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Identification of Environmental Factors Limiting Plant Uptake of Metaldehyde Seed Treatments under Field Conditions

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Slugs are serious pests of oilseed rape (canola) and wheat with most damage occurring just after sowing and seedling emergence. As an alternative to the use of bait pellets, molluscicidal seed treatments have been shown to protect seeds and seedlings from slug damage in laboratory and semi-field experiments. However, protection offered to plants in field trials was diminished and short-lived in comparison with laboratory experiments. To determine why field efficacy was reduced, we grew seedlings under a range of environmental conditions, with appropriate controls, that simulated differences between laboratory and field experiments. We then measured the metaldehyde content of plant seedlings using a previously unpublished methodology described herein, which, unlike previous methods, did not first depolymerize the metaldehyde to acetaldehyde. We confirmed that naturally abundant plant-derived acetaldehyde could not interfere with our measurements of metaldehyde, even if depolymerization took place within the column. Our data suggest that reduced field efficacy results from microbial breakdown and/or loss of active ingredient caused by percolating soil water. Once the seedlings had emerged, neither volatalization nor simulated rainwater reduced the metaldehyde content of seedlings. Our findings will help develop superior seed treatment formulations to overcome these constraints.

KEYWORDS: Metaldehyde; seed treatments; field efficacy; microbial degradation

INTRODUCTION

The development of new active ingredients (AI) for use as pesticides, or development of new formulations of currently available compounds, typically involves a series of preliminary laboratory and/or glasshouse experiments followed by small- and large-scale field experiments. A frequent phenomenon is that the new compounds/formulations do not perform as well under field conditions as the preliminary experiments would suggest (*1*−*5*). Determining the mechanisms by which field performance is compromised may allow development of improved formulations to overcome these constraints. The mechanisms will vary depending on the nature of the novel treatment, target pest, and crop and the environmental conditions under which laboratory and field experiments took place, and, as such, constraints will need to be determined on a case-by-case basis. The current paper describes one such study.

Slugs (Mollusca, Gastropoda) are major pests of oilseed rape (canola) (*Brassica napus* ssp. oleifera) and wheat (*Triticum aestivum* L.) in the U.K. and many parts of Northwest Europe (*6*, *7*). In addition, they are sporadic pests of corn (*Zea mays* L.) and soybean (*Glycine max* L.) in North America (*8*). The most serious damage to wheat occurs just after sowing when slugs hollow out grains resulting in plant death (*6*). For oilseed rape, corn and soybean slugs feed on the newly emerged seedling, and this is particularly serious for oilseed rape as slugs often destroy the apical meristem, thus killing the plant (*7*). In cases of lethal damage, the loss of plant stand often necessitates redrilling the crop. Current control methods rely on baited pellets containing metaldehyde, carbamates, or iron phosphate AIs, which often prove ineffective (*9*). Because the major economic damage done by slugs is to seeds or very young seedlings, an alternative approach would be to use repellent seed treatments. This would offer the potential benefits of reducing the amount of active ingredient applied to the environment, reducing the threat to nontarget organisms, and negating 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 (*10*), and we have previously shown that these compounds can protect oilseed rape from slug damage as effectively as bait pellets under laboratory conditions (*11*). In these cases, the action relies entirely on the strong repellence of metaldehyde taken up on or within the foliage (*12*), as only the foliage was available for slug consumption, and metaldehyde once free in soil depolymerizes into acetaldehyde (*13*). However, in mini-plot field experiments, the protection given by seed treatments to emerged wheat and oilseed rape seedlings was significantly less than that given by baited pellets and was short-lived (*14*). This is

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presumably because the active ingredient had been lost from the emerged seedlings.

If the factors responsible for limiting field efficacy of these seed treatments can be identified, it may be possible to use modern seed-treatment chemistry to overcome these constraints. Factors can be inferred by examining differences between conditions in the successful laboratory experiments (*11*) and unsuccessful field experiment (*14*). In the laboratory experiments of Simms et al. (*11*), seeds were grown in propagators with lids and no additional water was added to the soil, whereas in the field, plots were uncovered and were irrigated daily and exposed to rainfall. Thus, factors that could have possibly reduced field efficacy include loss of AI from leaves or the seed coat due to rain/irrigation or by volatilization from the emerged seedling leaves. In the laboratory experiments, soil was not sterilized but had been dried and stored for several months prior to use, probably causing a reduction in diversity and activity of the microbial community as compared to field soil. Thus, another possible cause of AI loss would be microbial breakdown. This last possibility seems particularly plausible for metaldehyde because it is known to depolymerize into acetaldehyde rapidly as a result of soil microbial activity (*13*).

Here, we describe experiments designed to determine which environmental factors might be responsible for reduced field efficacy. Metaldehyde-treated oilseed rape and wheat seeds were germinated and grown under different environmental conditions, and then the metaldehyde content in emerged seedlings was measured.

Previously published methods for quantifying metaldehyde in plant material involve removing naturally occurring acetaldehyde prior to depolymerization of metaldehyde and derivatization of the resulting acetaldehyde (*15*, *16*). However, due to the low expected concentrations of metaldehyde in harvested seedlings, we decided to use a method in which the intact parent compound was extracted and loaded without derivatization, thus avoiding background interference from plant-derived acetaldehyde, and also reducing the number of steps involved and hence potential loss of metaldehyde. This method involved an acetonitrile extraction and various cleanup steps followed by careful evaporation and analysis using gas chromatography (GC) and flame ionization detection (FID).

MATERIALS AND METHODS

Plant Material and Treatments. Oilseed rape (cv. Pronto) and winter wheat (cv. Savannah) seeds were coated in 20−50 g lots with metaldehyde (Aldrich, Milwaukee) mixed with a commercial seed adhesive, Sepiret (Agrichem, Whittlesey, U.K.). Control seeds had seed adhesive coating only, at the same application rate as treated seeds. Seeds were mixed until an even distribution of the colored 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. (*11*) for oilseed rape of 58 g a.i./kg seed. This rate was then adapted to wheat according to the thousand-grain weight, 6.6 g 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 in a fully randomized design with six replicate trays for each treatment.

Effect of Soil Microorganisms. Soil (sandy loam texture) taken from a local farm was sterilized by autoclaving for 1 h at 121 °C, in small batches (approx 5 kg). The soil was allowed to cool, mixed, and then re-autoclaved a further two times. All seeds were sown in seed trays containing sterilized soil and covered with a further 2 cm of sterilized soil. Soil was moistened with 200 mL of water that had been sterilized by autoclaving and either left sterile or re-inoculated with soil microorganisms from the same soil. Water was re-inoculated with soil microorganisms by vigorously mixing non-sterilized soil with an equal volume of tap water, and then allowing large soil mineral particles to settle for 5 min. Both water treatments were added 3 days before planting, on the day of planting, and then every 2 days until the end of the experiment. Plants (shoots and leaves) were harvested after 14 days, weighed, and stored at −20 °C.

Effect of Percolating Soil Water on Seeds. 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. The effect of percolating soil water was simulated by adding approximately 200 mL of tap water (equivalent to 5.5 mm rain/irrigation) with a small watering can at the time of planting and again 24 h later. Control trays received no additional water. Shoots and leaves were harvested 7 days after sowing, weighed, and stored at −20 °C.

Effect of Simulated Rain on Emerged Seedlings. Seeds were sown in seed trays containing John Innes No.2 compost and covered with a further 2 cm 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 100 mL of tap water either onto the emerged cotyledons/shoots or onto the soil adjacent. Water was added using a wash bottle every day for 5 days. Shoots and leaves were harvested 7 days after sowing, weighed, and stored at −20 °C.

Potential Volatilization of Metaldehyde from Seedlings. Seeds were sown in seed trays containing John Innes No.2 compost and covered with a further 2 cm of John Innes No.2 compost, moistened to field capacity with tap water, and covered with a propagator lid. Treatments to encourage volatilization within the propagators had a continuous flow of air provided by an aquarium pump (1 pump per 4 seed trays) attached with tubing to a 1 cm-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 × 1 mm diameter holes in the top of the propagator lids to allow for gaseous exchange. Shoots and leaves were harvested 14 days after sowing, weighed, and stored at −20 °C.

Metaldehyde Extraction and Analysis. Plant samples were homogenized in a pestle and mortar with liquid nitrogen. A sub-sample of 2.5 g was placed into a Wheaton vial, and 10 mL of acetonitrile was added to each vial and placed in an orbital shaker for 5 min. Oilseed rape and wheat control samples were each spiked with 0.001 g of metaldehyde to determine the rate of recovery. NaCl (1 g) was added to each vial and placed in an orbital shaker for a further 5 min. Samples were passed through a Whatman No. 1 filter paper, followed by an additional 10 mL of acetonitrile. The water phase at the bottom of each vial was then removed and discarded.

To clean the samples, three solid-phase extraction (SPE) tubes were connected in series and preconditioned with 6 mL of acetonitrile per tube, and the eluant was discarded. A further 6 mL of acetonitrile was added to all columns to eliminate air gaps. A C_{18} SPE column (Supelco, 6 mL, 1 g) was connected to the top of an Envicarb SPE column (Supelco, 6 mL, 500 mg) that was in turn connected to the top of an aminopropyl (NH_2) SPE column (Supelco, 6 mL, 1 g). Na_2SO_4 (1 g) was added to the Envicarb column. A 10 mL reservoir was connected to the top of the C_{18} column. The 20 mL sample was added and eluted using a vacuum manifold. Acetonitrile (5 mL) was then added to elute the sample fraction left in the C_{18} column. The C_{18} column was discarded, and 5 mL of 3:1 acetonitrile−toluene was added to the remaining columns to elute the extract. The samples were then evaporated under nitrogen in a water bath at 40 °C to just under 1 mL and brought up to 1 mL with toluene. Metaldehyde standards of between 1 and 200 mg/L in toluene were prepared for calibration. All samples were spiked with 10 μL of the internal standard hexachlorobenzene (10 g/L in toluene).

The metaldehyde samples were analyzed using a GC-FID (Thermoquest, GC8000). Samples (1 μL) were injected onto a capillary column (30 m × 0.32 mm internal diameter × 0.5 μm film thickness, ZB1 Phenomonex). The temperature program started at 90 °C for 1.5 min, increased to 280 °C at 15 °C/min, and was then held at this temperature for 10 min. The quantities of metaldehyde per plant were calculated using the peak area under the metaldehyde peak and quantified using the metaldehyde standard calibration curve. To confirm that there could be no peak interference from plant-derived acetaldehyde

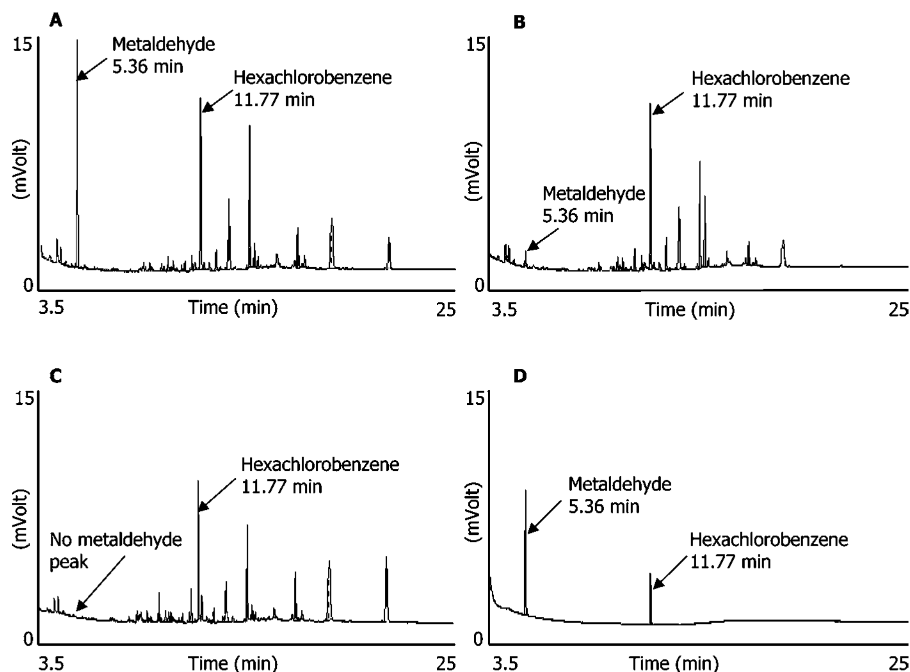

Figure 1. Typical chromatograms of shoot extracts from oilseed rape with (**A**) metaldehyde seed treatment grown in sterilized soil, (**B**) metaldehyde seed treatment grown in sterilized soil re-inoculated with soil microorganisms, (**C**) control seed treatment grown in sterilized soil re-inoculated with soil microorganisms, and (**D**) metaldehyde control (200 mg/L) with internal standard hexachlorobenzene.

with metaldehyde, we prepared a mixed standard of acetaldehyde, metaldehyde, and internal standard at similar concentrations described above, and ran the GC-FID under identical conditions. To test our recovery efficiency, six replicates of ground wheat or oilseed rape were amended with known quantities of metaldehyde equivalent to our experimental treatments. This material was then subjected to extraction and analysis as above.

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

RESULTS AND DISCUSSION

Metaldehyde Analysis. Metaldehyde calibration was carried out for each individual experiment both to confirm 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 5.4 and 11.8 min, respectively, in every sample (**Figure 1a−d**). In addition, there was no interference found between the metaldehyde and hexachlorobenzene peaks and other plant compound peaks. The sensitivity of this method was found to be excellent; metaldehyde residues in plant samples were detected down to a concentration of 1 mg/L (**Figure 2**), and there were no metaldehyde peaks present in any control plant samples (**Figure 1c**). Our extraction efficiencies were estimated to be 94.5% ± s.e. 7.8 (rape) and 70% ± s.e. 14.5 (wheat). It is not possible to be sure whether the metaldehyde that we measured was intact tetramer, or if it had been depolymerized to acetaldehyde within the column. The latter seems quite likely, as metaldehyde starts to depolymerize above 112 °C (*13*) and the column temperature when our peak was measured was >140 °C. However, we can be sure that we will not get interference from plant-derived acetaldehyde, because when a standard was run including both compounds, monomer and tetramer, it gave distinct peaks showing that the two compounds were separated within the column (**Figure 3**).

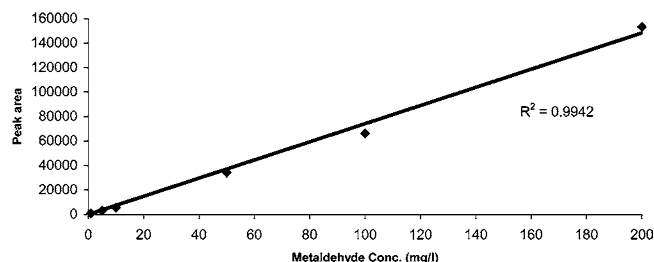

Figure 2. Calibration curve of metaldehyde in toluene analyzed by GC-FID.

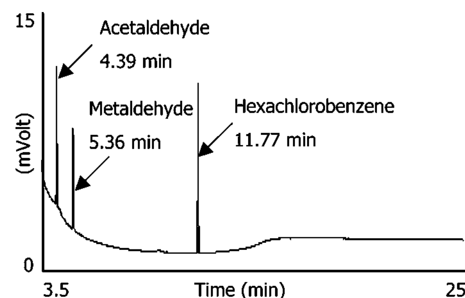

Figure 3. Gas chromatograms of standards of mixed metaldehyde and acetaldehyde with internal standard hexachlorobenzene, showing clear separation of monomer and tetramer.

The relationship between GC-FID peak area and metaldehyde concentration was found to be linear in all calibration experiments, with retention times being consistent among plant, calibration, and blank samples. The three-step cleanup process allowed the majority of plant compounds to be removed, resulting in clear metaldehyde and hexachlorobenzene peaks. This method involves fewer steps than previously reported methods (*15*, *16*), therefore reducing the risk of losing metaldehyde. In addition, by injecting metaldehyde directly into the

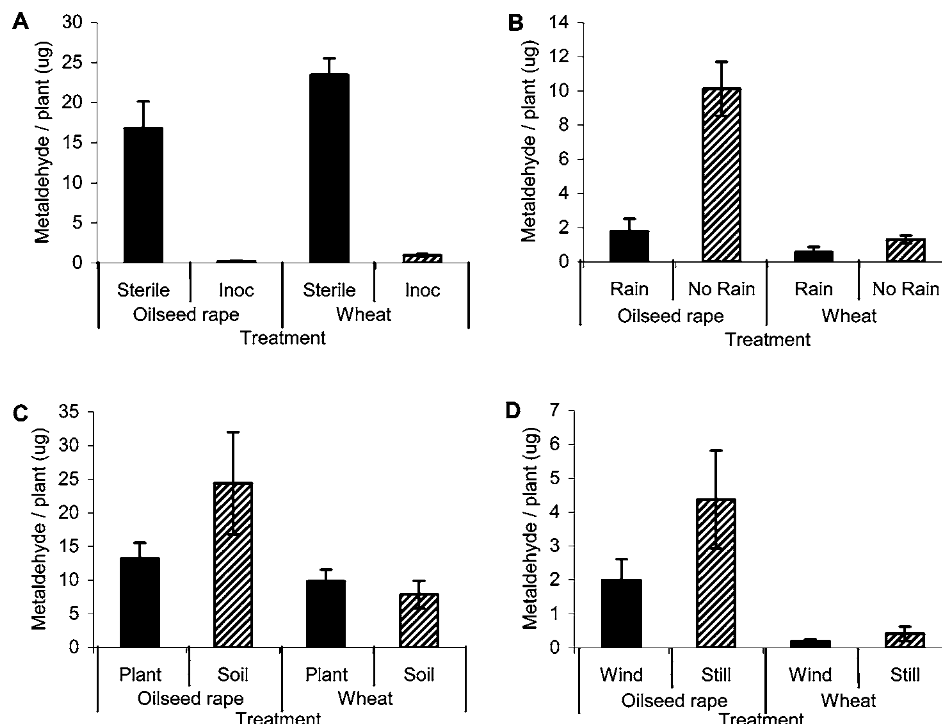

Figure 4. Mean amounts metaldehyde (μg per plant ± standard error) extracted from oilseed rape and wheat seedlings (**A**) grown in sterilized soil or sterilized soil re-inoculated with soil microorganisms, (**B**) with and without simulated rain on seeds, (**C**) with simulated rain applied directly to plants, or to the soil surrounding plants, and (**D**) with and without airflow to encourage volatalization as would be caused by wind under field conditions.

GC rather than derivatized acetaldehye, interference from background acetaldehyde commonly found in plant samples is avoided.

Effects of Environmental Factors on Metaldehyde Uptake. The presence of soil microorganisms significantly ($P < 0.001$) reduced the metaldehyde content of both wheat and oilseed rape seedlings when compared to seedlings grown in sterile soil (**Figure 4a**). The chemical structure of metaldehyde ($C_8H_{16}O_4$) represents an easily utilizable carbon source for microorganisms that rapidly depolymerize the tetramer into acetaldehyde. This rapid breakdown is considered a benefit in metaldehyde baited pellets because any AI that leaches from the pellets will not cause any environmental harm. However, for seed treatment, formulations containing antimicrobial compounds or a barrier layer to exclude microorganisms from the AI would be needed. Rapid microbial breakdown of metaldehyde is consistent with reported miniplot data, which show metaldehyde seed treatments offer short-lived protection from slug damage (*14*).

Percolating soil water at the time of sowing, as would occur following rain or irrigation, significantly reduced the metaldehyde content of emerged oilseed rape seedlings ($P < 0.001$) but not wheat seedlings ($P = 0.074$) (**Figure 4b**). The difference between rape and wheat is likely to result from the difference in surface texture of the two seed types. The rough surface texture of wheat seeds will allow the seed treatment to form a stronger bond with the seed in comparison with the shiny, smooth surface of oilseed rapeseeds.

Once the seedlings had emerged, neither water washing over the seedlings (**Figure 4c**) nor air movement through the seed trays (**Figure 4d**) influenced the amount of metaldehyde in oilseed rape or wheat seedlings. These data suggest that the metaldehyde is taken up by the root and transported systemically within plant tissue. If metaldehyde present on the seed simply adhered to the emerging shoots or cotyledons, we would expect these factors and in particular simulated rainfall to cause a greater loss of AI.

Modern seed-treatment chemistry may be able to overcome field loss of metaldehyde by incorporating antimicrobial compounds or by adding an additional barrier layer to the formulation that would prevent losses caused by microorganisms and percolating water. If commercialized, the benefits of such a product would be two-fold. Economically, avoiding the need for a separate field pass for bait pellet application could reduce the cost to farmers, and, environmentally, better targeting of the molluscicide allows the amount of active ingredient applied per unit area to be reduced.

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