risk of loosing metaldehyde. In addition, the analysis of metaldehyde directly, instead of acetaldehyde, removes the problem of interference from background acetaldehyde commonly found in control plant samples.

As anticipated, the presence of soil micro-organisms had the greatest effect on metaldehyde uptake. Both wheat and oilseed rape plants grown in sterile soil had significantly more metaldehyde than those grown in soil inoculated with naturally abundant soil micro-organisms. The chemical structure of metaldehyde ($C_8H_{16}O_4$) makes it an ideal carbon source for micro-organisms, which rapidly depolymerise the tetramer into acetaldehyde, which has no reported molluscicidal properties. Acetaldehyde in turn undergoes evaporation or oxidation to acetic acid. This rapid breakdown in soil makes metaldehyde an ideal choice for a seed treatment in comparison to other molluscicides, as it will have a limited effect on the environment. These results are also consistent with reported mini-plot data, which show metaldehyde seed treatments to offer short-lived protection from slug damage (Simms et al., in press). Rain on the seeds immediately after sowing reduced the uptake of metaldehyde in both plants, but results were only significant for oilseed rape. This is likely to be due to the difference in surface texture of the two different seed types. The rough surface texture of a wheat seed will allow the seed treatment to form a stronger bond with the seed in comparison with the shiny, smooth surface of an oilseed rape seed. Metaldehyde uptake was not significantly affected by either wind or rain on the seedling. This suggests that the metaldehyde is taken up by the root, transported and stored within the plant. If metaldehyde was simply adhered to the cotyledons as they emerged we would expect these factors, and in particular rainfall, to have had a greater effect. In addition, previously reported findings show metaldehyde continues to protect seedlings at least 4 weeks after sowing when many true leaves have emerged having not had contact with the seed yet still being protected from slug damage (Simms *et al*., 2002a; 2002b). It appears that temperature has a slight effect on the uptake of metaldehyde by oilseed rape reducing the amount of metaldehyde per plant at low temperatures. However, the use of this information is limited as it is unlikely that any amendments could be made to commercial seed formulations to overcome this. In addition, differences in plant physiology between plants grown at different temperatures make interpretation of the results difficult.

The new industry-produced seed treatment formulation has increased the efficacy of metaldehyde seed treatments in semi-field conditions in both the spring trials done in 2004 and 2005. In these trials all seeds treated with the new formulations had significantly more plants and less slug damage than control plants. In some cases the new metaldehyde seed treatment alone, performed as well as bait pellets (percentage damage to oilseed rape spring 2005, oilseed rape and wheat plant numbers spring 2004). In addition, when plots with the industry-produced metaldehyde seed treatment received a half dose of bait pellets plant numbers were more and slug damage less than the full dose of bait pellets applied at time of plants (Wheat plant numbers and plant damage spring 2005, wheat plant damage 2004 and oilseed rape plant numbers and plant damage spring 2004). In the trial done in the autumn of 2004, the new industry-produced metaldehyde seed treatment only successfully reduced damage to plants in conjunction with bait pellets. Immediately after the sowing of this trial, 2 extremely heavy rain events were experienced in Aberdeen, as shown in Figure 8. In addition, the rainfall for the month was approximately 3-fold that of an average year. It is suggested that the heavy and prolonged nature of the precipitation during the beginning of this trial had washed a sufficient amount of the metaldehyde seed treatment off the seeds before the seeds germinated leaving the plants open to damage by the introduced slug population.

Modern seed-treatment chemistry may be able to overcome the loss of metaldehyde from the seed due to microbial breakdown and rainfall to produce a seed treatment with greater efficacy in the field. If commercialised, the benefits of such a product would be 2-fold. Economically, the cost to farmers could be reduced by avoiding the need for a separate field pass for bait pellet application and reduce the loss of crops by protecting seeds and seedlings at their most vulnerable stage. Environmentally, better targeting of the molluscicide allows the amount of active ingredient applied per unit area to be reduced and if seeds are drilled reduces their availability to non-target organisms.

ACKNOWLEDGEMENTS

We thank J Fillion and C McLenaghan of The Pest Management Regulatory Agency, Canada, for help and technical advice.

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