Grain protection with admixed inert dusts (diatomaceous earth): exploring key parameters for improving the efficacy of wet application

Johnson, L.*#, Kumar, C., Hayles, J. Losic, D.*
School of Chemical Engineering, Engineering Building North, The University of Adelaide, Australia, 5005

*Corresponding, Email: lucas.johnson@adelaide.edu.au; dusan.losic@adelaide.edu.au
#Presenting author, Email: lucas.johnson@adelaide.edu.au;

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Abstract

Grain protectants based on inert dusts (such as Diatomaceous Earth, DE) are commonly used to supplement fumigation when protecting stored grain from pesticide resistant insects. While grain is most often treated with DE by wet application, this reduces dust efficacy, thereby requiring higher doses. The aim of this study was to explore the reasons for decreased DE efficacy with wet application. Bioassays were carried out using commercially available DE (Dryacide) with comparative dry and wet treatment of bulk grain (Katana hard winter wheat) at 30°C and 55% r.h. using adult *Rhyzopertha dominica* of mixed sex and age. Our results confirmed previous studies, showing significantly lower efficacy of wet treatment. To explain this finding, tests were first carried out to see if wet application of DE under these conditions (1 ml / 100 g grain) raises grain moisture compared to dry application, thereby facilitating insect survival by countering dehydration. This test showed little difference in grain moisture, ruling this out as a contributing factor. Likewise, surface application tests comparing the efficacy of dry and wetted DE against *R. dominica* showed no statistically significant difference in mortality (REGWQ test, $p < 0.05$), indicating that the presence of residual water on the DE particles following wet application does not reduce efficacy. Optical microscopy however, showed that wet application of DE on grain leads to substantially less uptake of DE particles onto insects compared to dry application. This observation was supported by SEM studies. Several possible causes of this low DE uptake were investigated. Aggregation of DE particles when applied onto grain wet (into clumps too heavy to be picked up by insects) was ruled out by SEM, which showed no difference in the coverage and dispersal of DE particles on grain when applied either wet or dry. However, evidence of stronger dust adhesion onto grain when applied wet (thereby restricting dust uptake onto insects) was found, as smaller quantities of unadhered DE were measured compared to grain treated with dry DE. This stronger adhesion of DE particles on grain with wet application was explained in terms of a capillary bridging effect, which was supported by increased uptake of dusts by insects and improved efficacy of wet delivery (56.0 % vs. 13.3 % after 14 days) with the addition of Cetyltrimethylammonium bromide surfactant (which weakens capillary forces).

Keywords: inert dust, wet application, grain protection, *Rhyzopertha dominica*, surfactant

1. Introduction

One of the main challenges facing the global grains industry is the protection of stored commodities from insect pests that have developed resistance to commonly used fumigants (such as phosphine or methyl bromide), while also abiding by strict limitations on pesticide residues (Subramanyam and Hagstrum, 1995).

One common approach to address this problem is to combine fumigation with the application of inert dusts materials that are usually comprised of amorphous silica particles derived mainly from natural (diatomaceous earth, DE) or synthetic (aerogel) sources. Inert dusts kill insects by adsorbing cuticular lipids that are responsible for water retention, leading to

desiccation (Ebeling, 1971). Owing to this physical mode of action, the probability of insect resistance emerging is low (Quarles and Winn, 1996). Inert dusts also offer long term effectiveness, are safe for consumption, and do not adversely affect grain quality (Desmarchelier and Dines, 1987; Korunic et al., 1996).

DE is typically applied to grain either as a capping treatment, where the dust is admixed with the uppermost layer of stored grain (usually employed by bulk handlers owing to the detrimental effect of inert dusts on bulk density and grain flow properties (Bridgemann, 1999) (Quarles, 1992)) or as an admixture with bulk grain (when the presence and effect of inert dusts is less problematic (Golob, 1997)). Most often, treatments of DE are applied by spraying as an aqueous suspension, in an effort to minimise the generation of nuisance airborne dust. However, the efficacy of inert dusts is substantially diminished with wet application, necessitating much larger application rates (Maceljski and Korunic, 1972; McLaughlin, 1994).

The reasons for this reduction in efficacy of wet applied DE are poorly understood, with no studies to date being dedicated to investigating the underlying causes. Therefore, the aim of this study is to examine the factors responsible for this reduction in efficacy, in an effort to inform new strategies for improving the wet delivery of DE. To achive this, we performed comparative bioassays using dry and wet application of DE for bulk treatment of grain, as well as surface tests to examine if residual water on DE particles following wet application is a factor. *R. dominica* was selected as the test species as it is amongst the most resistant to inert dust treatment. (Fields and Korunic, 2000). These studies were supported by measurements of grain moisture content and dust adhesion to grain, as well as optical and SEM characterization of DE particles on the surface of grain and insects, in order to better understand their adhesion on the surface and influence on insectidical properties.

2. Materials and Methods

2.1. Chemicals

A commercially available diatomaceous earth (DE) based grain protectant (Dryacide, supplied by Entosol Australia Pty Ltd) was selected as a model of DE material for use in this study. Cetyltrimethylammonium bromide (CTAB, ≥ 98%) was obtained from Sigma-Aldrich and used when received.

2.2. Grain

Katana hard winter wheat (Flinders Ranges Premium Grain) was used in this study. Prior to use, grain is cleaned by sieving (2 mm mesh), with larger dockage removed by hand. The grain is then sterilized at 60°C for 3 hours, and then pre-conditioned at 30°C / 55% r.h. for two weeks to achieve moisture content of 11.5 ± 0.3% (checked using a HE-50 grain moisture meter).

2.3. Insects

Adult *R. dominica* (F.) reared in laboratory cultures were selected for use in this study. Insects were reared on 300 g of clean, preconditioned Katana hard winter wheat (prepared as described above) at 30°C and 55% r.h. Adult insects are obtained from the cultures using a grain sieve (2 mm mesh), followed by a fine mesh sieve (0.5 mm mesh) to remove frass.

2.4. Bulk grain bioassays comparing dry and wet application

Bulk grain bioassays were carried out to compare the efficacy of wet and dry application of DE when admixed with hard winter wheat. Five replicates of each treatment, as well as a negative control to account for natural mortality, were tested per experiment. Equal

concentrations of DE (1,000 ppm) were used for all wet and dry treatments of grain. For dry DE application on bulk grain, 100 g of grain is evenly divided into 5 x 20 g replicates (stored in 60 ml glass jars), and DE (20 mg per replicate) was then added to give a concentration of 1,000 ppm in grain. For wet DE application, 100 g of grain (enough for all 5 replicates) is spread out as a monolayer in a shallow dish, and 1 ml of a sonicated suspension of DE (100 mg in 1 ml water) was sprayed evenly over the grain (< 15 sec) using a gravity fed compressed air sprayer, to give a total concentration of 1,000 ppm in grain. After spraying, the grain is evenly subdivided into 5 replicates and added to jars.

Following treatment, the grain jars were shaken/rotated for 1 min to evenly disperse the DE on the grain. The jar lids were then replaced with muslin cloth secured with rubber bands to provide ventilation. The jars were stored in a controlled environment cabinet at 30°C/55% r.h. for 3 days to allow the moisture content of wet applied samples to re-equilibrate. Thirty adult *R. dominica* of mixed sex and age were then randomly selected from laboratory cultures and added to each replicate jar. The jars were stored at 30°C / 55% r.h. for the duration of the bioassay. Counts of live and dead insects were carried out after 7 and 14 days by sieving insects from the grain using a grain sieve (2 mm mesh). Insect mortality was assessed by probing with tweezers. Insects that did not move were counted as dead.

2.5. Bulk grain bioassays comparing wet application from water and surfactant solution

These experiments were carried out in the same manner as bulk grain bioassays comparing wet and dry application of DE on bulk grain. Samples of DE in water were prepared by dispersing 100 mg of DE in 6 ml of water, and applying this whole volume to 100 g of grain. In the case of DE applied from a surfactant solution (CTAB, 4 mM), the surfactant (8.75 mg) is dissolved in 6 ml water by sonication prior to adding 100 mg of dust, and the total volume of prepared suspension is applied to 100 g of grain. Note that a more dilute solution is used in these experiments (6 ml vs. 1 ml) to avoid excessive foaming during spraying of the surfactant solution.

2.6. Monitoring of grain moisture content vs. time

The moisture content over time of wet treated grain was measured by spraying 100 g of grain with aqueous solution (without added dust) and subdividing into 5 jars sealed with muslin cloth as described for bulk grain bioassays. Equivalent samples of untreated grain were also prepared for comparison. The jars were stored in a controlled environment cabinet at 30°C / 55% r.h., with one jar for each treatment being removed for moisture content testing after 3, 7, 10, 14 and 17 days. Measurement of grain moisture content in each jar was carried out in duplicate using a HE-50 grain moisture meter.

2.7. Effect of residual water on DE efficacy

To investigate the influence of residual water on the efficacy of DE particles following wet application on grain and drying, surface application bioassays were carried out, which were designed to emulate wet application on grain while excluding confounding factors (such as dust aggregation and adhesion to test surfaces). For each treatment, 3 replicates were prepared.

Plastic petri dishes (84 mm diameter), serving as test surfaces, were first modified by cutting a hole in the lid, which was covered by tissue paper secured with glue. This provided ventilation while preventing insect escape. DE containing residual water (emulating wet application) was prepared by dispersing the DE in water (100 mg/ml), and then leaving to dry in a glass vial under the bioassay conditions (30°C / 55% r.h.) for 3 days. The solids were then ground up using a spatula, and 5.6 mg (1 g/m^2) of the resulting powder was added to

each dish and spread around evenly using a fine brush. For comparison, dishes treated with dry DE were also prepared. A negative control (clean dishes) was also included to account for natural mortality. 20 adult *R. dominica* of mixed sex and age were then randomly selected from laboratory cultures and added to each dish. Mortality was assessed each day in the same manner as with bulk grain bioassays.

2.8. Light and Scanning Electron Microscopy

Optical microscopy was conducted using a Nikon DXM1200 camera attached to a Leica MZ16 microscope (equipped with a 5x lens). Images were captured using Adobe Lightroom, and a composite image of photos taken at different focal planes was compiled using Zerene Stacker. Electron microscopy was conducted using a Phillips XL30 Field Emission Scanning Electron Microscope (SEM). Images were collected using the secondary electron detector at an accelerating voltage of 5 kV and a spot size of 3. The working distance was 10 mm. Prior to analysis, samples were mounted onto SEM stubs using double sided tape, and then sputter coated with a 30 nm thick layer of platinum to minimize charging effects.

2.9. The adhesion of DE particles to grain

Retention or binding of DE particles on the grain surface was assessed using the method described by La Hue (La Hue, 1972) using both wet and dry application. For each treatment, five replicates of clean, preconditioned grain (200 g per replicate) were first prepared as described for bulk grain bioassays, then stored under bioassay conditions (30°C / 55% r.h.) for 17 days (total bioassay time). Five replicates of untreated grain are also prepared. The retention of DE particles in each replicate was then determined by transferring a small portion of the grain (less than that needed to form a monolayer) to a grain sieve (2 mm mesh), and gently sieving the grain for 15 sec. Care is taken to prevent friction between grains and generation of airborne dust. The grain is then removed, and the process repeated with the remaining portions of grain. Dust was collected from the sieve pan using a brush, and then weighed to determine the proportion of free, un-adhered dust.

2.10. Data analysis

Proportional data among the treatments obtained from bioassays (percent mortality values) were first corrected for control mortality using Abbott's correction where necessary. (Abbott, 1925) Data was then transformed using an arcsine square root transformation and analysed using an analysis of variance (ANOVA), with treatment as the main effect. The Ryan-Einot-Gabriel-Welch q test (REGWQ) at $p < 0.05$ was used to identify significant differences amongst the treatments. Dust adhesion (percentage of free dust) was analysed as above, but without arcsine transformation. In all cases, non-transformed data are presented, with error bars representing sample standard deviation.

3. Results and Discussion

3.1. Bulk grain bioassays comparing wet and dry application

Bulk grain bioassays were first conducted in order to establish if the insecticidal efficacy of DE when applied to grain wet is reduced compared to dry application under the selected experimental conditions. The results of this bioassay are shown in Table 1. As can be seen, dry application of DE on bulk grain is substantially more effective than wet after 14 days. Furthermore, wet application at these doses (1000 ppm) yields no statistically significant increase in mortality compared to untreated controls. This confirms the results of previous studies that showed reduced efficacy of DE when applied on grain wet (Maceljski and Korunic, 1972; McLaughlin, 1994).

Table 1 Efficacy (mean ± standard deviation) of wet and dry application of DE for bulk treatment of wheat against *R. dominica* (30 insects/20 g grain per replicate, 5 replicates)[a].

Treatment	Mortality (%)	
	Day 7	Day 14
Negative Control	0.0 ± 2.7 *a*	0.0 ± 2.7 *a*
Wet Application	0.0 ± 4.5 *a*	13.3 ± 22.6 *a*
Dry Application	99.0 ± 2.2 *b*	99.0 ± 2.2 *b*

[a]Statistically significant means (REGWQ test, $p < 0.05$) are denoted by different letters in the table. Untreated grain is used as a negative control.

3.2. Monitoring of grain moisture content vs. time

One potential reason for this difference in efficacy is that wet application can raise the moisture content of the treated grain, thereby inhibiting the dehydrating effects of the DE by providing additional moisture to the insects from feeding. To this end, we monitored the moisture of grain for both dry and wet applications, which is presented in Figure 1. The graph shows that moisture of wet treated grain was slightly higher over the course of the assay, but the difference is not significant (< 0.2% r.h.) This indicates that the observed large differences in insect mortality are not related to increased grain moisture content following wet application (Aldryhim, 1993; Desmarchelier and Dines, 1987).

3.3.Effect of residual water on DE efficacy

Another possible factor that may cause the observed decrease in DE efficacy with wet application is the presence of residual water on the surface of the wet applied DE particles, which could interfere with the adsorption of insect epicuticle waxes. To study this, surface bioassays were carried out on DE that has been treated to introduce water onto the particle surfaces (emulating the DE present on grain following wet application in bulk bioassay experiments), and compared with tests using dry DE. A discriminatory dose of 1 g/m^2 was applied, as this is the minimum recommended dose for dry surface treatments with dryacide (so any significant reductions in efficacy due to residual water should be noticeable). The results from this surface treatment test are presented in Table 2, which shows that the presence of residual water on the DE particles has no statistically significant effect on efficacy, with both treatments yielding essentially complete mortality of insects after 1 day of exposure. These results indicate that of the presence of residual water on the DE does not play a role in reducing the efficacy when wet applied onto bulk grain, in line with the results of related studies on other DE materials (El-Awami and Dent, 1996).

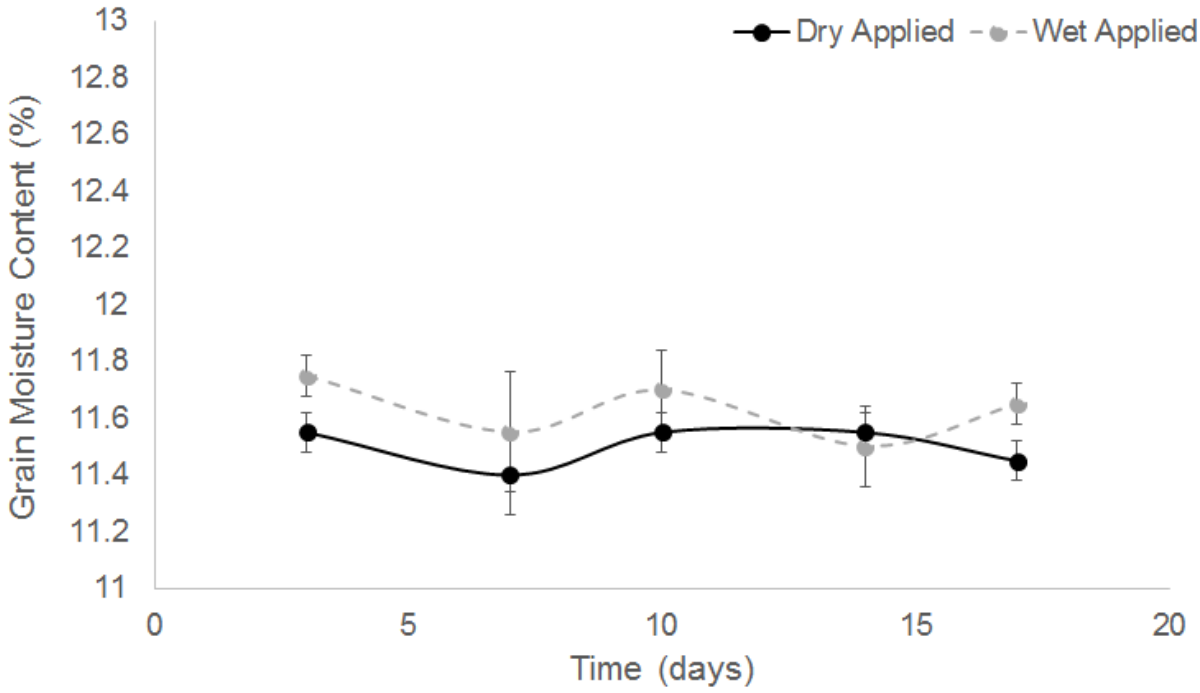

Figure 1 Moisture content of preconditioned grain following wet and dry application of DE dust in bulk wheat. Wet application yields a minor increase in grain moisture content (< 0.2% r.h.) compared to dry application.

Table 2 Surface bioassay results (mean ± standard deviation) comparing the effect of DE with residual surface water (emulating wet application on wheat) and dry DE against *R. dominica* (20 insects per replicate, 3 replicates)[a].

Treatment	Mortality (%)	
	Day 7	Day 14
Negative Control	0.0 ± 0.0 *a*	0.0 ± 0.0 *a*
Wetted Dust	98.3 ± 2.9 *b*	100.0 ± 0.0 *b*
Dry Dust	100.0 ± 0.0 *b*	100.0 ± 0.0 *b*

[a]Statistically significant means (REGWQ test, $p < 0.05$) are denoted by different letters in the table. Clean petri dishes are used as the negative control.

3.4. Examination of insects

In order to explain the observed difference in efficacy of wet and dry application of DE on bulk grain, insects obtained from bulk grain bioassays were examined using light microscopy and SEM, to obtain more information on the adsorption of DE particles on the insects. A selected series of light microscopy images showing the ventral surface of the insects' bodies is presented in Figure 2. Insects exposed to both dry and wet treatments demonstrate substantial differences in the level of dust coverage on their ventral surfaces. The insects exposed to grain treated with dry dusts having significantly greater dust coverage on their surface (Fig. 2C) compared to insects exposed to wet applied dust (Fig. 2b).

Figure 2 Optical microscope images of *R. dominica* from bulk grain bioassays showing the concentration of adsorbed DE particles: (A) untreated control (B) wet applied DE on bulk grain (C) dry applied DE bulk grain.

SEM images of these insects (Fig. 3) also show that there is a substantially higher density of DE particles on insects exposed to grain treated with dry applied DE, compared to wet applications of DE.

From these observations, it may be inferred that the degree of DE taken up by the insects is correlated with their efficacy, supporting observations made in other studies (Aldryhim, 1993; White and Loschiavo, 1989; McLaughlin, 1994). Logically, this implies that when dust is wet applied to grain, there is some subsequent restriction on the uptake of dusts onto the insects.

3.5. Causes of reduced DE uptake by insects

To further explain the reasons for reduced adsorption of DE particles onto the insects and the difference in their performance, we performed SEM examination of DE particles on the surface of treated grain. The amount of adhered of DE particles on grain surface is expected to have a significant impact on dust efficacy; providing a higher possibility to be taken by insects during their movement. Another reason was to confirm the possible aggregation of DE particles as suggested by Fields and Korunic (2000), who suggested that as silica dust particles clump together when wet, large aggregates may form during wet application and subsequent drying, which would be too heavy to remain stuck to the insects (Fields and Korunic, 2000). A series of SEM images showing the surface of grain with adsorbed DE particle from dry and wet application on bulk grain is presented in Figure 4. These SEM images suprisingly confirm no evidence of any noticeable difference in dust aggregation between wet and dry applied samples under these experimental conditions, with even coverages of dust over the grain surface. This result indicates that the difference in efficacy of wet and dry applied DE on bulk grain is not related to differences in DE coverage on the treated grain. Furthermore, it suggests that aggregation of DE into heavy clumps does not play a role.

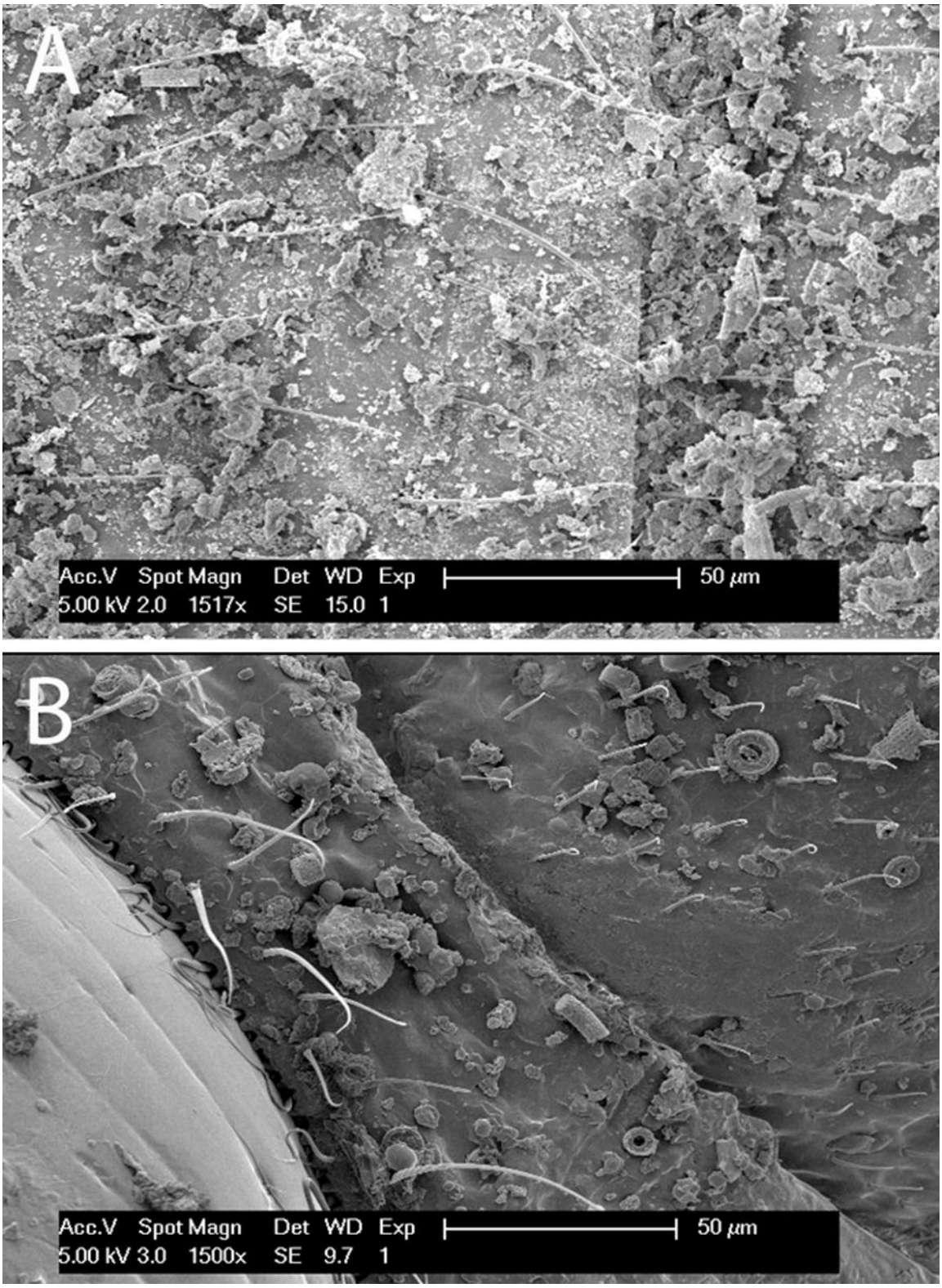

Figure 3 SEM images of the bodies of insects taken from bulk grain bioassays: (A) dry applied DE and (B) wet applied DE, showing significant differences in particle density.

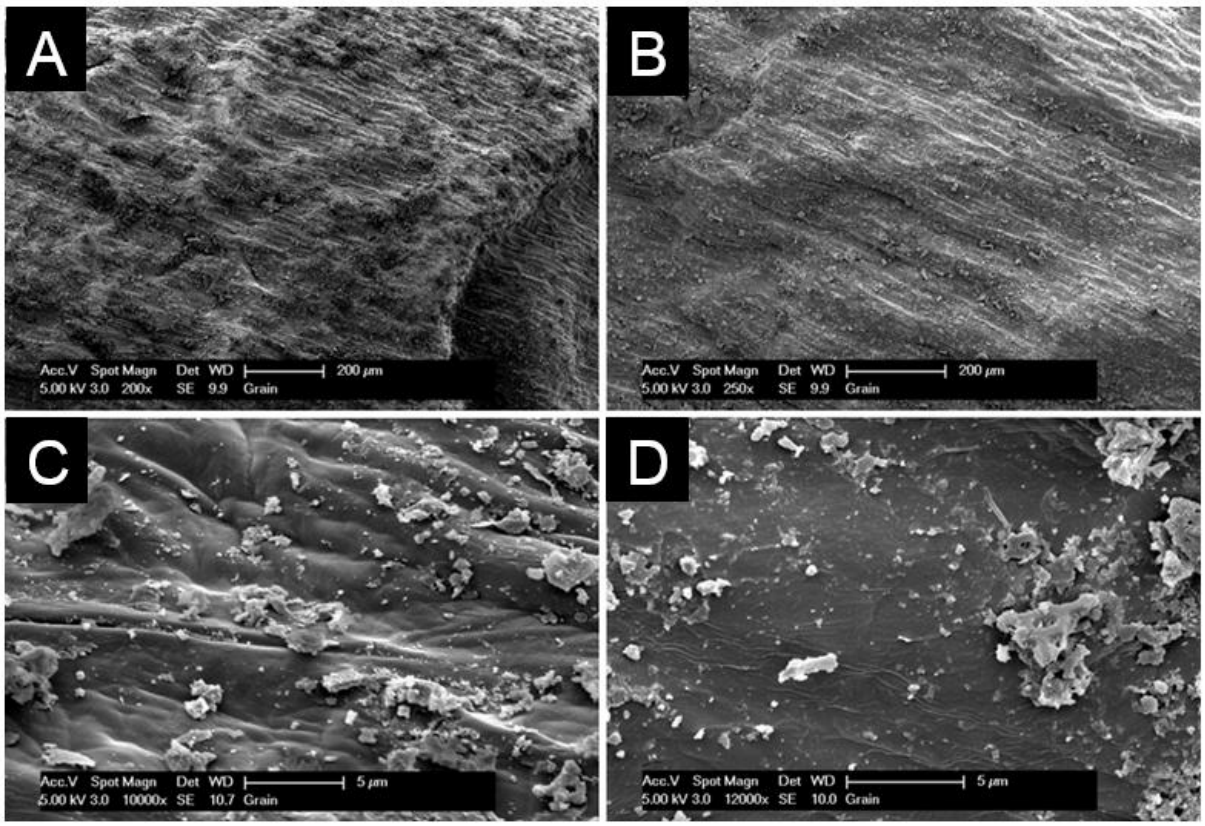

Figure 4 SEM images of wheat grain treated with (A, C) dry applied DE and (B, D) wet applied DE, showing no difference in dust aggregation or coverage between these treatments.

3.6. The adhesion of DE particles to grain

Another possible explanation for the decreased uptake of DE onto insects when applied wet onto bulk grain, is that the adhesion of dust to the grains is stronger when applied wet, thereby restricting the uptake of these dusts by the insects. To test this hypothesis, we performed an experiment to determine the adherence of dust to the grain from both wet and dry application, by weighing the quantity of loosely bound dust collected by sieving (La Hue, 1972). The results of this test (Table 3) support our explanation, as significantly smaller quantities of freely available dust are present on wet applied samples, indicating an overall stronger level of dust adhesion to the grain (and consequently that more dust is available for pickup by the insects). This result is in agreement with a related study by Aldryhim, who previously reported a correlation between the efficacy of dry applied DE and the extent of dust adhesion to different grain commodities (Aldryhim, 1993).

Table 3 Quantities of free dust (mean ± standard deviation) present in samples of bulk wheat following wet or dry application of 1000 ppm of DE (200 mg / 200 g grain per replicate, 5 replicates)[a].

Treatment	Free dust (%) [b]
Untreated	0.53 ± 0.49 *a*
Wet Application	1.92 ± 0.44 *b*
Dry Application	7.12 ± 1.30 *c*

[a]Samples are stored at 30°C / 55 % r.h. for 17 days prior to analysis.
[b]Statistically significant means (REGWQ test, $p < 0.05$) are denoted by different letters.

3.7. Addition of surfactants

One potential cause of stronger dust adhesion to the grain following wet application, is the formation of capillary bridges between the DE particles and the grain surface due to residual surface water (in much the same way as wet sand) (Israelachvili, 2011). A visual representation of this is given in Figure 5. Note that while water can condense on the surface of dry applied DE, the contribution to capillary bridging would be limited by comparison (under these test conditions), due to hysteresis between capillary condensation and evaporation (much more water remains on the DE particles following wet application, compared to the amount of water adsorbed from the atmosphere onto dry DE particles) (Feiler et al., 2007).

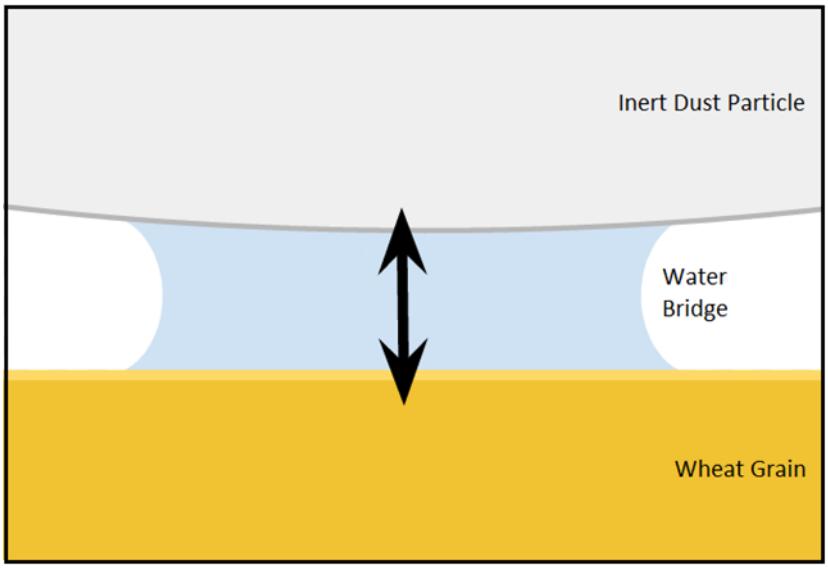

Figure 5 Diagram depicting a capillary bridge due to condensed water in the narrow space between a DE particle and the surface of grain.

Given that the adhesion force due to capillary bridging comes about largely from the surface tension of water, to prove this hypothesis we added surfactant into the DE formulation as this would act to reduce the surface tension of water, and would therefore reduce the strength of dust adhesion. The results of a bulk grain bioassay comparing dusts applied as a wet suspension in water and in a surfactant solution (CTAB, 4 mM) are presented in Table 4.

Table 4 Efficacy (mean ± standard deviation) of wet applied DE (from water or CTAB solution, 4 mM) on bulk wheat against *R. dominica* (30 insects per replicate, 5 replicates)[a].

Treatment	Mortality (%)	
	Day 7	Day 14
Control (CTAB solution)	0.0 ± 0.0 *a*	0.0 ± 0.0 *a*
Wet Application (water)	0.0 ± 4.5 *a*	13.3 ± 22.6 *a*
Wet Application (CTAB solution)	37.0 ± 27.8 *b*	56.0 ± 20.4 *b*

[a]Statistically significant means (REGWQ test, $p < 0.05$) are denoted by different letters in the table. Control samples as shown are comprised of grain treated with CTAB solution only (4 mM).

As can be seen, a statistically significant increase in insect mortality is observed after adding surfactant, although it remains lower than that previously measured for dry dust application. Importantly, a control sample of grain treated with the same dose and concentration of surfactant solution alone gives no insect mortality, showing that this increase is not due to any additional toxic effects from this additive.

The results of grain moisture content measurements for grain treated with DE from water and from CTAB solution is presented in Figure 6. As can be seen no significant differences in grain moisture content were measured, indicating that differences in water content are not a factor in the observed differences in efficacy.

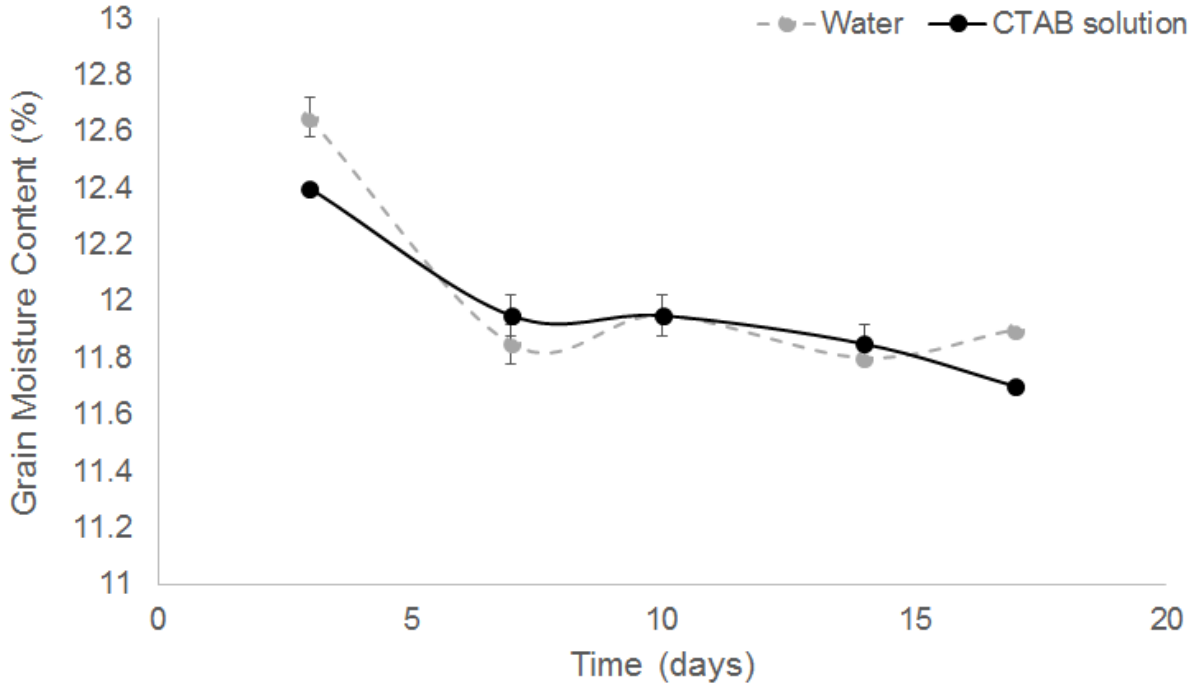

Figure 6 Moisture content of preconditioned wheat following wet application of DE from water (6 ml / 100 g grain) and CTAB solution (4 mM, 6 ml / 100 g grain).

Optical microscopy of the insects from these bioassays was also carried out to see if the use of surfactant has had an effect on the uptake of DE particles by the insects (Fig. 7). Clearly, there is a noticeable difference in dust uptake, with a higher density of DE particles on insects exposed to grain treated with DE from CTAB solution. This further supports the notion that dust adhesion (due to capillary bridging) is a decisive factor in the efficacy of wet applied inert dusts.

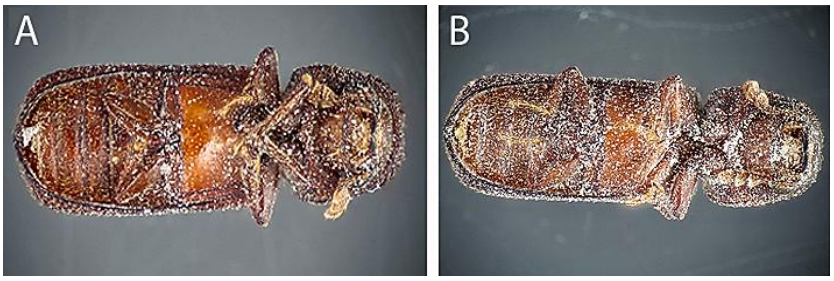

Figure 7 Optical microscope images of insects from bulk grain bioassays: (A) wet dust application from water (6 ml / 100 g grain) (B) wet dust application from CTAB solution (6 ml / 100 g grain, 4 mM).

4. Conclusions

In this study, the factors responsible for the reduced efficacy of DE inert dusts when applied wet were investigated. After controlling for effects on grain moisture content, it was found that wet delivery had no observable effect on the intrinsic efficacy of the dusts, but did result in less dust uptake by exposed insects. This was linked to stronger adhesion of the dusts to the grain, which was hypothesized to result from capillary bridging by residual surface water after drying. Evidence for this mechanism was presented through the effect of surfactants, which were shown to improve dust uptake by the insects. A concurrent increase in insect mortality was also observed, indicating that the use of surfactants is a promising approach towards improving wet delivery of inert dusts. Additional research is needed to provide more evidence for the proposed adhesion mechanism, such as studies by atomic force microscopy. Further work is also needed to explore and optimise the demonstrated beneficial effect of surfactants on the wet application of inert dusts on grain.

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