PROJECT REPORT No. 249

THE EFFICACY OF ALTERNATIVE
COMPOUNDS TO
ORGANOPHOSPHORUS PESTICIDES
FOR THE CONTROL OF
STORAGE MITE PESTS

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by

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ABSTRACT

Aims

This project aimed to find alternative compounds to organophosphorus (OP) pesticides for the control of storage mite pests from readily-available commercial formulations in a number categories, based on their activity; growth regulators, pyrethroids, diatomaceous earths, compounds of biological and novel control agents and botanicals. The initial aim was to screen 21 compounds for activity against mites, and then narrow the focus by ensuring that the most promising six were effective against stored product insects as well as mites under U.K. conditions. The final aim was to test the most successful candidates against mixed infestations on a larger scale culminating with a farm-scale trial and to ensure they were equally efficacious on rapeseed.

Conclusions

Most of the compounds assessed were very effective as protectants, inhibiting the development of pest populations. However, some had limitations in treating existing infestations, either because they worked rather slowly or because they were less effective against certain stages or species. The most successful were the chitin synthesis inhibitor, flufenoxuron, the plant extract, azadirachtin and the diatomaceous earth, 'Protect-it'. This last-named compound was successfully applied as a top dressing against an existing heavy mite infestation.

Implications

These experiments have enabled the benefits and limitations of the available alternatives to be clearly identified. They are effective in inhibiting the development of infestation but have limitations if a broad spectrum of pests require to be killed quickly. Their use may therefore lie in an integrated approach when applied as fabric treatments or as a top dressing, in conjunction with cooling and drying. However, if used as infestation treatments, for instance during loading at ports, careful selection of the compound may be required depending on the pest species present. The availability of any new compounds as grain protectants will be largely determined by the registration process, the small U.K. market and the cost of registration. Of the compounds tested, the nearest to the market are the diatomaceous earths which have the advantage of a physical mode of action and which have been extensively tested under U.K. conditions against insects and mites.

SUMMARY

Mites are very common pests of stored cereals and oilseed in the U. K. As well as the damage they cause through feeding, heavy infestations can taint grain making it unpalatable to livestock and unsuitable for milling. They also present a serious health risk through the development of allergies and have been implicated in the transmission of micro-organisms. In the U. K., stored grain protection in the past has relied heavily on bulk admixture with contact pesticides. Today only organophosphorus (OP) pesticides are approved for this use. None of these pesticides are particularly effective against field strains of mites and resistant populations have been detected. As well as concerns regarding the development of resistant pests, the costs and consumer resistance to toxic chemicals in food, have led to increasing pressures for a reduction in pesticide use. Cooling and drying effectively prevent infestations developing in the bulk, but the surface layers remain vulnerable to infestation and may require a pesticide treatment.

The aim of this project was to identify potential alternatives to OPs and to investigate their efficacy against storage mite pests. A literature review identified potential candidates and from this, 21 compounds were selected for initial screening. Selection was based on efficacy against other acarine pests and the availability of commercial formulations. The compounds included 4 or 5 representatives of diatomaceous earths (DEs), insect growth regulators (IGR), novel compounds, pyrethroids, biological control agents and botanicals. Efficacy was assessed, admixing each compound with wheat, against mixed stages of susceptible strains of *Acarus siro* L., *Lepidoglyphus destructor* (Schrank) and *Tyrophagus putrescentiae* (Schrank) at 15^{o}C and 75% relative humidity. Population inhibition was assessed after periods long enough to include the passing of at least 2 generations under control conditions. The most effective compounds from each group were found to be the DE 'Protect-it', the IGR, flufenoxuron, the novel compounds, sodium polyborate and benzyl benzoate, the pyrethroid, bifenthrin and the botanical, azadirachtin.

These six compounds were then further assessed against OP susceptible and resistant strains of mites and insects (*A. siro*, *L. destructor*, *T. putrescentiae*, *Sitophilus granarius* (L.), *Oryzaephilus surinamensis* (L.) and *Rhyzopertha dominica* (Fabricius). Four doses of each compound were applied to wheat which was stored at 10^{o}C or 25^{o}C at 75% r.h. and then sampled 1 day, 4 weeks and 12 weeks after treatment. Efficacy was evaluated by the effect on adult beetle mortality and on the ability of the compounds to inhibit development of the insect and mite populations at the two temperatures. The results indicated the range of tolerances of the different pest species and strains to the different compounds, with *Sitophilus granarius*, appearing the most tolerant species. In general, lower doses of the pesticides were required to inhibit the mite populations when exposed at 10^{o}C compared to 25^{o}C and adult insect mortality was higher at 25^{o}C than at 10^{o}C. The chitin synthesis inhibitor, flufenoxuron, and the plant extract, azadirachtin, were considered to be worthy of further investigation.

In the next stage of testing, batches of wheat were treated with flufenoxuron and azadirachtin, put into small bins, infested with mixed populations of mites and insects (*A. siro*, *L. destructor*, *S. granarius* and *O. surinamensis*) and stored at 15°C and 80% rh for 26 weeks. After a 2-week assessment, efficacy was evaluated at 4 weekly intervals by the ability of the compounds to inhibit the development and survival of the pest species. No *A. siro* or *L. destructor* were detected in the samples treated with flufenoxuron at or after 6 and 10 weeks respectively. In the azadirachtin-treated samples no mites were detected from week 2 up to week 26 when *A. siro* were found in one of the bins. However, no *L. destructor* were detected in any of the untreated samples after week 14 which may have been due to competition from the faster breeding *A. siro*. Adult beetles were caught in the traps throughout the experiment with lower numbers of *O. surinamensis* trapped than *S. granarius*. The treatments reduced the numbers of *S. granarius* by approximately 75 %.

In addition to wheat, it was also considered important to assess effectiveness on oilseed rape, as mites are common pests on this commodity, and from earlier this year, phosphine is the only pesticide approved for use. The efficacy of flufenoxuron, azadirachtin and the diatomaceous earth 'Protect-it' were assessed, when admixed with oilseed rape, against susceptible strains of *A. siro*, *L. destructor* and *T. putrescentiae*. Population inhibition was evaluated in experimental conditions of 15°C and 80% rh after the passing of at least one generation. 'Protect-it' appeared the most effective treatment against all the mite species with a dose of 3 g kg^{-1} inhibiting the populations by > 96 %. Flufenoxuron was highly effective at all doses against *L. destructor*, with azadirachtin appearing the least effective against all the species at the doses assessed.

Previous MAFF-funded research had found diatomaceous earths to be effective against insects and mites. However, information was lacking as to their efficacy against an existing mite infestation. Therefore in the final phase of this project the diatomaceous earth 'Protect-it' was assessed in a farm scale experiment. The surface of a mite-infested 20 t bin was divided into quadrants. Two were treated with 1g/kg of 'Protect-it' and two were left untreated. The mite population at the surface of the treated quadrants fell to near zero within a fortnight, although the effect at a depth of 0.25m was much less marked.

This project has demonstrated that there are potential alternative compounds to OPs that have proved effective against storage mite and insect pests. Most of the compounds were very effective as protectants, inhibiting the development of pest populations. However, some did have limitations in treating existing infestations, either because they worked rather slowly or because they were less effective against certain stages or species. They may prove suitable as admixture treatments to protect unventilated grain during prolonged storage or as a surface treatment in conjunction with cooling and drying, to combat the peculiar

problems of the British maritime climate. However, if used as infestation treatments, for instance during loading at ports, careful selection of the compound may be required depending on the infestation present.

The availability of any new compounds as grain protectants will be largely determined by the registration process, the small U.K. market and the cost of registration. Of the compounds tested, the nearest to the market are the diatomaceous earths which are registered for storage use in other countries, have the advantage of a physical mode of action and have been tested under U.K. conditions against insects and mites.

The future of any potential replacements to OPs lies in their ability to be incorporated into an integrated pest management programme. A targeted approach may be required where knowledge of the environment and pest biology are important factors in deciding the appropriate control measure, with a move away from sole reliance on one method.

BACKGROUND INFORMATION

Mites are very common pests of stored grain and oilseeds in the U.K. A survey of commercial grain stores during 1988/89 and oilseed stores in 1995, detected the presence of mites in 81.3 % and 89 % of the stores

respectively (Lynch et al., 1991; Prickett, 1997). The most common species in the grain stores were *Acarus siro, Lepidoglyphus destructor, Tyrophagus longior* and *Tyrophagus putrescentiae*, identified in 59%, 51.2%, 15.9% and 12% of stores respectively (Lynch et al., 1991). In the oilseed stores the predominant species were *Acarus siro* (67%), *Lepidoglyphus destructor* (37%), *Tyrophagus putrescentiae* (31%) and *Tyrophagus longior* (19%) (Prickett, 1997).

Some mites feed on the germ of cereals (Solomon, 1946) and hollow out rapeseed leaving only the seed coat (Anon, 1982), thus destroying germination capacity and decreasing the value for seed and malting. Heavy infestations can have a strong smell which taints the grain, making it unpalatable to livestock and unsuitable for milling (Wilkin and Stables, 1985). Research has found reduced growth rates in pigs fed a diet heavily infested with mites (Wilkin and Thind, 1984). Mites in finished cereal products are also a cause of concern. A recent investigation found 22% of cereal based products contained at least one mite (Anon, 1996). Infestations may arise as a result of mites entering the product during any stage in its manufacture, transport and storage (Anon, 1996). Mites are also highly allergenic and can pose a serious health risk to workers involved in grain and flour handling (Stengard Hansen et al., 1996). They have also been implicated in the transmission of micro-organisms, especially fungi, with recent suggestions of a role as vectors of prions (Sigrianskii, 1940; Griffiths et al, 1959; Wisniewski et al., 1996).

There is a requirement for traded grain to be pest-free; EC regulation 689/92 states in Article 2, that to be accepted for intervention, 'cereals must be free from live pests (including mites) at every stage of their development' (Intervention Board, 1996). In the U.K. stored grain protection has relied heavily on bulk admixture with insecticides. A survey of commercial grain stores in 1988/89 found that 67.5% of the sites had used contact pesticides on all or part of the grain, with two thirds treating for prophylactic reasons (Prickett, 1991). The only contact pesticides approved for use on stored grain are the organophosphorus (OP) compounds pirimiphos-methyl (Actellic), etrimfos (Satisfar) and chlorpyrifos-methyl (Reldan) (Whitehead, 2000). The survey of commercial stores found that pirimiphos-methyl, chlorpyrifos-methyl and etrimfos had been used in 73%, 21% and 12% of sites that treated grain, respectively (Prickett, 1991). With oilseeds, however, pirimiphos-methyl lost its approval for use on the commodity earlier this year, leaving the fumigant phosphine as the only pesticide available for oilseed treatment (Abel, 2000).

However, none of the OPs are particularly effective against field strains of mites. Results from recent surveys have found widespread resistance in populations of *Acarus siro* to one or more of these compounds. Resistance to twice the recommended rate of pirimiphos-methyl was detected in 15% of *Acarus siro* strains from farm stores (1987), 71% of strains from commercial stores (1988/89), 91% from animal feed mills (1992) and 93% from oilseed stores (1995) (Starzewski, 1991; Prickett, 1994; Prickett, 1997). In the latter survey resistance to chlorpyrifos-methyl was also detected in 63% of stores but only in mites from stores

where pirimiphos-methyl resistance was found (Prickett and Buckland, 1997). Resistance to etrimfos was also detected in two pirimiphos-methyl resistant strains (Prickett and Buckland, 1997). Unpublished data from the Central Science Laboratory by Binns and Buckland, found one *Acarus siro* population from a commercial store to be resistant to pirimiphos-methyl, etrimfos and chlorpyrifos-methyl.

Thind et al. (1996) also detected cross-resistance to chlorpyrifos-methyl and etrimfos in a pirimiphos-methyl resistant field strain of *Lepidoglyphus destructor*. This suggests that resistance to OPs in this case, has conferred cross-resistance to all three compounds, and it is possible that a single mechanism is responsible (Thind et al., 1996). Therefore, the use of any one compound may lead to selection for resistance to the others. Resistance to pirimiphos-methyl has been linked to an increase in esterase activity but other mechanisms may also be involved (Szlendak et al., 2000).

As well as concerns regarding the development of resistant pests, the costs and consumer resistance to toxic chemicals in food, have led to increasing pressures for a reduction in pesticide use. There are also strong pressures for OPs to be replaced, which can be based on fears that they may cause Chronic Fatigue Syndrome (CFS) following use as sheep dips, be involved in Gulf War Syndrome, have a causal link to spongiform encephalitis by causing prion mutation, or be a trigger for autoimmune diseases amid the continuing concerns over their undoubted presence as residues in foodstuffs (Vial et al., 1996; Wester et al., 1996; Stephens et al. 1996; Fairhall, 1996; Davies, 1997; Warden, 1996).

Cooling and drying can be used effectively to protect grain from pest infestation. By reducing the temperature of grain to below 5^{o}C mite breeding is prevented; and by drying grain to below 60% rh development can be inhibited (Cunnington, 1976). However, during the winter the surface layers may reabsorb moisture from the atmosphere (Burrell and Havers, 1976) locally increasing product moisture content and enhancing the development of surface mite infestations (Armitage, 1984). Surface grain cannot be kept cool or dry enough to limit mite numbers, so a surface pesticide treatment may be required.

There are a number of alternative compounds used effectively against acarine pests in field agriculture, veterinary and public health control programs, which may also prove effective against storage mites. These include insect growth regulators, inert dusts, botanicals, novel compounds and biological control agents. This project aims to identify potential alternatives to OPs and to investigate their efficacy against storage pests with a view to their use within an integrated pest management program.

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PART 1

INITIAL SCREEN OF 21 COMPOUNDS, WHEN ADMIXED WITH WHEAT, AGAINST SUSCEPTIBLE STRAINS OF STORAGE MITE PESTS

ABSTRACT

Twenty one compounds were assessed for their efficacy, when admixed with wheat, against mixed stages of susceptible strains of *Acarus siro* L., *Lepidoglyphus destructor* (Schrank) and *Tyrophagus putrescentiae* (Schrank) at 15^{o}C and 75% relative humidity. Population inhibition was assessed after periods long enough to include the passing of at least 2 generations. The compounds included 4 or 5 representatives of

diatomaceous earth (DE), insect growth regulators (IGR), novel compounds, pyrethroids and biological control agents and botanicals. The most effective compounds of each group were found to be the DE, 'Protect-it', the IGR, flufenoxuron, the novel compounds, sodium polyborate and benzyl benzoate, the pyrethroid, bifenthrin and the botanical, azadirachtin. These are to be included in future experiments in order to determine the most effective dose ranges against susceptible and resistant strains of mites and beetles under typical U. K. storage conditions.

INTRODUCTION

The aim of these initial experiments was to evaluate 21 compounds, identified from the review (HGCA Research Review No. 42), which appeared to be promising alternatives to OPs for the protection of stored grain against mite infestation. Because the aim was to identify possible replacements for OPs as admixture treatments, the most appropriate method of evaluation was to assess the compounds when applied directly to grain. Efficacy was therefore evaluated against three common mite pests found in stored grain under typical U.K. storage conditions. Also, because different mite stages may have different tolerances, the compounds were evaluated against mixed stages and assessed for periods long enough to include the passing of at least 2 generations.

MATERIALS

Wheat : The grain was pesticide-free, English milling wheat with a moisture content of about 15%, as determined using the oven method (BS4317), by drying in a ventilated oven at 130^{o}C for 2 hours. The wheat was stored in plastic bags in a freezer for at least 21 days prior to use to ensure any mites coming in on the grain were killed.

Mites : The mites used were laboratory susceptible strains of *Acarus siro* L., *Tyrophagus putrescentiae* (Schrank) and *Lepidoglyphus destructor* (Schrank). All have been reared at the Central Science Laboratory (CSL) in constant conditions of 15^{o}C and 75% relative humidity (rh) without exposure to pesticides. Mixed stages of unknown age were used.

Pesticides : The pesticides evaluated were those identified from the literature review which seemed to warrant further investigation for use as grain protectants. They include those that have shown to be effective against some storage mites, storage insects and other agricultural mite pests. Where possible commercially available formulations were used. Since few of the compounds had been evaluated against storage mites, the most effective dose range was difficult to determine. Therefore where little information was available, these initial experiments assessed a broad range of doses (to establish effective limits) which

can be narrowed down in further experiments. Table 1 shows the active ingredient, product name, formulation, active ingredient concentration and the doses applied of each compound evaluated.

METHOD

Preparation and treatment of grain : Batches of 493 g of whole wheat and 7 g of kibbled wheat were weighed out and mixed together into 1.5 l 'Kilner' jars, prior to application of the pesticides. The kibbled wheat served as an additional food supply for the mites to aid establishment on the whole grains and prevent population crashes.

The liquid formulations were prepared by serial dilution of the highest dose using an appropriate diluent. Each batch of grain was spread evenly over an enamelled tray in a single layer. The grain was then sprayed with 5 ml of the required dose, using a hand held 'De Vilbis' paint sprayer and returned back to the 'Kilner' jars. A batch of wheat was similarly treated with 5 ml of the diluent only, to act as a control.

The solid formulations were applied by weighing out the appropriate amount of product and adding directly to the grain in jars. One batch remained untreated to act as a control.

After treatment the batches of treated and control grain were mixed on a tumbler for 15 minutes to ensure an even distribution and stored at room temperature overnight. The jars were then tumbled for a further 10 minutes.

Each batch of treated and control grain was divided into approximately 50 g lots, put into 120 ml wide-necked bioassay jars and closed with filter paper lids. Six replicate jars were prepared for each treatment and each mite species. The jars were left to equilibrate in the test conditions of 15^{o}C and 75% rh for 24 hours.

Bioassay : The proportion of culturing medium to mites was adjusted so that a heaped spatula contained approximately 400-1200 mixed stage mites. This was achieved by taking a spatula from the culture flask and counting the mites under a binocular microscope using the Solomon's disc described below. The mite density was then diluted by adding more food to the culture. The added food was to serve as an initial food supply for the mites to aid establishment on whole grains and prevent population crashes. The numbers of mites in three heaped spatulas of adjusted mite culture were then counted. Table 2 shows the estimated range and mean mite numbers initially added to grain treated with each experimental group of compounds. A heaped spatula of adjusted mite culture was then placed into each jar and the jars were re-closed with a filter paper lid. The jars were incubated in the test conditions for periods long enough to include the passing

of at least two generations. Since the different mite species have different developmental rates from egg to adult, exposure periods varied for each species. Therefore, assessments of the F_1 and F_2 generations took place at approximately the following number of days after treatment :

Mite species	F_1 assessment	F_2 assessment
Acarus siro	30	70
Tyrophagus putrescentiae	50	110
Lepidoglyphus destructor	45	100

Three replicate jars were assessed at each assessment period. The contents of each jar were sieved over a 710 µm mesh and the sievings were examined under a low power binocular microscope. If many live mites were observed the dust was transferred to a petri dish over a 'Solomon's disc' (a disc divided into 64 sectors of which 8 are blacked - Solomon 1962). The dust was spread evenly over the surface of the dish using a seeker and the numbers of live mites on the blacked out sectors were counted. This number was then multiplied by 8 to provide an estimate of the total mite numbers. If fewer mites were initially observed, the dust was transferred to a petri dish, formed into a thin line and teased away using a seeker whilst counting the numbers of live mites.

After each assessment the moisture content of the grain was re-checked by drying in an oven for 2 hours at 130^{o}C (BS 4317).

The mean percentage inhibition of the mite populations was calculated for each treatment as a proportion of the controls and expressed as :

$$100 - \left[\frac{\text{Mean number of live mites per treatment}}{\text{Mean number of live mites in controls}}\right] \times 100$$

RESULTS AND DISCUSSION

Moisture content

In general the moisture contents of the control grain varied between 15.4 - 16.1%, with most samples recorded as being between 15.6 - 15.8% (Table 3). The exception to this was the grain used in the experiments with the silicaceous dusts, where the moisture contents varied between 14.7 - 14.9% (Table 3). As the rh in the exposure room was not found to be lower than during the rest of the experiments and the numbers of mites of all three species were generally as high at the F_1 and F_2 assessments as in the other

experiments, this difference is likely to be due to a different rh / moisture content relationship. The grain used in these experiments was of the same variety as that used in the others, but was from a different year's harvest. It was used only in the first set of experiments because of late delivery of the experimental batch of wheat.

The moisture contents of the wheat treated with the different pesticides were usually within 0.2% of the control moisture contents indicating little change over the experimental period.

Control numbers

In most cases the numbers of mites recorded at the F_1 assessment in the control replicates had increased from the estimation of the numbers originally introduced (Tables 2 and 3). However, the populations of all three mite species were seen to decline between the F_1 and F_2 assessments (Table 3). This was probably because the high populations of mites would have exhausted their limited food supply (Solomon, 1969). In these conditions it is likely that the mites hollow out the cereal germ and cannot access the endosperm through the dividing cellular layer (Parkinson, 1990). The numbers of mites initially introduced into the jars represented a very high infestation level compared to what would be found in field infestations (Table 2). The numbers ranged from 403 - 1285 in 50 g of grain which is equivalent to 8060 - 25700 mites per kg. It is therefore highly likely that the amount of grain in the jars would not have been able to support such large populations. Future experiments may aim to reduce the initial numbers of mites introduced.

The decline in numbers was particularly evident with *L. destructor* and *T. putrescentiae*, where in some cases < 5 live mites were recorded at the F_2 assessment (Table 3). In some of the control replicates it appeared that the population had crashed only a short time before the assessments as the bodies of the mites were turgid and not dried up. *T. putrescentiae* appeared the least successful at breeding under the experimental conditions. This species is more susceptible to lower temperatures than *A. siro*. It cannot breed much below 10^{o}C but breeds readily at temperatures above 30^{o}C which are lethal for *A. siro* (Cunnington, 1976). *T. putrescentiae* infests a wide variety of stored products particularly those rich in fat and protein while *A. siro* and *L. destructor* are found more frequently on stored cereals, cereal products and seeds (Cunnington, 1976).

It was observed throughout the experiment that some bioassay jars contained a higher proportion of kibbled wheat than others, and in these jars higher numbers of live mites were recorded. This extra kibbled wheat may have provided an additional food supply and prolonged the lives of the mites present.

In addition there were large variations in the numbers of live mites in the control replicates, particularly at the F_2 assessments (Table 3). This may have been due to the variation in the initial numbers introduced or variations in the moisture content of the grain. In future experiments the number of replicates will be increased in order to reduce the variation observed.

Silicaceous dusts

The population of *A. siro* was inhibited by > 99% at the F_1 assessment following exposure to doses of 1 g kg $^{-1}$ and above, of Dryacide and Insecto, and 0.5 g kg $^{-1}$ of Protect-it and Rid IP (Table 4, Figure 1). Against *T. putrescentiae*, 3 and 5 g kg $^{-1}$ of Dryacide and Insecto were required to inhibit the population by > 99%, with at least 1 and 0.5 g kg $^{-1}$ required of Rid IP and Protect-it respectively (Table 4, Figure 1). *L. destructor* consistently appeared the most tolerant species with a minimum dose of 5 g kg $^{-1}$ required of each compound required to reduce numbers by > 99%, at the F_1 assessment (Table 4, Figure 1).

Cook and Armitage (1999) also found *L. destructor* to be less susceptible than *A. siro* when exposed to wheat treated with Dryacide and suggested that 3 g kg $^{-1}$ may be an effective dose under U.K. conditions.

At the F_2 assessment, the *A. siro* population was inhibited by > 99 % after exposure to minimum doses of 0.5 g kg $^{-1}$ of Dryacide, Protect-it and Insecto with 1 g kg $^{-1}$ required of Rid IP (Table 4). Against *T. putrescentiae*, 1 g kg $^{-1}$ and above of all the compounds reduced numbers by > 99 % compared to those in the controls (Table 4), whereas against *L. destructor*, at least 3 g kg $^{-1}$ of Protect-it and Insecto was required compared to 5 g kg $^{-1}$ of Dryacide and Rid IP (Table 4).

Plate 1 shows scanning electron micrographs of the structure of the silicaceous dusts and the adherence of the dusts onto the mite cuticles.

Overall Protect-it appeared the most effective compound against all three mite species. However, the main disadvantage of using Protect-it as a grain protectant is that the diatoms present in the product are of marine origin, as is also the case with Insecto. Marine diatoms contain high quantities of crystalline silica which can result in silicosis and other respiratory diseases. However, Insecto has nevertheless been approved by the USA Environmental Protection Agency despite the fact that crystalline silica shows limited carcinogenic effect (Anon, IPDA cited in Quarles, 1992).

Dryacide is based on freshwater diatoms but coated with a silica aerogel (Subramanyan et al., in press). Silica aerogels are efficaceous at lower rates than diatom-based products. They are produced by drying

aqueous solutions of sodium silicate but their low dust density has prevented their use alone because of potential inhalation hazards (Golob, 1997).

'Rid IP' is amorphous precipitated silica. Compounds of this description usually have a SiO_2 content of 98% or more (Golob, 1997). According to IARC amorphous silica is not carcinogenic while crystalline silica shows limited evidence of carcinogenic effect. 'Rid IP' may be considered more environmentally friendly than the other products, however, it is more costly.

The results have shown all the silicaceous dusts to be effective under typical U.K. storage conditions, however, the high effective doses precludes use as an admixture treatment due to cost and effect on grain flow, but may be acceptable as a top-dressing as part of an integrated storage strategy or as a fabric treatment.

Novel Compounds

Against *A. siro* the population was inhibited by > 99% when exposed to 1 mg kg^{-1} and above of abamectin and 1000 mg kg^{-1} of sodium polyborate at the F_1 and F_2 assessments (Table 5, Figure 2). The highest dose of 100 mg kg^{-1} of benzyl benzoate only produced 67% and 79% inhibition for the spray and powder products respectively (Table 5, Figure 2).

Against *T. putrescentiae*, doses of 1 mg kg^{-1} and above of abamectin, 1000 mg kg^{-1} sodium polyborate and 100 mg kg^{-1} benzyl benzoate powder inhibited the mite population by > 99% at the F_1 assessment (Table 5, Figure 2). At the F_2 assessment exposure to 10 mg kg^{-1} abamectin and 1000 mg kg^{-1} sodium polyborate led to complete inhibition (Table 5).

Doses of at least 1 mg kg^{-1} and above of abamectin, 1000 mg kg^{-1} sodium polyborate and 100 mg kg^{-1} benzyl benzoate powder inhibited *L. destructor* by > 99% at the F_1 and F_2 assessments (Table 5, Figure 2).

Abamectin appeared the most effective pesticide producing greater mite inhibition at lower effective doses compared to the other compounds. However, abamectin is currently registered as an acaricide only for use on flowers and ornamentals and is not approved for use on food crops. It is unlikely that the product would ever be approved for use on stored grain, although it may have a use for residual treatments in empty stores.

Sodium polyborate is highly effective against house dust mites (Rosenkrantz and Griffin, 1995; Smith, 1997) and currently has HSE approval for household and veterinary fabric treatments. It has been suggested that sodium polyborate acts partly as a desiccant. However, compounds of a similar composition have been

shown to have chemosterilant activity. Alternative modes of action may explain why *A. siro* was the least susceptible mite for sodium polyborate, whereas for the silicaceous dusts (which act as desiccants) the least susceptible species was *L. destructor*. The doses required for complete inhibition of mites by sodium polyborate are generally lower than those required by some silicaceous dusts (Cook and Armitage, 1999).

Benzyl benzoate has FDA approval for use on foods (Kneist and Bischoff, 1995) and has proved effective against Astigmatid mites in domestic environments. In these experiments the powder formulation was more effective than the spray at the top dose.

Plate 2 shows the structure of the sodium polyborate and benzyl benzoate powder particles and the adhesion on the mite cuticles.

Insect growth regulators

The F_1 and F_2 populations of *A. siro* were inhibited by > 99 % when exposed to 1000 mg kg $^{-1}$ of methoprene, and following exposure to 100 mg kg $^{-1}$ flufenoxuron at the F_2 assessment (Table 6, Figure 3).

Against *T. putrescentiae*, 10 mg kg $^{-1}$ flufenoxuron was the only dose to reduce numbers by 99 % at the F_1 assessment, whereas at 100 mg kg $^{-1}$, < 95% inhibition was achieved (Table 6, Figure 3). At the F_2 assessment the numbers of live mites in the untreated controls had declined to < 4, therefore no real comment can be made on the efficacy of the pesticides (Table 3 and 6).

Flufenoxuron was also the most effective compound against *L. destructor*, with a > 99% reduction in mite numbers after exposure to 1 mg kg $^{-1}$ and above at the F_1 assessment (Table 6, Figure 3). Complete inhibition was also recorded with 1000 mg kg $^{-1}$ methoprene. However, as with *T. putrescentiae* the numbers of live mites in the controls at the F_2 assessment were very low (< 12) making pesticide effectiveness difficult to assess (Tables 3 and 6).

Flufenoxuron appeared the most effective compound against all three mite species. The compound acts as a chitin synthesis inhibitor and has been shown to be highly effective against a range of spider mites (Anderson et al., 1986). Methoprene appeared the next most effective compound although much higher doses were required compared to flufenoxuron.

Pyrethroids

A minimum dose of 2 mg kg $^{-1}$ of bifenthrin was found to inhibit *A. siro* populations by > 99 % at both the F_1 and F_2 assessments, with the other compounds requiring higher than 4 mg kg $^{-1}$ (Table 7, Figure 4).

Against *T. putrescentiae* none of the pesticides at the doses assessed reduced the mite population by > 99%. The most effective treatment appeared to be bifenthrin where inhibition by 89% was recorded at the F_1 assessment (Table 7, Figure 4).

Deltamethrin and flumethrin appeared the most effective pyrethroids against *L. destructor*. The mite populations were inhibited by > 99% when exposed to all doses at both the F_1 and F_2 assessments (Table 7, Figure 4). A dose of 2 mg kg $^{-1}$ of bifenthrin was required to produce the same degree of inhibition, whereas a dose of more than 4 mg kg $^{-1}$ of bioresmethrin was required (Table 7, Figure 4).

Overall bifenthrin appeared the most effective treatment against all three mites species, being least effective against *T. putrescentiae*.

Biological control agents and botanicals

None of the compounds effectively reduced the *A. siro* population by > 99%. The most effective pesticide was azadirachtin where 97% inhibition was recorded at a dose of 100 mg kg $^{-1}$ at the F_1 assessment (Table 8, Figure 5).

Against *T. putrescentiae* azadirachtin also appeared the most effective pesticide with 73% inhibition recorded when exposed to 100 mg kg $^{-1}$ at the F_1 assessment (Table 8, Figure 5). However, at the F_2 assessment, the numbers of mites were reduced by about 95% when exposed to azadirachtin at any dose level (Table 8). The *Bacillus thuringiensis* products also appeared more effective at the F_2 assessment, with inhibitions of 81-98 % with *Bt* var. *aizawa* and 91-97% when exposed to *Bt* var. *kurstaki* (Table 8). However, it is difficult to determine whether the reduction in the mite numbers was as a result of exposure to the pesticides or due to starvation from exhaustion of the food supply. At the lower doses, there appeared to be little effect on mite numbers at the F_1 assessment, but at the F_2 assessment there were very few live mites present with little difference in numbers between the doses.

Application of the fungal spores of *Beauveria bassiana* was the most effective treatment at inhibiting the population of *L. destructor*, with > 99% inhibition achieved after exposure to doses of 0.5 g kg^{-1} and above at both assessment periods (Table 8, Figure 5). Plate 3 shows the adhesion of the fungal spores on the mite cuticle. Azadirachtin was the next most effective with 93 % inhibition at the F_1 assessment (Table 8, Figure 5).

Although the fungal spores were not effective against all three species, the results are still encouraging as the fungal strain selected was a non-specific isolate. The lack of efficacy of this particular isolate against the other two species does not necessarily mean that other isolates would be ineffective.

The reduced activity of the *Bacillus thuringiensis* products against the mites is not unexpected as the products are specifically used for the control of caterpillars. Because the main advantage of biological control agents is their narrow host range and specificity, more work would be needed to identify pathogens and isolates that have specific activity against storage mites.

Linalool (a component of the essential oil of lavender) was also fairly ineffective against the mites even though other workers have shown high activity against other Astigmatid mites (Watanabe et al., 1989; Perrucci, 1995).

CONCLUSIONS

These preliminary experiments have shown that there are effective potential replacements to OPs for the protection of stored grain against storage mites. Of the 21 compounds assessed at least one from each experimental group was selected for further experiments. The compounds chosen were all judged to be effective in controlling storage mites at doses likely to allow them to be used cost effectively in the U. K. The chosen compounds are : Protect-It, flufenoxuron, sodium polyborate, benzyl benzoate, bifenthrin and azadirachtin.

The silicaceous dusts may represent the most near-market products available. They are already registered for storage use in some countries, are cost effective, have low mammalian toxicity and are effective against storage insects. Insect growth regulators have also been shown to be effective against insects within the storage environment. Their specificity to target pests and low toxicity to beneficial organisms and mammals suggest them to be ideal candidates for incorporation into an integrated pest management programme. Of the novel compounds, benzyl benzoate and sodium polyborate have been shown to be effective against Astigmatid mites in domestic environments and both have low mammalian toxicity. Pyrethroids are already registered for use on stored grain in some countries, they are effective against storage insects and degrade

slowly under U.K. storage conditions. Although none of the biological control agents were fully effective against all three mite species, these preliminary experiments indicate their potential and how further investigations are warranted to identify mite specific pathogens. This will be the subject of a more detailed study in a joint HGCA/MAFF/industry funded LINK project. Plant extracts have a wide spectrum of activity with some shown to be effective against storage insects.

It should also be considered that the potential of any replacements to OPs for stored grain protection is likely to lie in their ability to be incorporated into an integrated pest management programme rather than in their use as a separate control measure.

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Table 1 : Active ingredient, product name, formulation, active ingredient concentration and doses applied of pesticides

Experimental group	Active ingredient	Product name	Formulation	Active ingredient concentration	Doses applied
Silicaceous Dusts		Dryacide	Dust		0.5,1,3 and 5 g kg -1
		Protect-It	Dust		0.5,1,3 and 5 g kg -1
		Insecto	Dust		0.5,1,3 and 5 g kg -1
		RID	Dust		0.5,1,3 and 5 g kg -1
Insect growth regulators	Methoprene	Apex	Emulsifiable concentrate	600 g/l	1, 10, 100 and 1000 mg kg-1
	Fenoxycarb	Insegar	Wettable powder	25%	0.1, 1, 10 and 100 mg kg -1
	Diflubenzuron	Dimilin Flo	Suspension concentrate	480 g/l	1, 10, 100 and 1000 mg kg-1
	Flufenoxuron		Technical	100%	0.1, 1, 10 and 100 mg kg -1
Novel compounds	Abamectin	Dynamec	Emulsifiable concentrate	18 g/l	0.01, 0.1, 1 and 10 mg kg -1
	Sodium polyborate	Flea ban powder	Powder	100%	1, 10, 100 and 1000 mg k -1
	Benzyl benzoate	Acarosan	Spray	1.89%	0.1, 1, 10 and 100 mg kg -1
	Benzyl benzoate	Acarosan	Powder	5%	0.1, 1, 10 and 100 mg kg -1
Pyrethroids	Bifenthrin	Talstar	Emulsifiable concentrate	100 g/l	4,2,1 and 0.5 mg kg -1
	Bioresmethrin	Blade	Microemulsion	0.3% w/w	4,2,1 and 0.5 mg kg -1
	Deltamethrin	Decis	Emulsifiable concentrate	25 g/l	4,2,1 and 0.5 mg kg -1
	Flumethrin		Emulsifiable concentrate	6%	4,2,1 and 0.5 mg kg -1
Biological control agents and botanicals	*Bacillus thuringiensis* var. *aizawa*	Certan	Suspension concentrate	3500 GMU/mg	1.25, 2.5, 5 and 10 %
	Bacillus thuringiensis var. *kurstaki*	Dipel	Wettable powder	3.20%	0.1, 1, 10 and 100 mg kg -1
	Beauveria bassiana		Dried spores	1.03×10^{11} spores/g 95.5% germination rate	0.05, 0.5, 1 and 2 g kg -1
	Azadirachtin	Fortune AZA	Emulsifiable concentrate	3%	0.1, 1, 10 and 100 mg kg -1
	Linalool		Analytical	95 - 97%	0.1, 1, 10 and 100 mg kg -1

Table 2 : Estimated range and mean mite numbers initially added to grain treated with each experimental group of compounds

Experimental group	Species	Number range	Mean
Silicaceous dusts	*A. siro*	800 - 992	912
	T. putrescentiae	424 - 504	467
	L. destructor	376 - 560	440
Novel compounds	*A. siro*	352 - 656	485
	T. putrescentiae	416 - 608	499
	L. destructor	256 - 488	403
Insect growth regulators [1]	*A. siro*	1184 - 1408	1285
	T. putrescentiae	520 - 608	549
	L. destructor	896 - 1248	1056
Insect growth regulators [2]	*A. siro*	1080 - 1328	1219
	T. putrescentiae	560 - 672	632
	L. destructor	640 - 944	811
Pyrethroids	*A. siro*	1080 - 1328	1219
	T. putrescentiae	560 - 672	632
	L. destructor	640 - 944	811
Biological control agents and botanicals	*A. siro*	616 - 1016	933
	T. putrescentiae	528 - 624	584
	L. destructor	464 - 616	557

[1] Methoprene, Diflubenzuron and Flufenoxuron
[2] Fenoxycarb

Table 3 : Comparison of moisture contents (mc) and mean numbers of mites (n=3) in controls during tests to compare the efficacy of alternatives to OPs against mites.

Experimental group		*A. siro*		*T. putrescentiae*		*L. destructor*	
		F_1	F_2	F_1	F_2	F_1	F_2
Silicaceous dusts	**mc**	14.9	14.7	14.8	14.8	14.9	14.8
	mean	4875	1981	870	317	2472	435
	range	(4088-5752)	(1464-2512)	(51-1360)	(72-616)	(1464-3944)	(376-472)
Novel compounds	**mc**	15.6	15.6	15.7	15.7	15.7	15.4
	mean	1709	744	203	22	952	72
	range	(416-2808)	(416-1048)	(72-320)	(8-40)	(128-1416)	(16-144)
IGRs [1]	**mc**	16.0	15.9	15.7	15.8	15.9	15.7
	mean	3688	547	765	1	805	3
	range	(3432-3848)	(368-904)	(184-1120)	(1-3)	(640-984)	(1-4)
IGRs [2]	**mc**	15.7	15.8	15.6	15.7	15.8	15.6
	mean	3437	936	1109	4	1613	12
	range	(2616-4032)	(144-1568)	(304-1720)	(2-8)	(1016-2016)	(6-16)
IGRs [3]	**mc**	15.8	16.1	15.8	15.8	15.8	15.7
	mean	1736	973	379	85	1165	37
	range	(1080-2584)	(744-1144)	(128-808)	(40-120)	(1048-1320)	(24-56)
Pyrethroids	**mc**	15.8	16.1	15.8	15.8	15.8	15.7
	mean	1736	973	379	85	1165	37
	range	(1080-2584)	(744-1144)	(128-808)	(40-120)	(1048-1320)	(24-56)
Biologicals[4]**/ botanicals**	**mc**	15.8	15.7 / (15.3)	15.8	15.5	15.8	15.5
	mean	1948	491 / (880)	285	85	1083	43
	range	(1704-2192)	(376-632) / (608-1224)	(104-392)	(32-128)	(656-1528)	(16-80)
Biologicals[5]**/ botanicals**	**mc**	15.6	15.8	15.8	15.4	15.8	15.5
	mean	3928	851	893	48	1715	61
	range	(2952-4784)	(584-1296)	(640-1280)	(32-64)	(1424-2016)	(15-104)

[1] Methoprene, Diflubenzuron (water as diluent)
[2] Flufenoxuron (acetone as diluent)
[3] Fenoxycarb (water as diluent)
[4] Bt, Neem (water as diluent) / (Bt var *aizawa* repeat test at 10 % and 5 % vs. *A. siro* at F_2 assessment)
[5] Linalool (acetone as diluent)

Table 4 - Mean % inhibition of each mite species exposed to silicaceous dusts

Pesticide	Dose (g kg -1)	Mite species	F_1 Inhibition (%)	F_2 Inhibition (%)
Dryacide	5	*A. siro*	100	100
		L. destructor	100	99.8
		T. putrescentiae	100	100
	3	*A. siro*	100	100
		L. destructor	87.9	64.6
		T. putrescentiae	100	100
	1	*A. siro*	99.6	100
		L. destructor	42.3	0
		T. putrescentiae	95.1	99.9
	0.5	*A. siro*	91.5	100
		L. destructor	0	85.9
		T. putrescentiae	99	97.6
Protect-It	5	*A. siro*	100	100
		L. destructor	100	100
		T. putrescentiae	100	100
	3	*A. siro*	99.9	100
		L. destructor	98.2	99.7
		T. putrescentiae	100	100
	1	*A. siro*	99.9	100
		L. destructor	74.1	44.2
		T. putrescentiae	100	99.8
	0.5	*A. siro*	99.8	99.9
		L. destructor	37.9	50.9
		T. putrescentiae	99.1	92.4
Insecto	5	*A. siro*	100	100
		L. destructor	99.9	99.8
		T. putrescentiae	100	100
	3	*A. siro*	100	100
		L. destructor	97.9	100
		T. putrescentiae	100	100
	1	*A. siro*	99.7	99.9
		L. destructor	57.2	43.2
		T. putrescentiae	98.6	99.7
	0.5	*A. siro*	92.9	100
		L. destructor	0	91.4
		T. putrescentiae	69.6	97.8
RID powder	5	*A. siro*	100	100
		L. destructor	100	100
		T. putrescentiae	100	100
	3	*A. siro*	99.99	100
		L. destructor	97.3	78.5
		T. putrescentiae	99.9	100
	1	*A. siro*	99.9	100
		L. destructor	70.8	79.5
		T. putrescentiae	99.4	100
	0.5	*A. siro*	99.8	98.5
		L. destructor	58.5	39.9
		T. putrescentiae	98.8	55.5

Table 5 - Mean % inhibition of each mite species exposed to novel compounds

Pesticide	Dose (mg kg -1)	Mite species	F_1 Inhibition (%)	F_2 Inhibition (%)

Abamectin	10	*A. siro*	100	counted early in error
		L. destructor	100	100
		T. putrescentiae	100	100
	1	*A. siro*	100	100
		L. destructor	100	100
		T. putrescentiae	99.6	98.6
	0.1	*A. siro*	45.6	0
		L. destructor	92.7	11.1
		T. putrescentiae	46.1	0
	0.01	*A. siro*	0	0
		L. destructor	9.5	0
		T. putrescentiae	0	0
Sodium polyborate	1000	*A. siro*	99.9	100
		L. destructor	100	100
		T. putrescentiae	100	100
	100	*A. siro*	0	0
		L. destructor	99.7	100
		T. putrescentiae	35.5	81.6
	10	*A. siro*	0	20.4
		L. destructor	0	8.3
		T. putrescentiae	1.3	0
	1	*A. siro*	0	0
		L. destructor	0	0
		T. putrescentiae	0	0
Benzyl benzoate spray	100	*A. siro*	67.2	0
		L. destructor	84.3	0
		T. putrescentiae	71.5	0
	10	*A. siro*	0	0
		L. destructor	7	48.2
		T. putrescentiae	0	0
	1	*A. siro*	0	0
		L. destructor	3.1	0
		T. putrescentiae	0	26.3
	0.1	*A. siro*	0	0
		L. destructor	2.8	77.8
		T. putrescentiae	0	15.7
Benzyl benzoate powder	100	*A. siro*	79.2	0
		L. destructor	100	100
		T. putrescentiae	100	27.6
	10	*A. siro*	0	0
		L. destructor	0	3.7
		T. putrescentiae	31.6	0
	1	*A. siro*	0	6.5
		L. destructor	0	25.9
		T. putrescentiae	25	0
	0.1	*A. siro*	0	14.3
		L. destructor	0	0
		T. putrescentiae	0	0

Table 6 - Mean % inhibition of each mite species exposed to insect growth regulators

Pesticide	Dose (mg kg -1)	Mite species	F_1 Inhibition (%)	F_2 Inhibition (%)
Methoprene	1000	*A. siro*	99.8	100

		L. destructor	100	100 *
		T. putrescentiae	96.2	76.9 *
	100	*A. siro*	96	70.7
		L. destructor	55.9	0 *
		T. putrescentiae	66.2	0 *
	10	*A. siro*	61.7	0
		L. destructor	0	0 *
		T. putrescentiae	12.9	0 *
	1	*A. siro*	69.6	0
		L. destructor	0	0 *
		T. putrescentiae	0	0 *
Fenoxycarb	100	*A. siro*	54.5	32.9
		L. destructor	32.9	29.5 *
		T. putrescentiae	58.5	98.8 *
	10	*A. siro*	8.8	23.8
		L. destructor	39.8	1.6 *
		T. putrescentiae	21.8	85.6 *
	1	*A. siro*	0	14.5
		L. destructor	13.3	11.5 *
		T. putrescentiae	0	96.1 *
	0.1	*A. siro*	0	31.8
		L. destructor	0	69.7 *
		T. putrescentiae	0	92.6 *
Diflubenzuron	1000	*A. siro*	96.3	95.3
		L. destructor	4.9	0 *
		T. putrescentiae	68.3	0 *
	100	*A. siro*	93.6	70.7
		L. destructor	0	0 *
		T. putrescentiae	43.2	0 *
	10	*A. siro*	69.5	0
		L. destructor	0	0 *
		T. putrescentiae	0	0 *
	1	*A. siro*	56.7	0
		L. destructor	0	0 *
		T. putrescentiae	5.2	0 *
Flufenoxuron	100	*A. siro*	97	99.1
		L. destructor	100	100 *
		T. putrescentiae	94.9	100 *
	10	*A. siro*	97.2	98.8
		L. destructor	100	100 *
		T. putrescentiae	99.8	100 *
	1	*A. siro*	98.4	97.6
		L. destructor	99.1	100 *
		T. putrescentiae	98.9	100 *
	0.1	*A. siro*	91	98.6
		L. destructor	97.2	100 *
		T. putrescentiae	40.9	0 *

Table 7 - Mean % inhibition of each mite species exposed to pyrethroids

Pesticide	Dose (mg kg -1)	Mite species	F_1 Inhibition (%)	F_2 Inhibition (%)
Bifenthrin	4	*A. siro*	99.7	99.8
		L. destructor	99.9	100

		T. putrescentiae	88.6	47.2
	2	*A. siro*	99.3	99.2
		L. destructor	99.6	100
		T. putrescentiae	78.8	0
	1	*A. siro*	90.9	95.3
		L. destructor	93.2	78.5
		T. putrescentiae	0	0
	0.5	*A. siro*	61.7	83.6
		L. destructor	67.7	0
		T. putrescentiae	0	0
Bioresmethrin	4	*A. siro*	98.5	89.6
		L. destructor	97.1	0.8
		T. putrescentiae	50	0
	2	*A. siro*	96.6	84.4
		L. destructor	92.7	28.4
		T. putrescentiae	0	0
	1	*A. siro*	73.4	70.7
		L. destructor	53.8	0
		T. putrescentiae	0	0
	0.5	*A. siro*	46.5	9.9
		L. destructor	17.8	11.5
		T. putrescentiae	0	21.8
Deltamethrin	4	*A. siro*	84.3	85.2
		L. destructor	100	100
		T. putrescentiae	78.3	92.1
	2	*A. siro*	79.7	79.7
		L. destructor	100	100
		T. putrescentiae	35.2	98.8
	1	*A. siro*	68.2	50.1
		L. destructor	100	100
		T. putrescentiae	0	95.3
	0.5	*A. siro*	51.5	57.8
		L. destructor	100	100
		T. putrescentiae	0	99.2
Flumethrin	4	*A. siro*	67.4	35.1
		L. destructor	100	100
		T. putrescentiae	52.8	90.6
	2	*A. siro*	44.4	66.3
		L. destructor	100	100
		T. putrescentiae	0	83.6
	1	*A. siro*	0	74.5
		L. destructor	99.9	100
		T. putrescentiae	0	94.1
	0.5	*A. siro*	44.9	68.8
		L. destructor	100	99.2
		T. putrescentiae	0	91.4

Table 8 - Mean % inhibition of each mite species exposed to biological control agents and botanicals

Pesticide	Dose	Mite species	F_1 Inhibition (%)	F_2 Inhibition (%)
Bacillus	10%	*A. siro*	0	18.2
thuringiensis		*L. destructor*	34.2	25
subsp. *aizawa*		*T. putrescentiae*	37.4	97.6

	5%	*A. siro*	0	10.9
		L. destructor	0	12.6
		T. putrescentiae	0.9	95.7
	2.50%	*A. siro*	0	0
		L. destructor	0	0
		T. putrescentiae	14	98.8
	1.25%	*A. siro*	0	7.1
		L. destructor	41.1	74.9
		T. putrescentiae	0	81.2
Bacillus thuringiensis subsp. *kurstaki*	100 mg kg -1	*A. siro*	0	0
		L. destructor	41.9	11
		T. putrescentiae	5.6	97.3
	10 mg kg -1	*A. siro*	0	0
		L. destructor	0	29
		T. putrescentiae	0	93
	1 mg kg -1	*A. siro*	0	0
		L. destructor	0	40.7
		T. putrescentiae	35.5	94.5
	0.1 mg kg -1	*A. siro*	0	0
		L. destructor	0	7.9
		T. putrescentiae	0	91.4
Beauveria bassiana	2 g kg -1	*A. siro*	29	5.4
		L. destructor	99.9	100
		T. putrescentiae	0	0
	1 g kg -1	*A. siro*	22.9	1.6
		L. destructor	99.7	100
		T. putrescentiae	0	25
	0.5 g kg -1	*A. siro*	4.5	0
		L. destructor	99.1	99.3
		T. putrescentiae	0	25
	0.05 g kg -1	*A. siro*	0	0
		L. destructor	31.5	0
		T. putrescentiae	0	37.5
Neem	100 mg kg -1	*A. siro*	96.9	78.8
		L. destructor	93.1	12.6
		T. putrescentiae	72.9	95
	10 mg kg -1	*A. siro*	0	0
		L. destructor	0	12.6
		T. putrescentiae	0	93.3
	1 mg kg -1	*A. siro*	0	31.5
		L. destructor	0	0
		T. putrescentiae	0	96.5
	0.1 mg kg -1	*A. siro*	0	51.6
		L. destructor	0	18.7
		T. putrescentiae	25.2	94.5

Table 8 continued - Mean % inhibition of each mite species exposed to biological control agents and botanicals

Pesticide	Dose	Mite species	F_1 Inhibition (%)	F_2 Inhibition (%)
Linalool	100 mg kg -1	*A. siro*	26.4	10
		L. destructor	0	24.6
		T. putrescentiae	0	71.5
	10 mg kg -1	*A. siro*	11.6	18.5
		L. destructor	0	43.1
		T. putrescentiae	0	73.5
	1 mg kg -1	*A. siro*	0	4.7
		L. destructor	0	35.6
		T. putrescentiae	0	81.3
	0.1 mg kg -1	*A. siro*	15.9	0
		L. destructor	0	69.3
		T. putrescentiae	0	60.4

* - F2 control populations had crashed

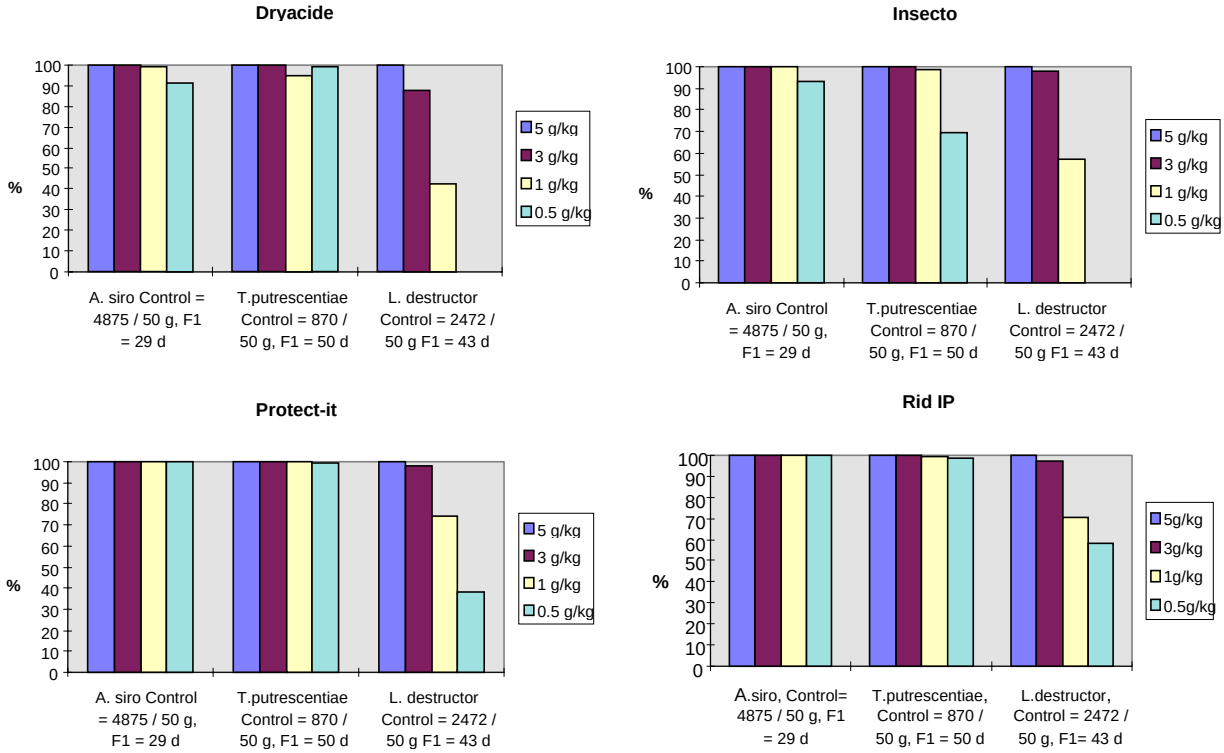


Figure 1. The percent inhibition (expressed as a proportion of populations on untreated grain) at 75% rh, 15oC of four diatomaceous earths against three mite species. F 1 assessment

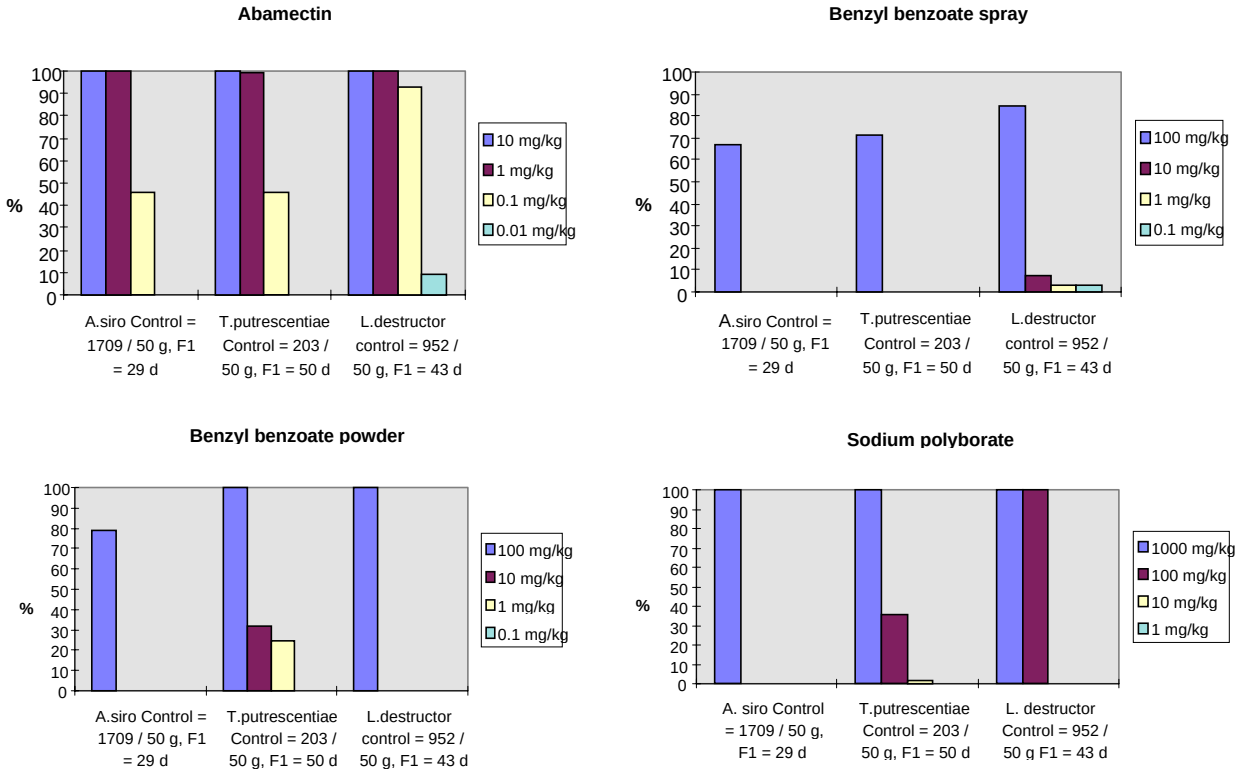


Figure 2. The percent inhibition (expressed as a proportion of populations on untreated grain) at 75% rh, 15oC of four novel compounds against three mite species. F 1 assessment

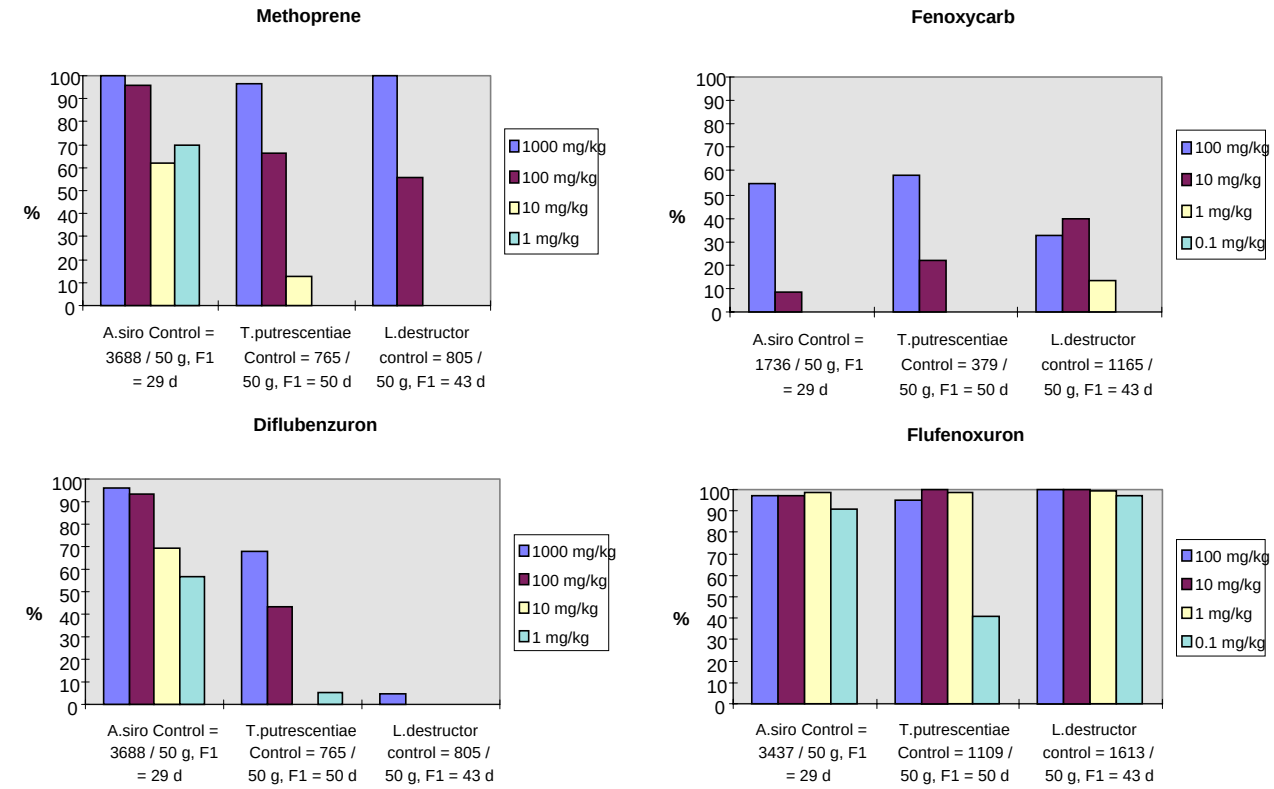


Figure 3. The percent inhibition (expressed as a proportion of populations on untreated grain) at 75% rh, 15oC of four insect growth regulators against three mite species. F 1 assessment

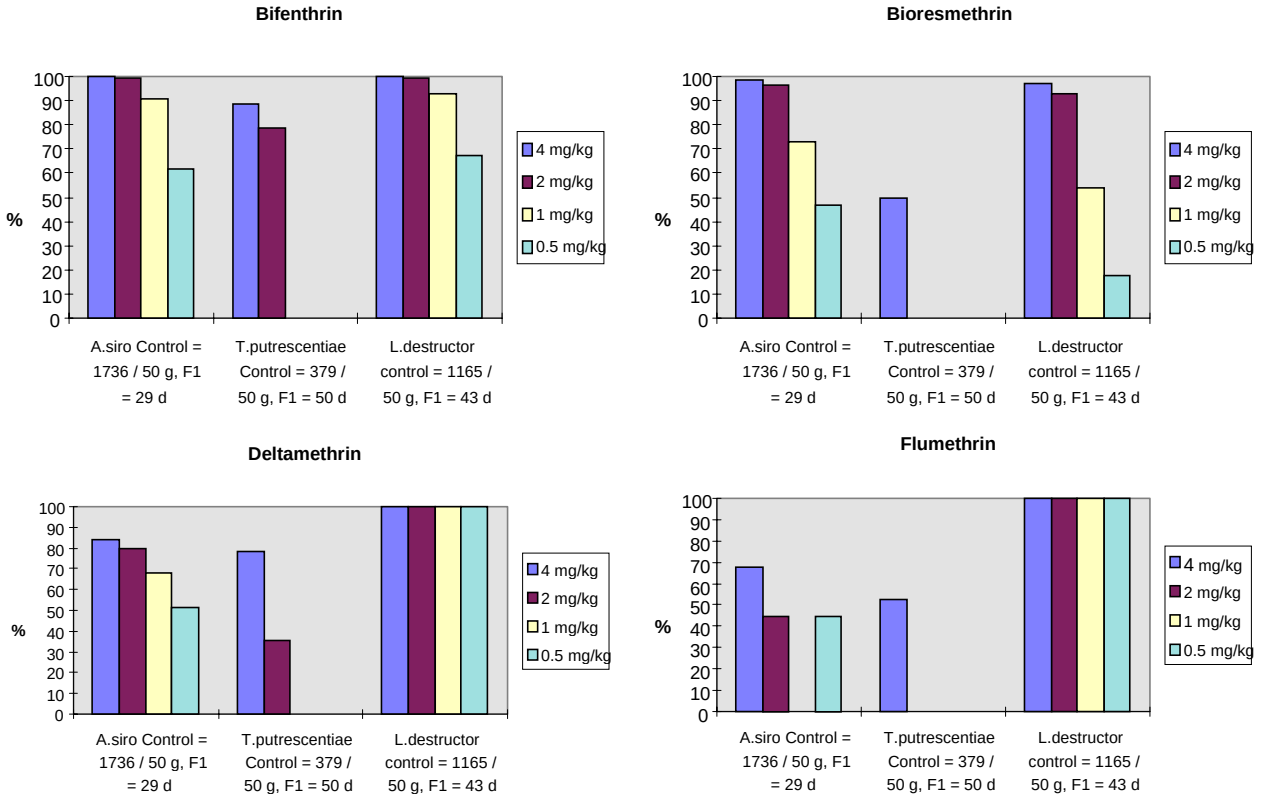


Figure 4. The percent inhibition (expressed as a proportion of populations on untreated grain) at 75% rh, 15oC of four pyrethorids against three mite species. F 1 assessment

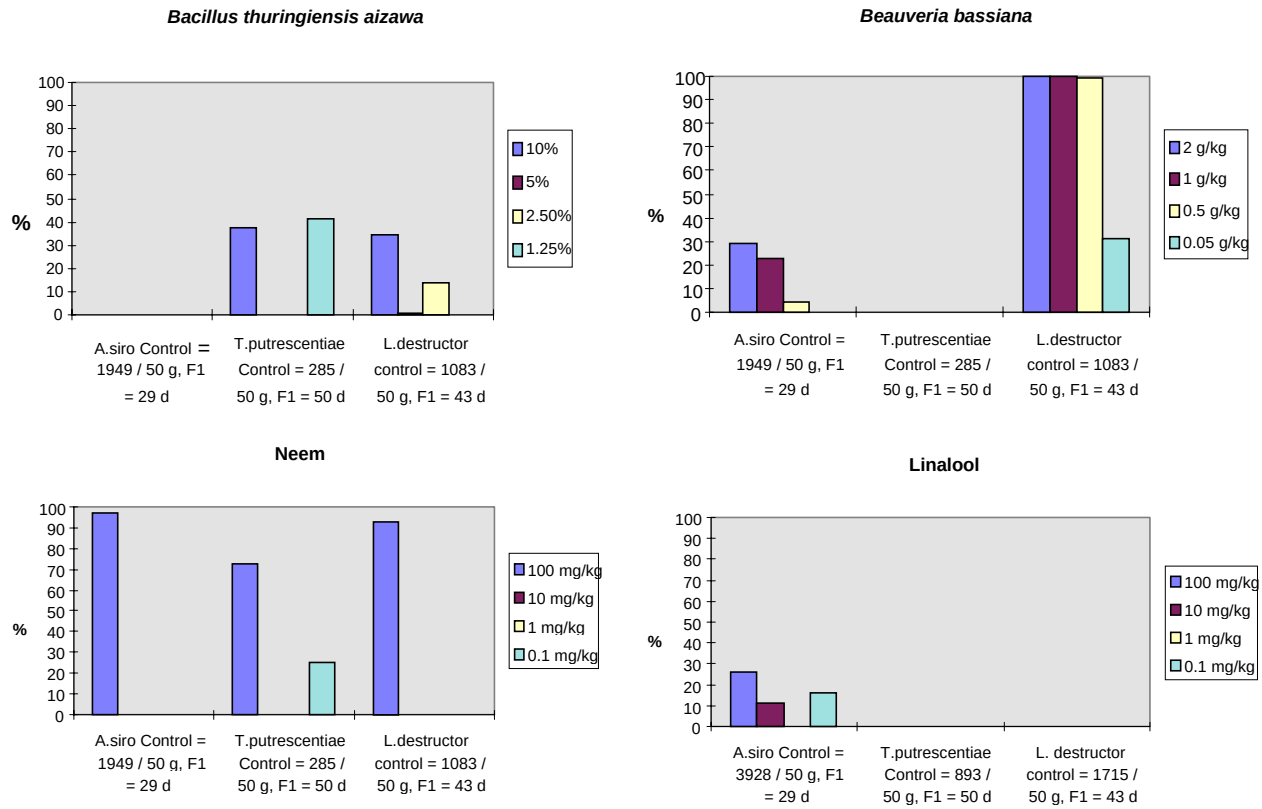
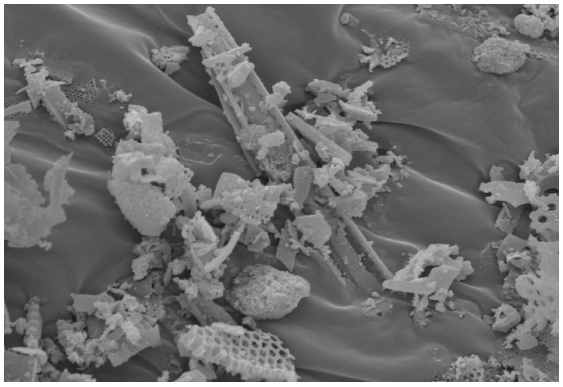
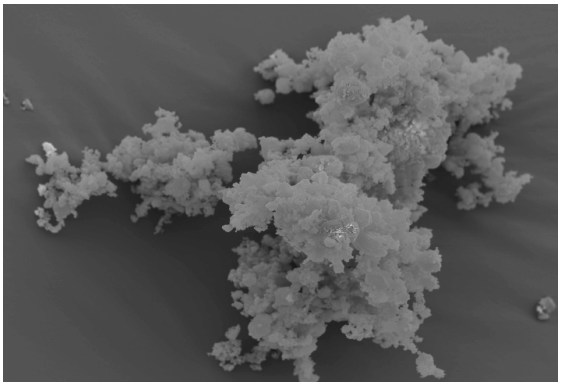


Figure 5. The percent inhibition (expressed as a proportion of populations on untreated grain) at 75% rh, 15oC of two biocontrol agents and two plant extracts against three mite species. F 1 assessment

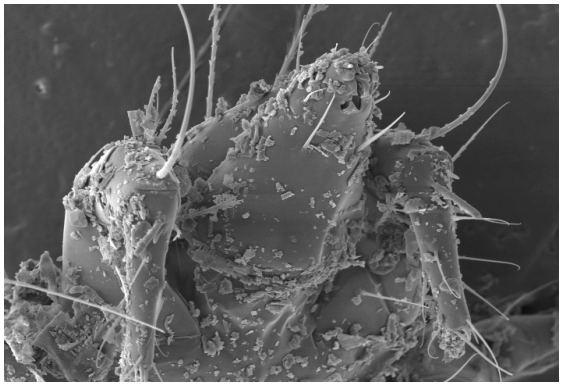
PLATE 1. SEM images showing structure of silicaceous dust and the adhesion to mite cuticles.



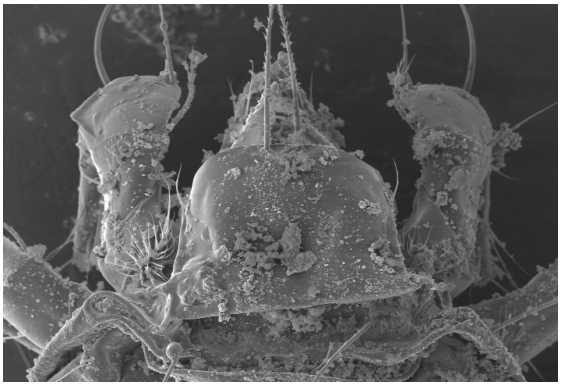
Protect-it particles x 705



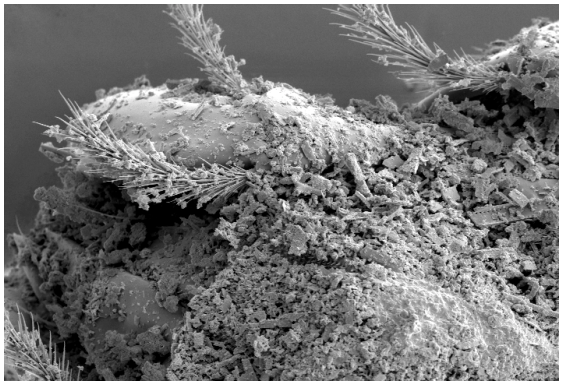
Rid IP particles x 187



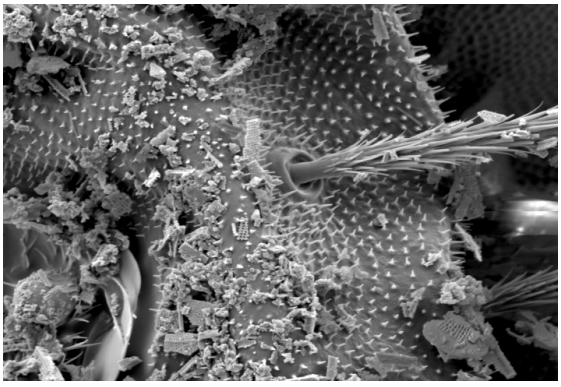
T. putrescentiae exposed to 1 g/kg Insecto x 106



A. siro exposed to 1 g/kg Rid IP x 125

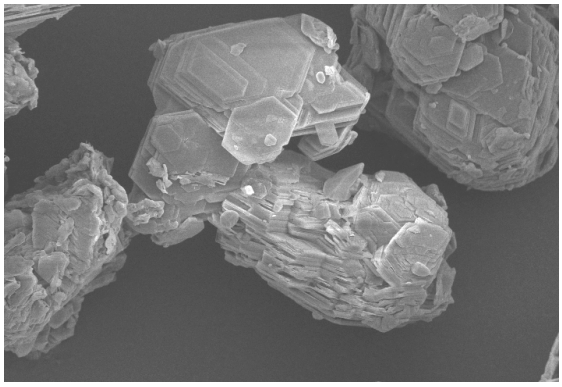


Detail of particles adhering to cuticle of *L. destructor* exposed to 1 g/kg Protect-it x 257

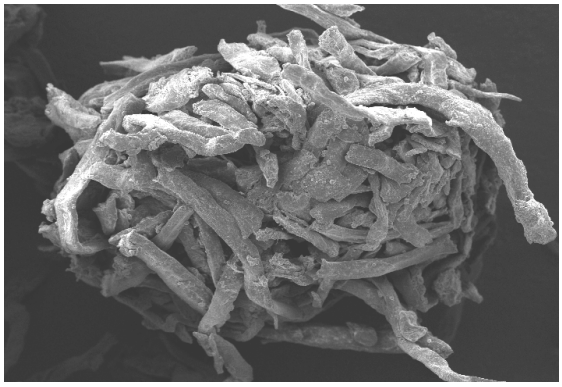


Detail of particles adhering to cuticle of *L. destructor* exposed to 1 g/kg Dryacide x 509

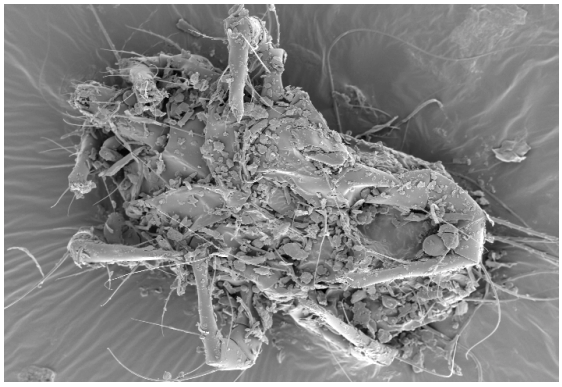
PLATE 2. SEM images showing structure of Sodium polyborate and benzyl benzoate particles and the adhesion to mite cuticles.



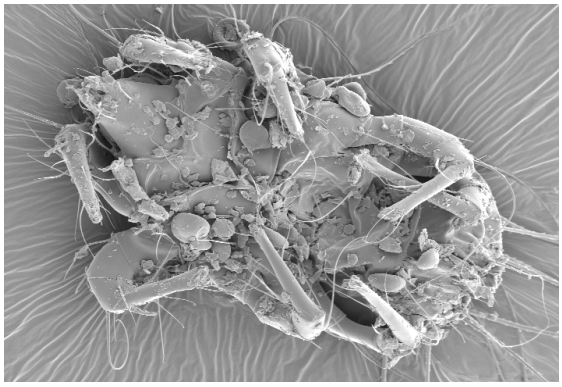
Sodium polyborate particles x 533



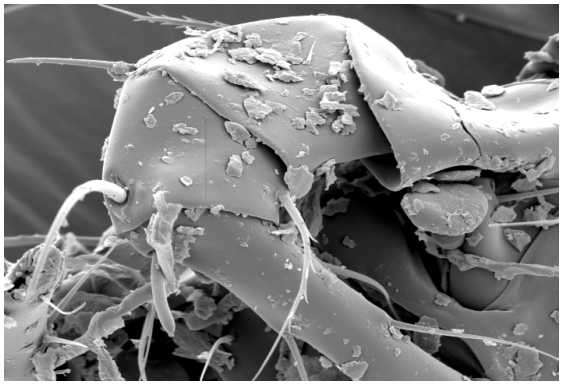
Benzyl benzoate particles x 72



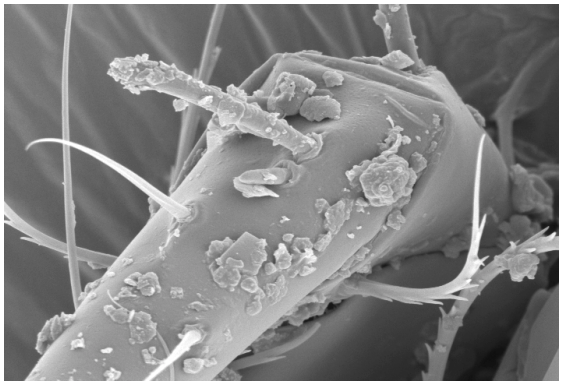
T. putrescentiae exposed to 10 mg/kg Sodium polyborate x 89



T. putrescentiae exposed to 10 mg/kg benzyl benzoate x 99

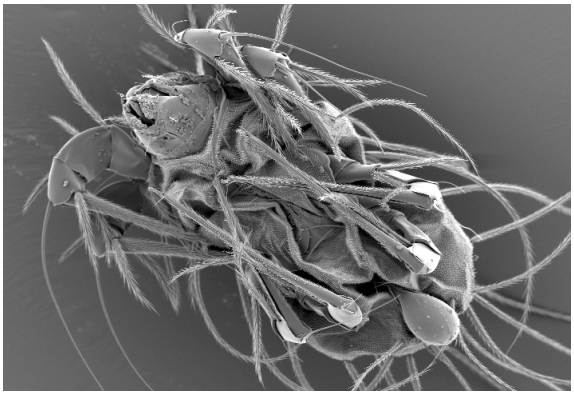


Detail of particles adhering to leg of *T.putrescentiae* exposed to 10 mg/kg Sodium polyborate x 403

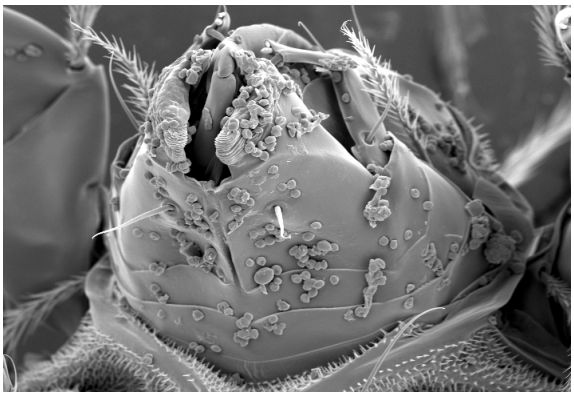


Detail of particles adhering to leg of *T.putrescentiae* exposed to 10 mg/kg benzyl benzoate x 772

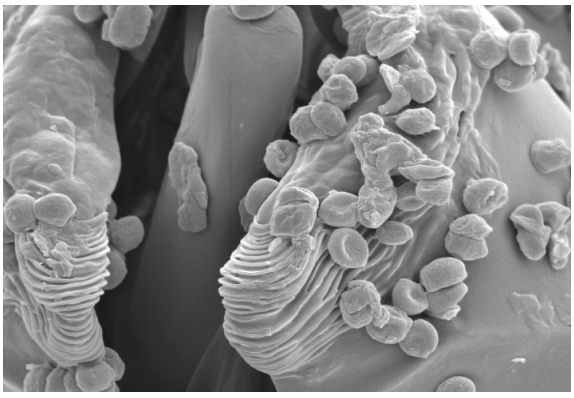
PLATE 3. SEM images of *Beauveria bassiana* spores adhering to cuticle of *Lepidoglyphus destructor* exposed to 2 g/kg.



x 64



x 256



x 1024

PART 2

THE EFFICACY OF 6 COMPOUNDS, WHEN ADMIXED WITH WHEAT, AGAINST SUSCEPTIBLE AND RESISTANT STRAINS OF MITES AND INSECTS, OVER 12WEEKS STORAGE IN TWO DIFFERENT CONDITIONS

ABSTRACT

Six compounds were assessed for their efficacy, when admixed with wheat, against organophosphate susceptible and resistant strains of storage mites and insects. Wheat was stored at 10°C and 25°C at 75% r.h. and then sampled 1 day, 4 weeks and 12 weeks after treatment. Efficacy was evaluated by the effect on adult beetle mortality and on the ability of the compounds to inhibit development of the insect and mite populations. The results indicated the range of tolerances of the different pest species and strains to the different compounds, with *Sitophilus granarius*, appearing the most tolerant species. In general, lower doses of the pesticides were required to inhibit the mite populations when exposed at 10°C compared to 25°C and adult insect mortality was higher at 25°C than at 10°C. Flufenoxuron and azadirachtin were considered to be worthy of further investigation and are to be included in future experiments.

INTRODUCTION

Previous experiments evaluated 21 compounds for their potential as possible replacements to organophosphorus compounds (OPs) for the control of storage mites on grain (Part 1). From those experiments, 6 compounds were identified as being judged to be effective in controlling mites at doses likely to allow them to be used cost-effectively in the U.K. However, for compounds to be considered as potential alternatives to OPs as well as being effective against storage mites, they must also be effective against storage insects, against resistant strains, provide prolonged protection over extended storage periods, be effective under typical U. K. storage conditions and be able to be incorporated into an integrated pest management program.

The aim of these experiments was to extend the scope of previous investigations by assessing efficacy :

1. against susceptible and OP resistant strains.
2. against storage mite pests.
3. against storage insect pests.
4. at temperatures covering the immediate post-harvest and long term storage conditions.
5. using narrower dose ranges.

MATERIALS

Wheat : Pesticide-free, English milling wheat of the 'Mercia' variety was used. The wheat was stored in plastic bags in a freezer for at least 21 days prior to use to ensure any mites coming in the grain were killed. The moisture content (mc) was determined by drying in a ventilated oven at 130^{o}C for 2 hours (ISO 712).

Pest species : Details of the pest species used in the experiments are given in table 9 with details of the strain, resistance status, culturing conditions, test age and stage. All have been reared at the Central Science Laboratory (CSL) in constant conditions without exposure to pesticides.

Pesticides : Table 10 shows the active ingredient, product name, formulation, active ingredient concentration and the doses of each compound evaluated.

METHOD

Preparation and treatment of grain : For each pesticide ten batches of 15kg of wheat were weighed out and put into plastic bags prior to treatment. The mc of five batches of grain were adjusted to 17 % and five batches to 16 % which are in equilibrium with 75% rh at 10^{o}C and 25^{o}C respectively (as taken from CSL's Integrated Grain Store Manager software and based on work of Pixton, 1982). The grain was left for 7 days in sealed plastic bags at 5^{o}C to thoroughly equilibrate. The mcs were then confirmed by drying a sample in a ventilated oven at 130^{o}C for 2 hours (ISO 712).

Each pesticide was applied at four doses (Table 2) to batches of grain at each mc and temperature. The liquid formulations were prepared by serial dilution of the highest dose using distilled water as a diluent. Each batch of grain was placed into a concrete mixer and 12 ml of the required dose (starting with the lowest) was applied using a hand held ('De Vilbis') paint sprayer whilst the grain was tumbling in the mixer. A batch of grain was similarly treated with 12 ml of distilled water alone to act as a control.

The doses of the solid formulations were applied by adding the required weight of compound directly to the grain whilst in the concrete mixer. A batch of grain was left untreated to act as a control.

The opening of the mixer was covered with a piece of plastic to ensure that there was no loss of the compound during the mixing process. The grain was then tumbled in the mixer for 15 minutes to ensure thorough mixing. Each batch of grain was then put into galvanised steel bins, measuring 58 cm high and 46 cm diameter, and covered with a muslin lid. The five bins containing grain at 17% mc were then stored in a CE room set at 10^{o}C and 75% rh, and the five bins with grain at 16% mc were stored in a CE room set at 25^{o}C and 75% rh.

Sampling of grain : One day, 4 weeks and 12 weeks after treatment, samples of approximately 4 kg of each dose of treated and control grain were removed from the bins, using a metal scoop. Approximately 100 g of each sample were put into plastic bags and stored in a freezer to await moisture content analysis. The remainder was used in the bioassays.

Mite bioassay : Each batch of treated and control grain was divided into approximately 50 g lots, put into wide-necked 120 ml bioassay jars and closed with filter paper lids. Five replicate jars were prepared for each treatment and each mite species. The jars were left to equilibrate in the appropriate test conditions for 24 hours.

The mite cultures were prepared in the following way : The initial culture population density was assessed by taking a level microspoonful of culture and counting the numbers of live mixed stage mites under a binocular microscope using the Solomon's disc method described below. Food medium was then added to each culture flask, to adjust the proportion of food to mites, so that a level microspoon contained in the range of 400 - 600 mixed stage mobile mites. This was confirmed by re-counting and recording those numbers (n=3). The added food served as an initial food supply for the mites to aid establishment on whole grains and prevent population crashes.

A level microspoon of adjusted mite culture of each species was then placed into separate jars and the jars were re-closed with a filter paper lid. The jars were incubated in the test conditions for periods long enough to include the passing of at least one generation. Since the different mite species had different developmental rates from egg to adult in different conditions, exposure periods varied for each species. Therefore, assessments of the F_1 generation took place at approximately the following number of days after exposure to the grain :

Mite species	25°C	10°C
Acarus siro	14	56
Tyrophagus putrescentiae	16	- (will not breed in conditions)
Lepidoglyphus destructor	19	84

The contents of each jar were then sieved over a 710 µm mesh and the sievings were examined under a low power binocular microscope. If many live mites were observed (> approximately 80) the dust was transferred to a petri dish over a 'Solomon's disc' (a disc divided into 64 sectors of which 8 are blacked -

Solomon 1962). The dust was spread evenly over the surface of the dish using a seeker, and the numbers of live mites on the blacked out sectors were counted. This number was then multiplied by 8 to provide an estimate of the total mite numbers. The accuracy of this method in estimating mite numbers has been validated by a series of experiments where known numbers of mites were added to a 'Solomon's disc' and then recounted on the disc (See Appendix).

If lower numbers of mites were initially observed (about one per black segment), the dust was transferred to a petri dish, formed into a thin line and teased away using a seeker whilst counting the numbers of live mites. After each assessment, the grain from each jar was combined, put into a plastic bag and stored in a freezer to await moisture content analysis.

The mean percentage inhibition of each mite population was calculated for each treatment as a proportion of the numbers of mites in the controls.

Insect bioassay : Each batch of treated and control grain was divided into approximately 50 g lots, put into wide-necked 120 ml bioassay jars and closed with filter paper lids. The inner rim of the jars used to contain *Oryzaephilus surinamensis* (L.) and *Sitophilus granarius* (L.) were previously coated with 'fluon' (PTFE) to prevent escapes. Five replicate jars were prepared for each treatment and each insect species. The jars were left to equilibrate in the appropriate test conditions for 24 hours.

Batches of 25 insects were then removed from the laboratory cultures and placed into each jar. The jars were re-closed with a filter paper lid and left in the test conditions for 7 days. The contents of each jar were then emptied on to an enamel tray and the numbers of live, knocked down and dead insects were recorded. An insect was considered knocked down if it was on its back and unable to right itself even when aided with a small brush. It was considered dead if no visible movement was detected. The mean percentage adult mortality was calculated for each species exposed to each treatment.

The grain from jars held at 25^{o}C was then returned to the jars and incubated for a further 6, 7 and 10 weeks for *S. granarius*, *O. surinamensis* and *Rhyzopertha dominica* respectively, to allow for development of the F_1 generation. The grain and insects were then separated using a 2mm sieve and the insects were emptied on to an enamel tray. The numbers of live and dead F_1 adults were then counted. The grain was also emptied on to the tray and any adults inside or emerging from the grains were counted.

The mean percentage inhibition of each insect population was then calculated for each treatment as a proportion of the numbers of insects in the controls.

RESULTS AND DISCUSSION

Moisture content : One day after treatment, grain stored at 10°C and 75 % r.h., with an intended mc of 17 %, was found to vary between 16.7 - 17.4 % (Table 11a). However, after 4 weeks the levels were found to have decreased to between 16.3 - 16.7 %, and after 12 weeks levels of 15.9 - 16.3 % were recorded (Table 11a). These results indicate that over a period of 12 weeks the grain had dried by approximately 1 %. Investigation of the CE room conditions found that the r.h. of the room was approximately 5 % lower than the reading given on the control panel. In response to this, the r.h. was increased to give a reading of 80 % in order to compensate for any irregularities.

The mc of grain stored at 25°C and 75% r.h., with an intended mc of 16%, was seen to remain fairly constant throughout the storage period with levels seen to vary between 15.7 and 16.6 %, indicating little fluctuation in the storage conditions (Table 11a).

Control numbers : On grain stored at 10°C, low numbers of mites in the control samples were consistently observed throughout the experiment, particularly with *L. destructor*. Table 11b shows the m.c. of the grain used in the bioassays after the counts were complete. Compared to the mcs of the samples taken from the bins at each assessment period (Table 11a), it can be seen that during the exposure periods in the bioassay jars the mc of the grain decreased by between 0.03 - 1.5 % (Table 11b). Therefore as the r.h. of the CE room was lower than intended, the grain lost moisture when held in the jars and bins. In general, where the mc of the grain was approximately 15.8 % or below, low mite numbers in the controls were recorded. At 10°C, this mc is in equilibrium with approximately 68 % r.h. (as taken from CSL's Integrated Grain Store Manager software and based on work of Pixton, 1982). For *L. destructor*, these conditions are just outside the limits for this mite to complete its development and there is also known to be heavy juvenile mortality at 70% r.h. (Cunnington, 1976). *Lepidoglyphus destructor* has been found to be less tolerant of low humidity than *A. siro* in the laboratory (Cunnington, 1976), which may explain why such low numbers of this species were recorded in some of the experiments.

On grain stored at 25°C, the mc of the grain was seen to fluctuate only slightly during the experimental period (Table 11a), however, low control numbers were again observed, particularly at the 12 week assessment, mainly with *L. destructor* and *O. surinamensis*. During the assessments a noticeable amount of fungal growth, identified as *Eurotium spp*, was observed on the grain. Some storage fungi are known to be toxic to insects and have detrimental effects on development (Sikorowski, 1964 and David et al., 1974). *Oryzaephilus surinamensis* has been found to be unable to complete development on diets comprising of different *Aspergillus* spp. (Sikorowski, 1964). Storage fungi have also been found to have detrimental effects on mite growth and some have been shown to be pathogenic (Solomon et al, 1964).

Protect-it

Mites : At 25°C, inhibition of each mite species and strain was seen to increase when exposed to grain that had been stored for the longest period. At 3 g kg $^{-1}$, mean inhibitions were seen to range from 55 - 82 % on grain treated one day previously, and increased to 94 - 99.8 % following exposure to grain that had been treated for 12 weeks (Table 12, Figures 6 and 7). This may have been due to gradual permeation of the dust through the grain during storage, so that grain sampled at 12 weeks contained more dust than that sampled one day after treatment. In general, a dose of between 1 and 3 g kg $^{-1}$ was effective at inhibiting all mite populations by > 95% when exposed to grain that had been treated for at least 4 weeks.

At 10°C, a lower dose range was effective with, in general, 0.5 to 1 g kg $^{-1}$ found to inhibit all mite populations by > 95 % (Table 13, Figures 8 and 9). The top dose of 3 g kg $^{-1}$ produced mean inhibitions of > 99 % throughout the experiment. The increased efficacy at the lower temperature may have been due to the extended exposure periods of the mites to the treated grain compared to those held at 25°C. However, Cook and Armitage (1999) also found increased efficacy of another inert dust, 'Dryacide', against mites on grain stored at 10°C compared to experiments at 25°C after the same exposure periods. This is encouraging, as mite populations in stored grain usually peak in the winter in the UK, when surface layers absorb moisture and levels rise towards 17%.

Previous experiments have found *L. destructor* to be more tolerant to inert dusts than other mite species (Collins et al, 1999; Cook and Armitage 1999). However, these experiments have found relatively little difference between species in efficacy. There was also little difference observed in the effects between the susceptible and resistant strains, although in the 10°C experiments, the resistant strain of both mites appeared slightly less tolerant than the susceptible strain at the lower doses.

Insects : At 25°C, complete mortality of both strains of adult *O. surinamensis* was achieved after 7 days exposure to 3 g kg $^{-1}$, with mortalities decreasing to < 10 % at 0.25 g kg $^{-1}$ (Table 14). Against *S. granarius*, mortalities of approximately 50 % were achieved at 3 g kg $^{-1}$ with < 10% recorded at the other doses (Table 14). Against the susceptible strain of *R. dominica*, mortalities of approximately 30% were recorded at 3 g kg $^{-1}$, with 90% mortality of the OP resistant strain at weeks 4 and 12 (Table 14). At 10°C, lower mortalities were recorded for all species after 7 days exposure with maximum mortalities of 98, 18 and 10% recorded for *O. surinamensis, S. granarius* and *R. dominica* respectively (Table 15). The decreased efficacy at the lower temperature is not unexpected and may be due to reduced insect activity resulting in a decrease in pick-up of the inert dust. Aldryhim (1990, 1993) found *S. granarius* and *R. dominica* to be more susceptible to 'Dryacide' at 30°C than at 20°C, and suggested this may have been due to the reduced mobility of the

beetles at the lower temperature, leading to a reduction in the accumulation of the silica dust particles on the insects.

Doses of between 0.5 and 1 g kg $^{-1}$ produced 90 - 100 % inhibition of both strains of *O. surinamensis* throughout the experiment (Table 16; Figures 10 and 11). At 3 g kg $^{-1}$, > 95 and 99 % inhibition of the susceptible and OP resistant strains of *R. dominica* was respectively achieved. At the lower doses, the resistant strain of *R. dominica* was also inhibited to a greater extent than the susceptible strain. This may have been due in part to the greater efficacy against the parent beetles following the 7-day exposure and may also indicate difference in tolerance between strains. Against *S. granarius*, maximum mean inhibitions of around 73 % were recorded (Table 16; Figures 10 and 11). Previous studies have shown *S. granarius* to be among the most tolerant insects to inert dusts (LePatourel, 1986, Desmarchelier and Dines, 1987) with longer exposure times needed for control compared to *S. oryzae* and mite species (McLaughlin, 1994; Cook and Armitage, 2000). Lack of efficacy against *S. granarius* is an important consideration as it is a common pest in U.K. grain stores.

Bifenthrin

Mites : At 25°C, both strains of *L. destructor* were inhibited by > 99.6 % at 2 mg kg $^{-1}$ at all assessment periods, and against *A. siro* at one day and 4 weeks after treatment (Table 17, Figures 12 and 13). Against *T. putrescentiae*, the highest inhibition recorded was 78%, one day after treatment. With all the species the OP resistant strains appeared slightly more tolerant of bifenthrin than the susceptible strains.

At 10°C, > 95% inhibition of the susceptible strain of *A. siro* was achieved with all but one dose throughout the experiment (Table 18, Figures 14 and 15). Against the resistant strain doses of 1 mg kg $^{-1}$ and above produced the same degree of inhibition. With the susceptible strain of *L. destructor* > 99% inhibition was achieved following exposure to doses of 1 mg kg $^{-1}$ and above, whereas with the resistant strain doses of 0.5 mg kg $^{-1}$ and above produced the same level of inhibition (Table 18, Figures 14 and 15). The greater efficacy of bifenthrin on wheat stored at 10^{o}C may have been due to the extended periods of exposure at the lower temperature. It is also known that pyrethroids have negative temperature coefficients i.e. toxicity is greater when insects are held at lower temperatures (Subramanyam and Cutkomp, 1987; Thaung and Collins, 1986; Longstaff and Desmarchelier, 1983; Watters et al., 1983).

Binns (1989) recorded respective mortalities of 100% and 50% of *A. siro* following 14 days exposure to wheat treated with 1 mg kg $^{-1}$ bifenthrin, one day and 4 weeks after treatment at 17.5^{o}C and 75% rh, although by 12 weeks the treatment was ineffective. The present experiments demonstrate a higher level of inhibition.

Insects : Complete mortality of susceptible *O. surinamensis* adults was achieved throughout the experiment after 7 days exposure on wheat at 25°C, apart from the lowest dose at 12 weeks, where 99.2% died (Table 19). Against the OP resistant strain, mortalities of between 90 and 100% were achieved at 0.5 - 2 mg kg $^{-1}$ (Table 19). Against *S. granarius*, maximum mortalities of 94 % and 59 % were recorded at 2 mg kg $^{-1}$ for the susceptible and resistant strains respectively (Table 19). The resistant strain of *R. dominica* appeared less tolerant than the susceptible strain. Complete mortality was achieved throughout the experiment other than at 0.25 mg kg $^{-1}$ at 4 weeks, whereas with the susceptible strain mortalities ranged from 57 - 100% (Table 19). At 10°C the mortalities recorded were lower than those at 25°C. Against *O. surinamensis* mortalities ranged from 47 - 100 % and 16 - 100 % for the susceptible and resistant strains respectively (Table 20). With *R. dominica* there appeared little difference between strains with mortalities of 40 - 100 %. Lower mortalities were recorded with *S. granarius* with maxima of 46 and 18 % recorded for the susceptible and resistant strains respectively (table 20).

Almost complete inhibition of both strains of *O. surinamensis* and *R. dominica* was achieved following incubation of wheat at 25°C at all doses (Table 21, Figures 16 and 17). However, low numbers of progeny were recorded in the control samples at the 12-week assessment with both species, which may have been due to an effect of the fungi detected on the wheat (Table 21). With *S. granarius*, exposure to 2 mg kg $^{-1}$ inhibited the susceptible strain by > 95% and the resistant strain by > 89 %, 4 weeks after treatment.

Flufenoxuron

Mites : At 25°C, the susceptible and resistant strains of *A. siro* were inhibited by 71 - 92 % and 60 - 88 % respectively, following exposure to 2 mg kg $^{-1}$, over the experimental period (Table 22, Figures 18 and 19).

The compound was most effective against *L. destructor* with, in general, >99 and 97 % inhibition of the susceptible and resistant strains respectively at 0.5 mg kg $^{-1}$ and above at one day and 4 weeks after treatment (Table 22, Figures 18 and 19). At 12 weeks the efficacy had decreased slightly with a lack of inhibition at 1 and 0.1 mg kg $^{-1}$ with the resistant strain. At this assessment, the numbers of live mites in the controls were very low, with means of 65 and 11 for the susceptible and resistant strains respectively, making efficacy assessments difficult (Table 22). The compound was least effective against *T. putrescentiae*, with the susceptible and resistant strains inhibited respectively by 40 - 66 % and 48 - 92 % at the top dose (Table 22, Figures 18 and 19).

The compound appeared more effective against the mites at 10°C than at 25°C which may have been due to the extended exposure periods at the lower temperature. Against susceptible *A. siro*, > 85 % inhibition at

0.5 mg kg $^{-1}$ was recorded at one day and 4 weeks after treatment, with a reduction in efficacy at 12 weeks (Table 23, Figure 20). Against the resistant strain there was between 73 - 92 % inhibition at 0.5 mg kg $^{-1}$ and above, at one day and 4 weeks and again a reduction at 12 weeks (Table 23, Figure 24).

Against *L. destructor*, there was between 92 - 99.5 % and 91 - 99 % inhibition of the susceptible and resistant strains respectively at all doses and assessment periods (Table 23, Figures 20 and 21). Low control numbers of both species were recorded at the 4 and 12 weeks assessments.

Flufenoxuron acts as an chitin synthesis inhibitor. The cuticle in mites acts as an attachment site for muscles, protects against physical damage, penetration of pathogens and desiccation with the procuticle comprising of mainly chitin and protein. Acylureas, of which flufenoxuron is one, affect the immature stages of mites and also prevent hatching of eggs. They interfere with the moulting process by inhibiting chitin incorporation into the mite cuticle but their precise mode of action is not known, although they do not appear to inhibit the enzyme chitin synthetase. Against spider mites flufenoxuron has been found to be principally effective against immature stages undergoing moults, and it has also been found that treated females lay sterile eggs (Anderson et al., 1986).

It was observed throughout the mite assessments that there appeared to be a noticeable number of unhatched eggs present in the treated replicates. These observations would suggest that the compound has a similar mode of action against storage mites as observed in spider mites. Also, complete inhibition of the mite populations was rarely achieved as some mites were always present. These are most likely to have been the adult mites initially introduced which may have been unaffected by the compound.

Insects : Low mortalities of < 3% were recorded after 7 days exposure (Tables 24 and 25). Exceptions were with *O. surinamensis* on grain stored at 10^{o}C, where mortalities of 10, 14 and 11% were recorded respectively at 2, 1 and 0.5 mg kg $^{-1}$ at 12 weeks after treatment (Table 25).

With the susceptible strains of *O. surinamensis* and *R. dominica*, development was inhibited by 92 - 100 % and 89 - 96 % for the respective species at doses of 0.5 mg kg $^{-1}$ and above (Table 26, Figure 22). The compound was less effective against *S. granarius*, with the highest inhibition of 49 % recorded at the 12 weeks assessment at the lowest dose, although the ranges were seen to overlap between the doses (Table 26, Figure 22).

Against the resistant strains, similar patterns of inhibition were seen. Against *O. surinamensis* and *R. dominica* population development was inhibited by 95 - 100 % and 85 - 95 % respectively, at doses of 0.5 mg kg $^{-1}$ and above (Table 26, Figure 23). Whereas against *S. granarius* the compound was again less

effective with a maximum inhibition of 52 % recorded at 2 mg kg $^{-1}$, one day after treatment although again the ranges overlapped (Table 26, Figure 23).

Other workers have also found that the dose required to control *Sitophilus* species with other insect growth regulators, is often high (Bengston, 1987). McGregor and Kramer (1976) reported that another chitin synthesis inhibitor, diflubenzuron, exerted a delayed effect on many stored product insects. It is therefore likely that insects exposed continuously would not produce progeny, except in the short period immediately after exposure when parent adults are unaffected by the pesticide. These delayed effects may not have been detected in these experiments as the parent adults were only exposed to treated grain for 7 days. Therefore the progeny produced may have resulted from adults that had not been affected by the pesticide in the short term of the exposure. Other workers have found that extending the exposure period of adult beetles to insect growth regulators increases the inhibition of the F_1 progeny (Desmarchelier and Allen, 1992; Eisa and Ammar, 1992; Elek and Longstaff, 1994). This effect will be investigated in the next stage of this project, where adults will be continuously exposed to treated grain over a six month experimental period.

Sodium polyborate

Mites : At 25^{o}C against susceptible *A. siro*, 2 g kg $^{-1}$ produced the greatest inhibition of 99.9% one day after treatment with reduced efficacy at the other assessment periods (Table 27, Figure 24). Against the resistant strain, doses of at least 0.5 g kg $^{-1}$ produced > 96% inhibition at one day after treatment, and thereafter, 2 g kg $^{-1}$ produced 99 and 97 % inhibition at 4 and 12 weeks after treatment respectively (Table 27, Figure 25).

Against both strains of *L. destructor*, > 99% inhibition was achieved at doses of at least 0.5 g kg $^{-1}$ at one day, 1 g kg $^{-1}$ at 4 weeks and 2 g kg $^{-1}$ at 12 weeks after treatment (Table 27, Figures 24 and 25).

Against *T. putrescentiae* doses of at least 0.5 g kg $^{-1}$ produced > 99% inhibition at one day with 2 g kg $^{-1}$ effective 4 weeks after treatment. At 12 weeks after treatment 2 g kg $^{-1}$ produced approximately 92 and 98% inhibition of the susceptible and resistant strains respectively (Table 27, Figures 24 and 25).

At 10^{o}C against *A. siro*, the compound again appeared more effective against the resistant strain than against the susceptible. A dose of 2 g kg $^{-1}$ produced 100% inhibition of the resistant strains throughout the experiment, whereas against the susceptible strain the same dose produced a mean inhibition of 99.9 % only at one day after treatment (Table 28, Figures 26 and 27).

Against *L. destructor*, doses of at least 0.5 g kg $^{-1}$ produced complete inhibition of the susceptible strain throughout the experiment, whereas against the resistant strain doses of at least 1 g kg $^{-1}$ were required for the same degree of inhibition (Table 28, Figures 26 and 27).

These results agree well with previous work which found that 1 g kg $^{-1}$ produced approximately 96% inhibition of a susceptible strain of *A. siro*, and doses of at least 400 mg kg $^{-1}$ produced complete inhibition of *L. destructor*, when tested one day after treatment and exposed to similar conditions (CSL Contract report no. FN3600).

Insects : At 25^{o}C, 100% mortality of susceptible *O. surinamensis* was achieved following 7 days exposure to 2 g kg $^{-1}$ one day after treatment which decreased to 50%, 12 weeks after treatment (Table 29). Against the resistant strain, mortality decreased from 74 to 5 %. In the same time against *S. granarius* and *R. dominica* low mortalities were achieved throughout the experiment with maxima of 1.6 and 8 % recorded for the respective species (Table 29). At 10^{o}C, low mortalities of < 3.2% were recorded for each species and strain throughout the experiment (Table 30).

Against *S. granarius*, a dose of 2 g kg $^{-1}$ produced complete inhibition of both strains throughout the experiment (Table 31, Figures 28 and 29). With *O. surinamensis* and *R. dominica*, 1 and 2 g kg $^{-1}$ produced inhibitions of 96 - 100 % and 98 - 100 % of both strains respectively throughout the experiment (Table 31, Figures 28 and 29).

Previous experiments also found sodium polyborate to be ineffective at killing adult insects following a short term exposure of 7 days (CSL Contract report no. FN3600). It appears to work more effectively at preventing population development. It has been suggested that sodium polyborate acts partly as a desiccant, although compounds of a similar composition are normally considered to have chemosterilant activity.

Benzyl benzoate

Mites : Against *A. siro* at 25^{o}C, maximum inhibitions of 98 and 100 % were recorded against the susceptible and resistant strains respectively at 200 mg kg $^{-1}$, but only at one day after treatment, with efficacy decreasing after this time (Table 32, Figures 30 and 31). Against *L. destructor* almost complete inhibition was achieved against both strains at all doses at one day after treatment. After this time efficacy decreased with the top dose inhibiting population development by between 94 and 99.6 % (Table 32, Figures 30 and 31). Against *T. putrescentiae*, the resistant strain was inhibited by > 99% at 200 and 150 mg kg $^{-1}$ at one day after treatment, whereas against the susceptible strain only the top dose provided the same degree of inhibition (Table 32, Figures 30 and 31). Large variations in populations were observed between the doses

so that in many cases the ranges overlapped. This may have been due to uneven distribution of the compound on the grain so that some samples contained more or less active ingredient than others.

At 10°C, 99.9 and 100% inhibition of the susceptible strain of *A. siro* was achieved at 200 mg kg $^{-1}$ at one day and 4 weeks after treatment respectively (Table 33, Figures 32 and 33). Against the resistant strain 100% inhibition was achieved after exposure to at least 150 mg kg $^{-1}$, one day after treatment, although efficacy declined thereafter. Against *L. destructor* both strains were completely inhibited at all doses at one day after treatment and then by >99 % at 200 mg kg $^{-1}$, 4 weeks after treatment (Table 33, Figures 32 and 33). However, the control numbers of *L. destructor* were very low so that the high inhibitions recorded may have been due to a combined effect of the pesticide and natural mortality.

Commercial products containing benzyl benzoate have proved to be effective against house dust mites when used to treat carpets, furnishings and upholstery (Bischoff et al., 1989; Schober et al., 1987)

Insects : Low adult mortality was observed of insects exposed for 7 days on grain stored at 25°C. Maximum mortalities of 25, 1.6 and 3.2 % were recorded for *O. surinamensis*, *S. granarius* and *R. dominica* respectively (Table 34). Higher mortalities were recorded on grain stored at 10°C, with maximum mortalities of 99, 8 and 45 % recorded for the respective species one day after treatment (Table 35).

Low inhibitions of both strains of *S. granarius* and *R. dominica* were achieved with maximums of approximately 25 and 40 % for the respective species. Also in most cases, the inhibition ranges overlapped between doses (Table 36, Figures 34 and 35). Against *O. surinamensis*, the highest dose of 200 mg kg $^{-1}$ produced inhibitions of 96 and 84 % for the susceptible and resistant strain respectively at one day after treatment. There was a decrease in efficacy at 4 weeks and an increase at 12 weeks with inhibitions of 92 and 80 % recorded for the respective strains (Table 36, Figures 34 and 35). Again there was some degree of overlap between doses.

Azadirachtin

Mites : Against the mites at 25°C, little difference in efficacy was initially observed between species and strains, with maximum mean inhibitions of 97, 93 and 95 % recorded for *A. siro, L. destructor* and *T. putrescentiae* respectively at 100 mg kg $^{-1}$. The ranges were, however, seen to overlap between the top dose and 75 mg kg $^{-1}$ (Table 37, Figures 36 and 37). Efficacy then declined at 4 and 12 weeks after treatment.

At 10°C against *A. siro*, little difference in efficacy was observed between the strains or at the different times after treatment. Mean inhibitions ranging from 86 - 91 % and 84 - 90 % were recorded for the

susceptible and resistant strains respectively at 100 mg kg $^{-1}$ over the experiment (Table 38, Figures 38 and 39). Against *L. destructor*, 100 mg kg $^{-1}$ inhibited the susceptible strain by 96, 89 and 84 % at one day, 4 weeks and 12 weeks after treatment respectively; and the resistant strain by 96, 79 and 76 % at the respective times (Table 38, Figures 38 and 39).

Insects : Low mortalities (generally <5 %) of the adult beetles were recorded following the 7 days exposure on grain stored at 10 and 25^{o}C (Tables 39 and 40). This lack of efficacy on adult mortality is not unexpected as the known effects of azadirachtin are predominantly on growth regulation, reproduction and feeding (Mordue-Luntz and Blackwell, 1993). The effects on feeding may not have been demonstrated in the short term of the bioassay, however a longer exposure period may provide more of an indication of this effect and whether starvation would lead to an increase in adult mortality. This will be investigated in future experiments.

Almost complete inhibition of both strains of *O. surinamensis* was achieved at all doses and assessment periods (Table 41, Figures 40 and 41). Where <100% inhibition was recorded, only single adults were found all of which were dead. During the assessments dead small larvae were observed in the jars indicating that the parent adults were able to mate and lay eggs, but that the larvae were unable to survive the treatment.

Little difference in efficacy was observed between the two strains of *R. dominica*, with >98 % inhibition at 100 and 75 mg kg $^{-1}$ at one day and 4 weeks after treatment, with reduced efficacy at 12 weeks (Table 41, Figures 40 and 41). Little difference was also observed between these two doses with ranges seen to overlap. The same results were reported by Rahim (1998) who noted that the efficacy of an azadirachtin-enriched neem kernel extract did not differ at application rates of 75 mg kg $^{-1}$ and above, although efficacy was seen to be more prolonged in those experiments.

Against *S. granarius*, little difference between the strains was observed with the susceptible and resistant populations inhibited by 66 - 79 % and 63 - 75 % respectively, following exposure to 100 mg kg $^{-1}$ over the experimental period (Table 41, Figures 40 and 41). During the assessments, a number of grains were dissected and found to contain dead, disfigured pre-adults. This may suggest that larval development was relatively protected when inside the grain but as development increased there may have been greater contact with the pesticide in the outer layers thereby having more of an effect. It was also noted that at the doses used in this experiment, there was a noticeable odour from the compound that did appear to taint the grain.

Table 42 summarises the results of the inhibition experiments and shows the doses required of each pesticide to inhibit the development of each pest population by > 95%. The results show the different

tolerances of the different species and strains to the compounds, with *S. granarius*, being the most tolerant species. Efficacy against *S. granarius* is an important consideration as it is one of the common insect pests encountered in U.K. grain stores and is often tolerant of control measures. In general, lower doses of the pesticides were required to inhibit the mite populations by > 95% when exposed at 10^{o}C compared to 25^{o}C. This is probably due to the extended exposure periods when held at the lower temperature. In a field situation, exposure is unlikely to be limited to certain periods of time and so efficacy may be greater in more practical situations. This will be investigated in future experiments.

CONCLUSIONS

In order for compounds to be considered as effective alternatives to OPs for use as grain protectants in the U.K., there are several criteria that must be met. They must be effective against a range of pest species and strains, ideally achieving a rapid kill of existing infestations as well as inhibition of population development. Pest infestations can only develop in stored grain if all steps in reproduction - mating, oviposition, immature development, adult emergence and survival to reproductive age, are able to be completed. Therefore the ability of a compound to prevent population development may be considered as a more important criterion of effectiveness than a rapid kill effect on adults.

Of the compounds tested, flufenoxuron, sodium polyborate, benzyl benzoate and azadirachtin, showed low efficacy against adult beetles after the 7-day exposure period but were effective at inhibiting population development. This may have been due to the short period of exposure or inactivity against the adults stages. In a field situation, exposure is unlikely to be limited to certain periods of time and so efficacy may be greater in more practical situations. Lack of efficacy against adults is an important consideration when assessing potential grain protectants, as the presence of insects can lead to rejection of the grain. In unaerated grain stores in the U.K., adult beetles can survive for considerable periods of time. Cooling grain to below 5^{o}C kills insects and prevents mite breeding, but the surface layers may remain vulnerable to infestation.

These experiments have demonstrated the range of efficacies of the different compounds to the different pest species and strains. *Sitophilus granarius* appeared the most tolerant species with sodium polyborate, azadirachtin and bifenthrin shown to be the most effective at the doses assessed. It was difficult with some of the pesticides to accurately determine effective dose ranges against the different species as little previous work had been done under similar experimental conditions, therefore higher doses of some of the compounds may be required for effective control.

Potential alternatives must also be effective at different temperatures and mcs. Grain can enter the store from the field at temperatures and mcs above 20°C and 20% respectively. It is then cooled and dried ideally to below 5°C and 15% mc to prevent mite and insect development. However, conditions at the grain surface can vary according to the ambient temperature and rh of the store environment. In these experiments, in general, lower doses of the pesticides were required to inhibit the mite populations when exposed at 10°C compared to 25°C. This is probably due to the extended exposure periods at the lower temperature. It is, however, encouraging that all the compounds were effective in these conditions as mite populations in stored aerated grain usually peak in the winter in the UK, when surface layers absorb moisture and levels rise towards 17%. Against the adult insects, mortality was higher on grain stored at 25°C compared to 10°C, apart from with benzyl benzoate which was more effective at the lower temperature. The decreased efficacy at the lower temperature is not unexpected and may be due to reduced insect activity resulting in a decrease in pick-up and metabolism of the pesticide.

In the U.K., grain is often stored for extended periods. These can last from a few months to a season, which runs from about August to May, or in the case of intervention grain, several years. However, there is still the need for high quality pest-free grain. Any potential alternatives must therefore provide prolonged protection. In general, higher doses of the compounds were required to achieve the same degree of protection as storage time increased, apart from with Protect-it, where efficacy appeared to increase with time. This may have been due to gradual movement of the dust through the grain during storage.

Ideally any compound to be considered as a replacement for OPs should already be registered for use on stored grain, but in the U.K. only OPs are approved for application to grain. Protect-it and bifenthrin are already registered for use in grain stores in some countries, with commercial products of sodium polyborate and benzyl benzoate approved for use in domestic environments. A commercial product of flufenoxuron has HSE approval for professional use in the U.K., and although commercial products of azadirachtin are available, they are not registered for use in this country.

Considering the overall efficacy of the compounds and in view of the extent of previous research, it was decided that future experiments should include azadirachtin and flufenoxuron. These compounds were effective against most of the pest species under the conditions of the experiment, and appeared to warrant further investigation. 'Protect-it' was also shown to be effective, corroborating the previous findings with the diatomaceous earth (Cook and Armitage, 1999 and 2000). Experiments in part 5 of this project aimed to gain information on efficacy against an existing infestation.

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Table 9 : Details of pest species used in experiments.

Species	Strain	Culturing conditions	Age at test	Stage at test	Resistance factor	Experimental methods used in resistance testing
Acarus siro	A47	15oC and 90% r.h.	Unknown age	Mixed stages	OP susceptible	
	A45	15oC and 90% r.h.	Unknown age	Mixed stages	x 14.7 (KD 99)	Pirimiphos-methyl treated filter papers 72 hr count - 1995
Lepidoglyphus destructor	G6	15oC and 90% r.h.	Unknown age	Mixed stages	OP susceptible	
	4016/2	15oC and 90% r.h.	Unknown age	Mixed stages		Survival on wheat treated with 8 mg/kg p-m 1988/89
Tyrophagus putrescentiae	Ex - Salami	15oC and 90% r.h.	Unknown age	Mixed stages	OP susceptible	
	T 96	15oC and 90% r.h.	Unknown age	Mixed stages		Survival on wheat treated with 8 mg/kg p-m 1992
Oryzaephilus surinamensis	Lab. susceptible	25oC and 70% r.h.	0 - 2 weeks	Adults	OP susceptible	
	8518B Faceby	25oC and 70% r.h.	0 - 2 weeks	Adults	x 2.9 (KD 99.9)	P-m treated filter papers 24 hr knock-down 1998
Sitophilus granarius	Windsor	25oC and 70% r.h.	2 - 4 weeks	Adults	OP susceptible	
	Gainsborough	25oC and 70% r.h.	2 - 4 weeks	Adults	x 2.79 (KD 99.9)	P-m treated filter papers 24 hr knock-down 1998
Rhyzopertha dominica	Salisbury	25oC and 70% r.h.	2 - 4 weeks	Adults	OP susceptible	
	915	25oC and 70% r.h.	2 - 4 weeks	Adults	x 0.5 (KD 99.9)	P-m treated filter papers 24 hr knock-down 1984

Table 10 : Details of experimental pesticides

Active ingredient	Product name	Formulation	Active ingredient concentration	Doses applied
Silicaceous dust	Protect-it	Dust	100%	0.25, 0.5, 1 and 2 g kg^{-1}
Bifenthrin	Talstar	Emulsifiable concentrate	100 g/l	0.25, 0.5, 1 and 2 mg kg^{-1}
Flufenoxuron	Motto	Suspension concentrate	30 g/l	0.1, 0.5, 1 and 2 mg kg^{-1}
Sodium polyborate	Flea ban	Powder	100%	0.25, 0.5, 1 and 2 g kg^{-1}
Benzyl benzoate	Acarosan	Powder	5%	50, 100, 150 and 200 mg kg^{-1}
Azadirachtin	Fortune AZA	Emulsifiable concentrate	3%	25, 50, 75 and 100 mg kg^{-1}

Table 11a - Moisture content (mc) and equilibrium relative humidity (erh) of wheat treated with each
compound at each assessment period

Compound	Storage temperature (^{o}C)	Mean percent mc and (erh) at each assessment period (n=10) One day	4 weeks	12 weeks
Protect-it	10	16.7 (74.1)	16.3 (72.3)	16.1 (71.3)
	25	15.8 (73.4)	15.8 (73.4)	16 (74.3)
Bifenthrin	10	16.8 (74.6)	16.3 (72.3)	16.1 (71.3)
	25	15.9 (73.9)	16.1 (74.8)	16.2 (75.2)
Flufenoxuron	10	16.7 (74.1)	16.5 (73.1)	16.3 (72.3)
	25	15.8 (73.4)	15.7 (72.9)	15.8 (73.4)
Sodium polyborate	10	16.8 (74.6)	16.6 (73.7)	15.9 * (70.3)
	25	15.9 (73.9)	-	16.3 * (75.7)
Benzyl benzoate	10	16.7 (74.1)	16.4 (72.7)	16.2 (71.8)
	25	15.9 (73.9)	15.8 (73.4)	15.8 (73.4)
Azadirachtin	10	17.4 (77.2)	16.7 (74.1)	16.1 (71.3)
	25	16.6 (77)	16.1 (74.8)	16.5 (76.5)

* - n = 2

Table 11b - Moisture content (mc) and equilibrium relative humidity (erh) of wheat used in mite bioassays,

at 10°C,
for each assessment period

Compound	Mite species	Mite strain	Mean percent mc and (erh) at each assessment period		
			One day	4 weeks	12 weeks
Protect-it	*A. siro*	susceptible	16.1 (71.3)	16.1 (71.3)	15.6 (68.8)
		resistant	16 (70.8)	16 (70.8)	15.7 (69.3)
	L. destructor	susceptible	15.9 (70.3)	16.1 (71.3)	15.8 (69.8)
		resistant	16 (70.8)	16.1 (71.3)	15.8 (69.8)
Bifenthrin	*A. siro*	susceptible	15.8 (69.8)	16.2 (71.8)	16 (70.8)
		resistant	15.8 (69.8)	16.2 (71.8)	15.9 (70.3)
	L. destructor	susceptible	15.7 (69.3)	16 (70.8)	15.8 (69.8)
		resistant	15.7 (69.3)	16.1 (71.3)	15.8 (69.8)
Flufenoxuron	*A. siro*	susceptible	16.1 (71.3)	15.8 (69.8)	15.8 (69.8)
		resistant	16.1 (71.3)	15.9 (70.3)	15.7 (69.3)
	L. destructor	susceptible	16.2 (71.8)	15.9 (70.3)	15.8 (69.8)
		resistant	16.2 (71.8)	15.8 (69.8)	15.9 (70.3)
Sodium polyborate	*A. siro*	susceptible	16.2 (71.8)	16.2 (71.8)	15.9 (70.3)
		resistant	16.1 (71.3)	16 (70.8)	15.9 (70.3)
	L. destructor	susceptible	15.9 (70.3)	15.9 (70.3)	15.7 (69.3)
		resistant	15.8 (69.8)	15.7 (69.3)	15.8 (69.8)
Benzyl benzoate	*A. siro*	susceptible	15.7 (69.3)	16.1 (71.3)	16.5 (73.2)
		resistant	15.6 (68.8)	16.2 (71.8)	16.5 (73.2)
	L. destructor	susceptible	15.8 (69.8)	16.1 (71.3)	16.2 (71.8)
		resistant	15.8 (69.8)	16 (70.8)	16.1 (71.3)
Azadirachtin	*A. siro*	susceptible	15.9 (70.3)	16.7 (74.1)	16.7 (74.1)
		resistant	15.9 (70.3)	16.6 (73.68)	16.8 (74.6)
	L. destructor	susceptible	16.4 (72.7)	16.4 (72.7)	16.7 (74.1)
		resistant	16.2 (71.8)	16.4 (72.7)	16.7 (74.1)

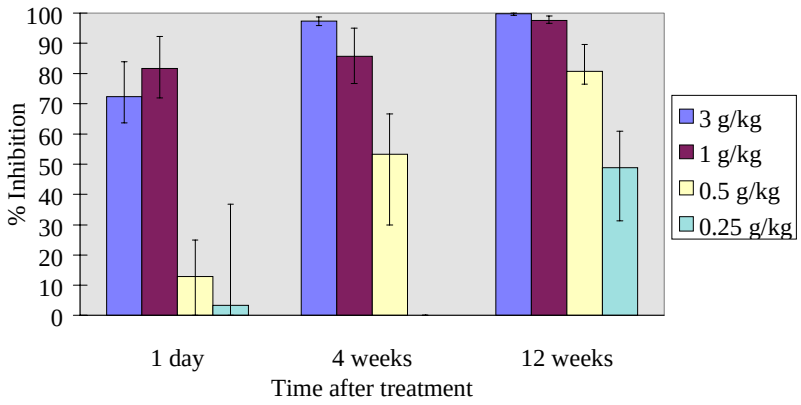
Bold line indicates when r.h. in CE room was increased to 80 %

Table 12 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Protect-It at different times after treatment on wheat stored at 25oC and 75% rh (n=5)

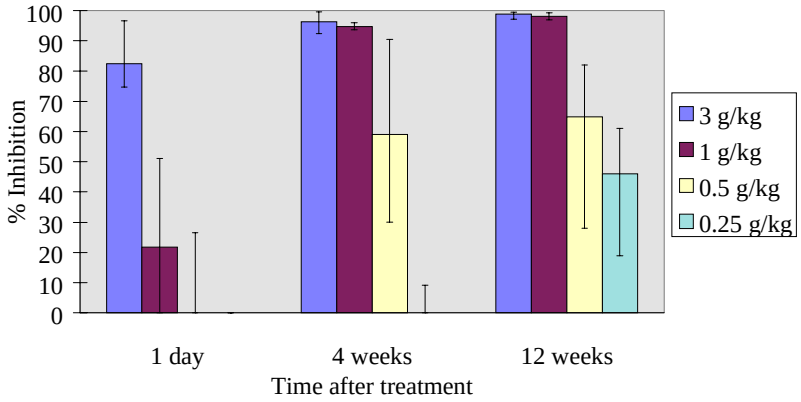
Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
A. siro susceptible	3924.8 (3496 - 4544)	72.3 (63.7 - 83.9)	81.7 (71.9 - 92.3)	12.9 (0 - 25)	3.3 (0 - 36.8)	240 (64 - 496)	97.4 (95.8 - 98.7)	85.7 (76.7 - 95)	53.3 (30 - 66.7)	0	920 (688 - 1056)	99.8 (99.2 - 100)	97.6 (96.6 - 99)	80.7 (76.5 - 89.6)	48.9 (31.3 - 60.9)
A. siro resistant	1604.8 (1128 - 2032)	75.9 (57.6 - 91)	56.4 (49.2 - 69.6)	0	0 (0 - 66.6)	1529.6 (1192 - 1768)	98.8 (97.8 - 99.4)	81.2 (65.5 - 88)	68.5 (59.7 - 79.1)	4.4 (0 - 40.4)	161.6 (88 - 312)	93.9 (90.7 - 96.9)	73.4 (40.6 - 92)	24.8 (0 - 55.4)	0
L. destructor susceptible	1896 (1480 - 2120)	82.4 (74.7 - 96.6)	21.8 (0 - 51.1)	0 (0 - 26.6)	0	1990.4 (1512 - 2752)	96.3 (92.4 - 99.6)	94.7 (93.6 - 96)	59.1 (30.1 - 90.4)	0 (0 - 9.2)	800 (592 - 976)	98.8 (97.1 - 99.5)	98.1 (96.9 - 99.3)	64.8 (28 - 82)	46 (19 - 61)
L. destructor resistant	364.8 (280 - 456)	55.3 (16.7 - 75.9)	4.4 (0 - 82.5)	0	0	542.4 (216 - 856)	99.4 (98.7 - 100)	99.2 (98.3 - 99.6)	48.1 (0 - 88.2)	43.4 (0 - 88.2)	356.8 (224 - 560)	99.8 (99.4 - 100)	98.5 (97.2 - 99.2)	70.9 (59.6 - 75.3)	44.4 (23.8 - 64.1)
T. putrescentiae susceptible	480 (144 - 592)	67 (33.3 - 81.7)	0 (0 - 80)	0	0	108.8 (40 - 328)	98 (96.3 - 100)	89.7 (81.6 - 97.2)	70 (19.1 - 99.1)	2.9 (0 - 77.9)	699.2 (504 - 760)	99.5 (99 - 100)	95.1 (91.6 - 98.3)	75.3 (64.5 - 85.1)	69.1 (58.8 - 84)
T. putrescentiae resistant	505.6 (216 - 728)	74.8 (32 - 99.8)	0 (0 - 46.2)	0	0	1201.6 (952 - 1432)	99.4 (99.3 - 99.6)	78.7 46.7 - 93.3)	69.9 (22.1 - 92.7)	0 (0 - 4.8)	99 (26 - 192)	99.4 (98 - 100)	87.1 (75.8 - 97)	51.9 (32.3 - 70)	38.2 (0 - 77.8)

Figure 6 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Protect-It on wheat stored at 25°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae

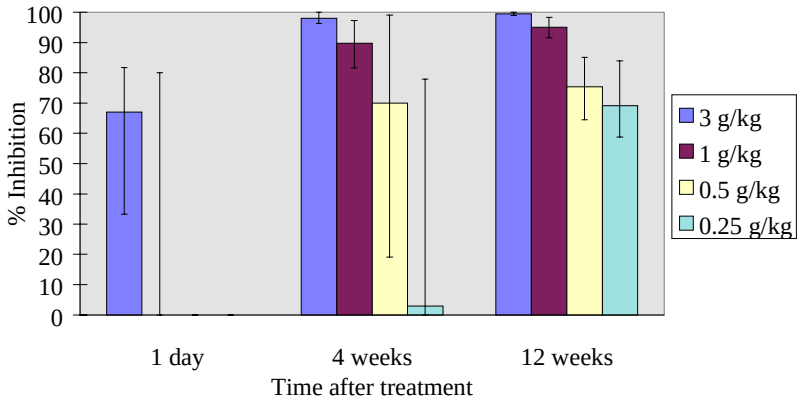
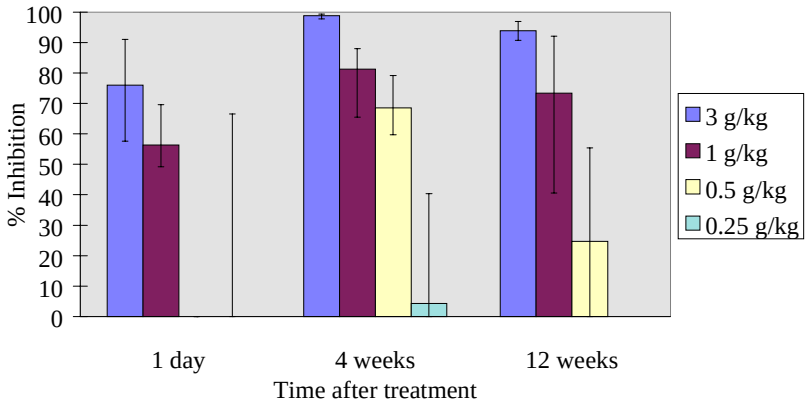
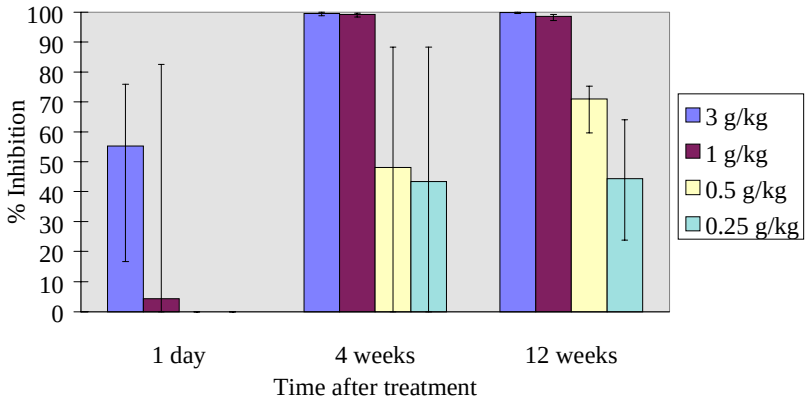


Figure 7 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Protect-It on wheat stored at 25°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae

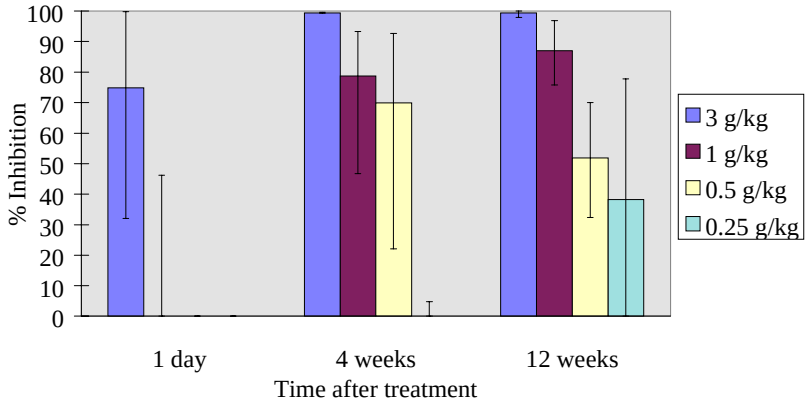
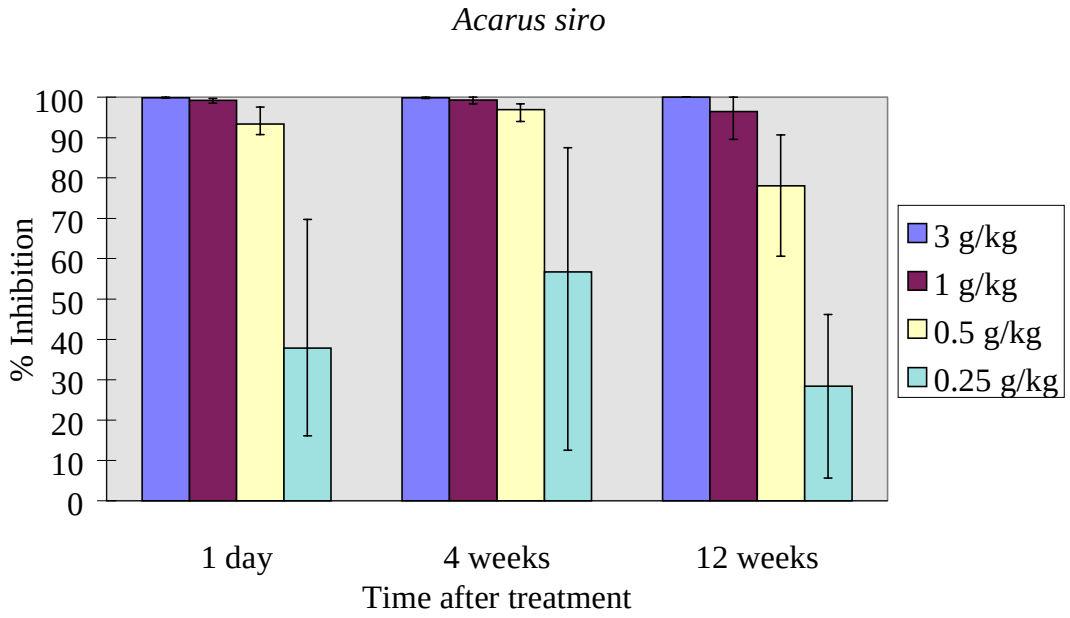


Table 13 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Protect-It at different times after treatment on wheat stored at 10oC and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
A. siro susceptible	2355.2 (1640 - 3168)	99.9 (99.8 - 100)	99.3 (98.6 - 99.7)	93.4 (90.8 - 97.6)	37.8 (16.1 - 69.8)	384 (248 - 432)	99.9 (99.7 - 100)	99.4 (98.4 - 100)	97 (94 - 98.4)	56.7 (12.5 - 87.5)	96.4 (82 - 122)	100	96.5 (89.6 - 100)	78 (60.6 - 90.7)	28.4 (5.6 - 46.1)
A. siro resistant	2428.8 (2008 - 2832)	99.9 (99.7 - 100)	98.4 (97.4 - 99.3)	94.3 (90.8 - 96.7)	50.1 (22.6 - 63.4)	332.8 (240 - 400)	100	99.8 (99.4 - 100)	97.2 (95.2 - 98.8)	72.9 (51.9 - 92.2)	183.2 (120 - 320)	100	99.5 (97.3 - 100)	99.3 (97.8 - 100)	93.9 (90.2 - 97.8)
L. destructor susceptible	1246.4 (728 - 1800)	99.8 (99.4 - 100)	99.9 (99.8 - 100)	93.3 (81.4 - 99.7)	61.4 (43.5 - 84)	1064 (216 - 1904)	99.9 (99.5 - 100)	91.2 (74.4 - 96.6)	87.5 (70.7 - 94)	2.6 (0 - 27.1)	155.2 (104 - 184)	100	99.9 (99.4 - 100)	93.7 (86.5 - 100)	83.2 (66.5 - 98.1)
L. destructor	497.6 (328 - 752)	98.6 (96.4 - 99.6)	98.8 (96 - 100)	86.5 (59.8 - 97)	47.3 (22.8 - 69.5)	299.2 (152 - 536)	100	98.7 (97.7 - 99.3)	94.6 (89.6 - 96.7)	40 (0 - 87.3)	47.4 (27 - 72)	100	100	98.7 (95.8 - 100)	94.5 (91.6 - 97.9)

Figure 8 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Protect-It on wheat stored at 10°C and 75% rh (n=5)



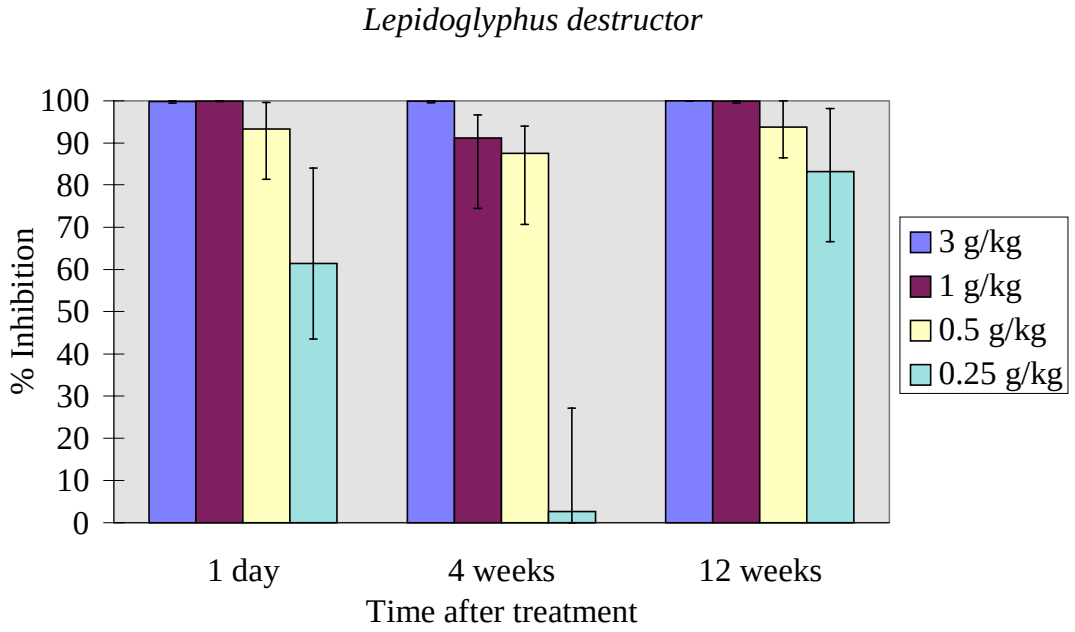
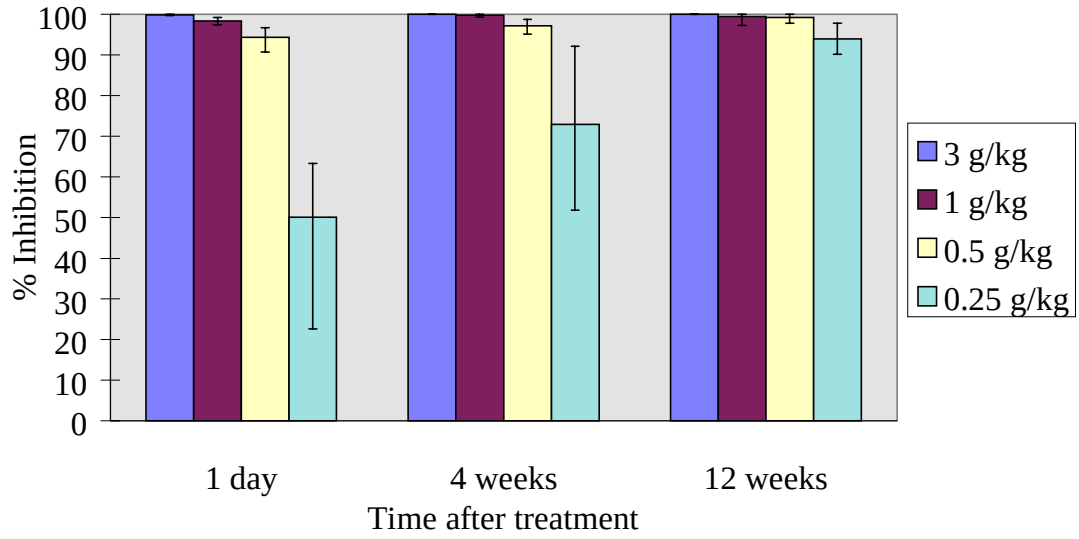


Figure 9 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Protect-It on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

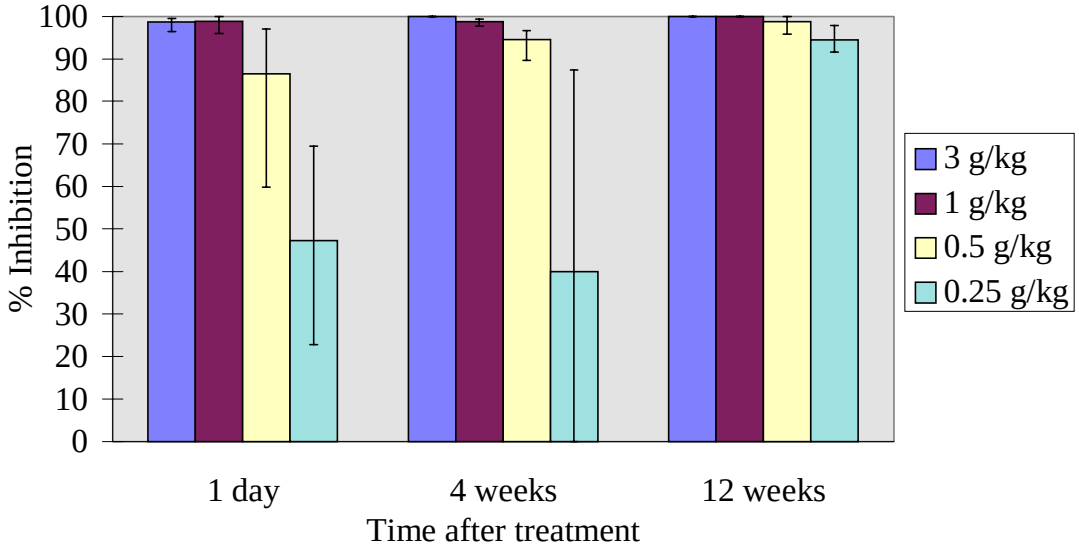


Table 14 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Protect-It at different times after treatment on wheat stored at 25oC and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
O. surinamensis susceptible	100	94.3	33	3.3	100	96.8	46	8.7	100	89.4	28.5	1.6
O. surinamensis resistant	100	80.5	18.9	4	100	41.6	8	2.4	100	70.4	13.6	1.6
S. granarius susceptible	49.2	0	0	0	56.9	4.1	2.4	0	41.7	0	0.8	0
S. granarius resistant	50.4	9.6	0	0	61.2	3.2	0.8	0.8	56.5	2.4	0	0
R. dominica susceptible	25.4	1.6	1.7	0	27	0	0	0	35.5	2.5	0	0
R. dominica resistant	60.8	19	0.9	0.9	90.1	17.4	0	0	91.1	6.5	1.7	0

Table 15 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Protect-It at different times after treatment on wheat stored at 10oC and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
O. surinamensis susceptible	53.2	8.6	3.2	0.8	96.9	32.8	8	4	98.3	62.7	0	0
O. surinamensis resistant	66.6	14.2	2.4	0	91	31.5	0	0	89.5	9	0.8	1.6
S. granarius susceptible	2.4	0.8	0.8	0	4.9	0	0.8	0	5.6	1.6	0.8	0
S. granarius resistant	0	4.8	2.5	0	12.8	4	1.6	0	17.7	10.5	4	0.8
R. dominica susceptible	0	0	0	0	0	0	0.9	0	5.6	6.5	0	0.8
R. dominica resistant	0	0.8	1.6	0	7.2	4	3.2	2.4	7.4	1.7	0	0

Table 16 : Mean % population inhibition and ranges of each beetle species exposed to 4 doses of Protect-it at different times after treatment on wheat stored at 25oC and 75% rh

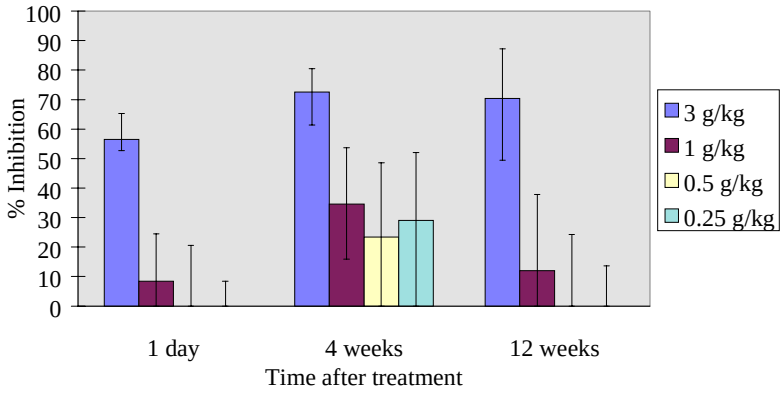
Figures in square brackets are the mean % of the F1 adults that were dead (n=5*)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		3 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
O. surinamensis susceptible	95.4 [6] (61 - 119)	100	100	92.9 [61.9] (84.3 - 99)	39.6 [10.6] (21.4 - 53.9)	105.4 [12.8] (60 - 122)	100	100	93.1 [77] (84.8 - 97.1)	45.5 [20.5] (22.2 - 68.7)	55 (45 - 74)	100	100	98.2 [100*] (92.7 - 100)	46.5 [20.6] (18.2 - 76.4)
O. surinamensis resistant	80.6 [6.7] (66 - 98)	100	100	90.1 [44.4] (83.9 - 96.3)	58.8 [22.5] (44.2 - 76.4)	72 [16.8] (55 - 86)	100	99.7 [100 *] (98.6 - 100)	96.4 [81.3 *] (94.4 - 100)	64.2 [24.2] (30.6 - 87.5)	27.4 (20 - 38)	100	100	98.5 [0*] (92.7 - 100)	86.9 [47.6] (74.5 - 100)
S. granarius susceptible	255.6 [0.3] (123 - 321)	56.5 [13.3] (52.7 - 65.2)	8.5 [0.4] (0 - 24.5)	0 [0.2] (0 - 20.6)	0 [0.1]	354.4 [0.1] (145 - 442)	72.6 [23.7] (61.3 - 80.5)	34.5 [0.5] (15.9 - 53.7)	23.4 [0] (0 - 48.6)	29.1 [0.1] (0 - 52)	273.2 [0.4] (216 - 335)	70.4 [6.6] (49.5 - 87.2)	12 [0.2] (0 - 37.8)	0 [0.2] (0 - 24.2)	0 [0.3] (0 - 13.6)
S. granarius resistant	208.6 [0] (173 - 288)	45.3 [2.1] (37.7 - 55.4)	2.6 [0.3] (0 - 40.6)	0 [0.2] (0 - 19.5)	0 [0] (0 - 35.3)	248.4 [0.2] (99 - 355)	61.3 [4.2] (55.3 - 75.4)	17.5 [0.4] (0 - 37.6)	34.5 [0.9] (8.2 - 64.6)	18.4 [0.3] (0 - 72.6)	320.2 [0.1] (296 - 350)	72.8 [1.3] (64.4 - 82.8)	35.6 [0.3] (19.7 - 47.5)	1.7 [0.1] (0 - 14.1)	28.2 [0.3] (6.3 - 62.2)
R. dominica susceptible	571.6 [0.2] (493 - 650)	96.9 [35] (96.2 - 97.4)	76.1 [0.9] (68.9 - 82.7)	45.2 [0.6] (38.2 - 59.1)	6.7 [0.4] (0 - 9.9)	537.2 [0.4] (547 - 652)	97.1 [46.2] (96.6 - 97.4)	72 [0.7] (68.9 - 77.7)	46.2 [0.7] (36 - 64.6)	5 [0.3] (0 - 14.4)	548.8 [0.3] (474 - 624)	95 [10.5] (93.1 - 96.9)	60.1 [0.3] (53.7 - 65.6)	19.5 [0.2] (11.8 - 34.6)	3.7 [0.1] (0 - 11.6)
R. dominica resistant	530.8 [1.1] (466 - 614)	99 [85.4] (97.6 - 99.6)	94.6 [13] (91.9 - 97)	57.9 [1.6] (54.2 - 64.6)	38.1 [0.7] (35.9 - 42.7)	540.8 [0.8] (424 - 672)	99.6 [90] (99.3 - 99.8)	93.1 [7.6] (91.1 - 95.2)	60 [1.4] (55.8 - 65.8)	24.3 [1.4] (15.5 - 37.5)	507 [0.5] (429 - 640)	99.4 [92] (99 - 99.6)	80 [1.6] (69.8 - 90.3)	42.3 [1.5] (30.4 - 62.3)	5.2 [0.6] (0 - 40.8

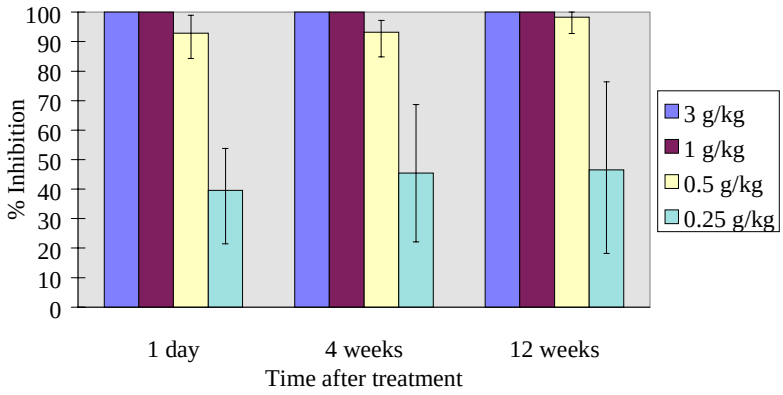
* - n = < 5 where 100% inhibition occurred in one or more replicates

Figure 10 : Mean % population inhibition (and ranges) of susceptible strains of insects exposed to 4 doses of Protect-It on wheat stored at 25ºC and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

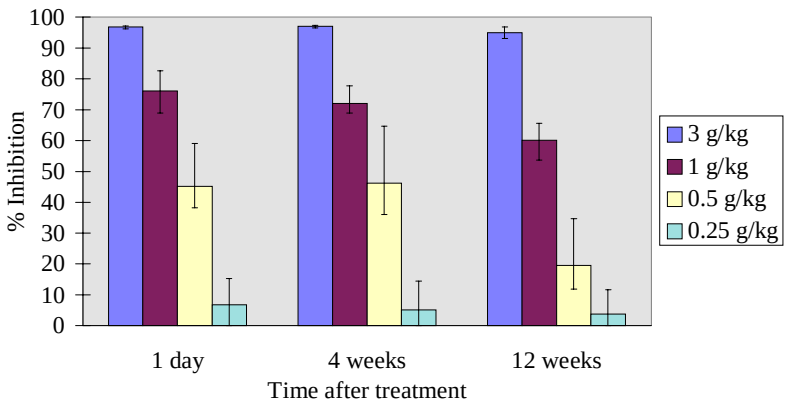
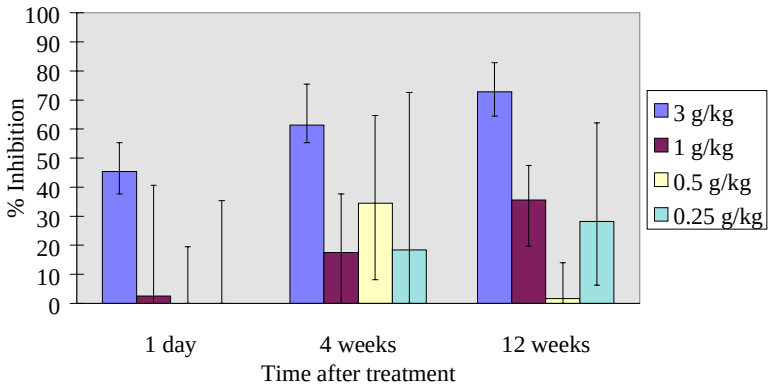
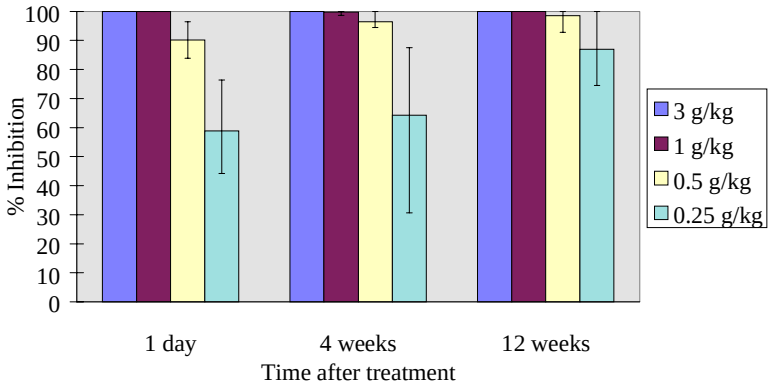


Figure 11 : Mean % population inhibition (and ranges) of OP resistant strains of insects exposed to 4 doses of Protect-It on wheat stored at 25°C and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

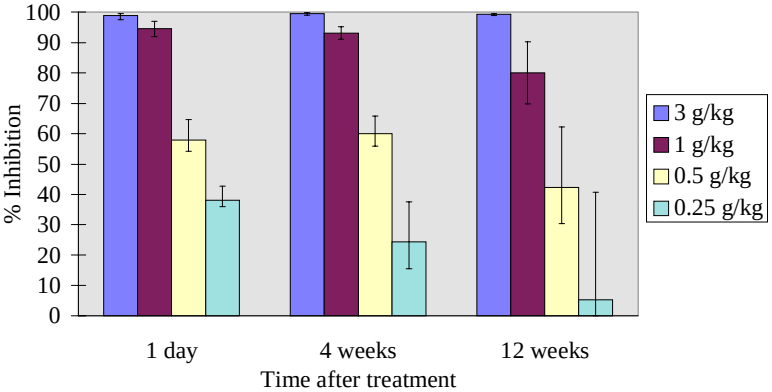


Table 17 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Bifenthrin at different times after treatment on wheat stored at 25oC and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
A. siro susceptible	2160 (1808 - 2664)	99.9 (99.9 - 100)	99.3 (99.2 - 99.5)	92.2 (85.9 - 95.2)	67.7 (36.6 - 78.9)	1510.4 (1184 - 1744)	99.9 (99.9 - 100)	98.9 (98.5 - 99.5)	91.8 (87.8 - 97.7)	49.9 (40.7 - 58.2)	2156.8 (1848 - 2360)	98.4 (97.5 - 99.3)	84.6 (80.3 - 90)	43.2 (26.9 - 54)	16.7 (0 - 63.6)
A. siro resistant	2532.8 (2264 - 3056)	99.8 (99.6 - 99.9)	89.5 (85.2 - 94.9)	66.9 (56.4 - 72.8)	24.1 (13.1 - 33.4)	904 (712 - 1056)	99.6 (99 - 100)	82.7 (67.3 - 92)	72.4 (61.9 - 81.4)	41.6 (35.4 - 47.8)	1942.4 (1776 - 2080)	91.4 (87.2 - 95.1)	52.9 (11.9 - 69.5)	46.4 (38.2 - 53.5)	4.3 (0 - 18)
L. destructor susceptible	915.2 (680 - 1176)	99.8 (99.6 - 99.9)	96.6 (94.2 - 98.7)	78.5 (71.2 - 85.1)	34.6 (23.1 - 42.3)	561.6 (496 - 728)	100	98.9 (98.2 - 99.5)	78.3 (70.1 - 85.8)	11.7 (0 - 40.2)	571.2 (352 - 672)	100	89.7 (87 - 96)	93.7 (88.1 - 97)	68.9 (46.8 - 81.8)
L. destructor resistant	328 (256 - 400)	99.8 (99.1 - 100)	92.6 (89 - 94.8)	62.4 (46.3 - 87.8)	3.4 (0 - 31.7)	384 (320 - 448)	100	97 (93 - 98.4)	40 (22.9 - 52.1)	0 (0 - 10.4)	521.6 (328 - 784)	99.9 (99.8 - 100)	93.1 (97.7 - 91.2)	42.3 (4.9 - 69.3)	45.7 (23.3 - 73.9)
T. putrescentiae susceptible	1560 (1256 - 2064)	78.6 (70.3 - 83.1)	43.6 (30.8 - 53.8)	30.1 (24.1 - 42.6)	11.9 (0 - 30.3)	896 (752 - 1072)	77.7 (70.5 - 85.7)	13.4 (0 - 30.4)	12 (0 - 33.9)	0 (0 - 23.2)	1736 (1408 - 2088)	58.4 (49.8 - 66.8)	16.1 (3.2 23)	9.4 (1 - 16.1)	8.6 (0 - 18.9)
T. putrescentiae resistant	539.2 (408 - 744)	77.4 (70.3 - 83.7)	48.8 (25.8 - 73.3)	29.1 (0 - 59.9)	22.6 (5 - 36.2)	1174.4 (1088 - 1376)	61.8 (49.6 - 78.9)	0 (0 - 40.1)	12.5 (0 - 25.1	0 (0 - 27.8)	1848 (1384 - 2472)	59 (40.3 - 70.6)	28.1 (20.8 - 34.2)	17.7 (6.5 - 39.8)	15.3 (0 - 24.7)

Figure 12 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Bifenthrin on wheat stored at 25°C and 75% rh (n=5)

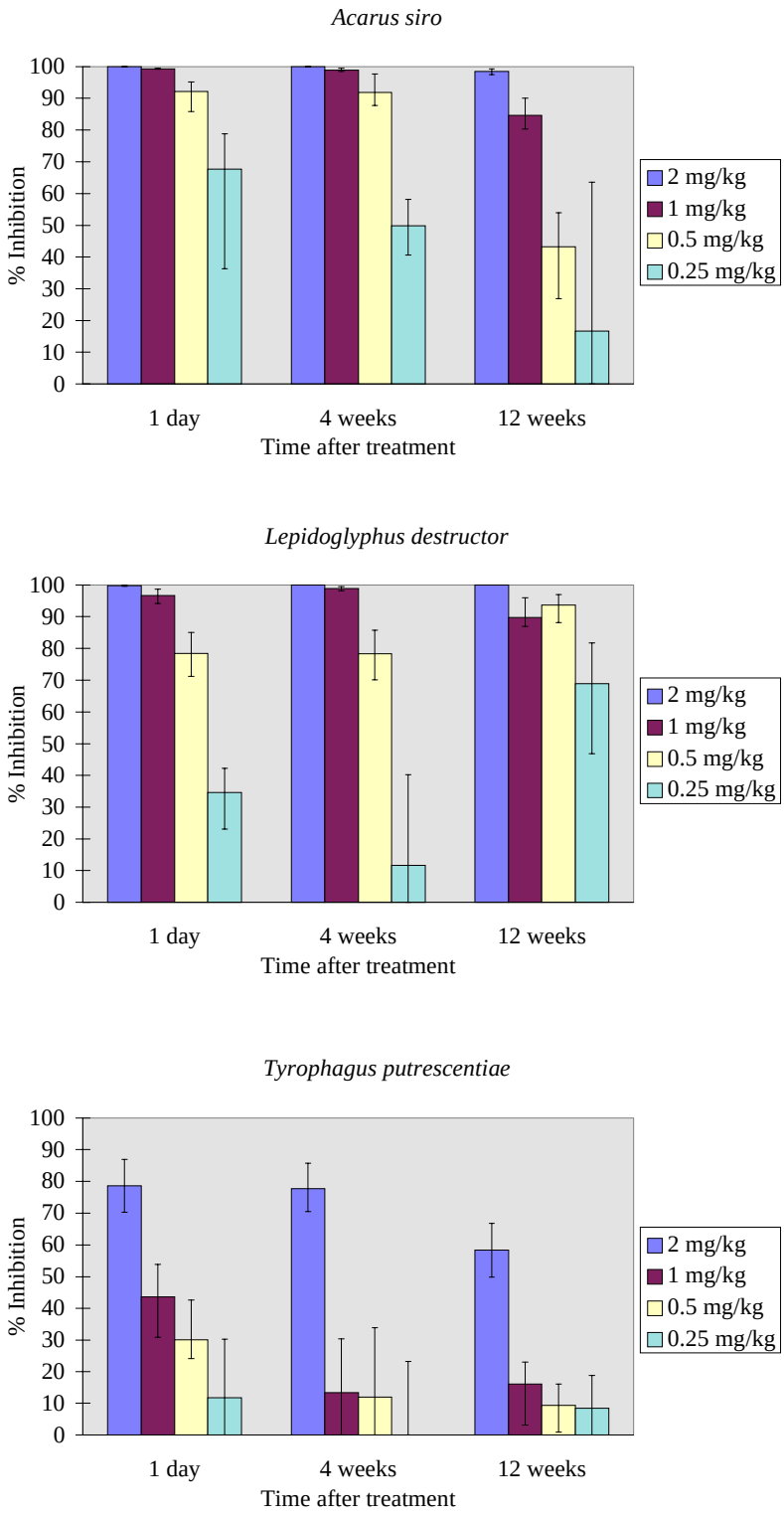


Figure 13 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Bifenthrin on wheat stored at 25°C and 75% rh (n=5)

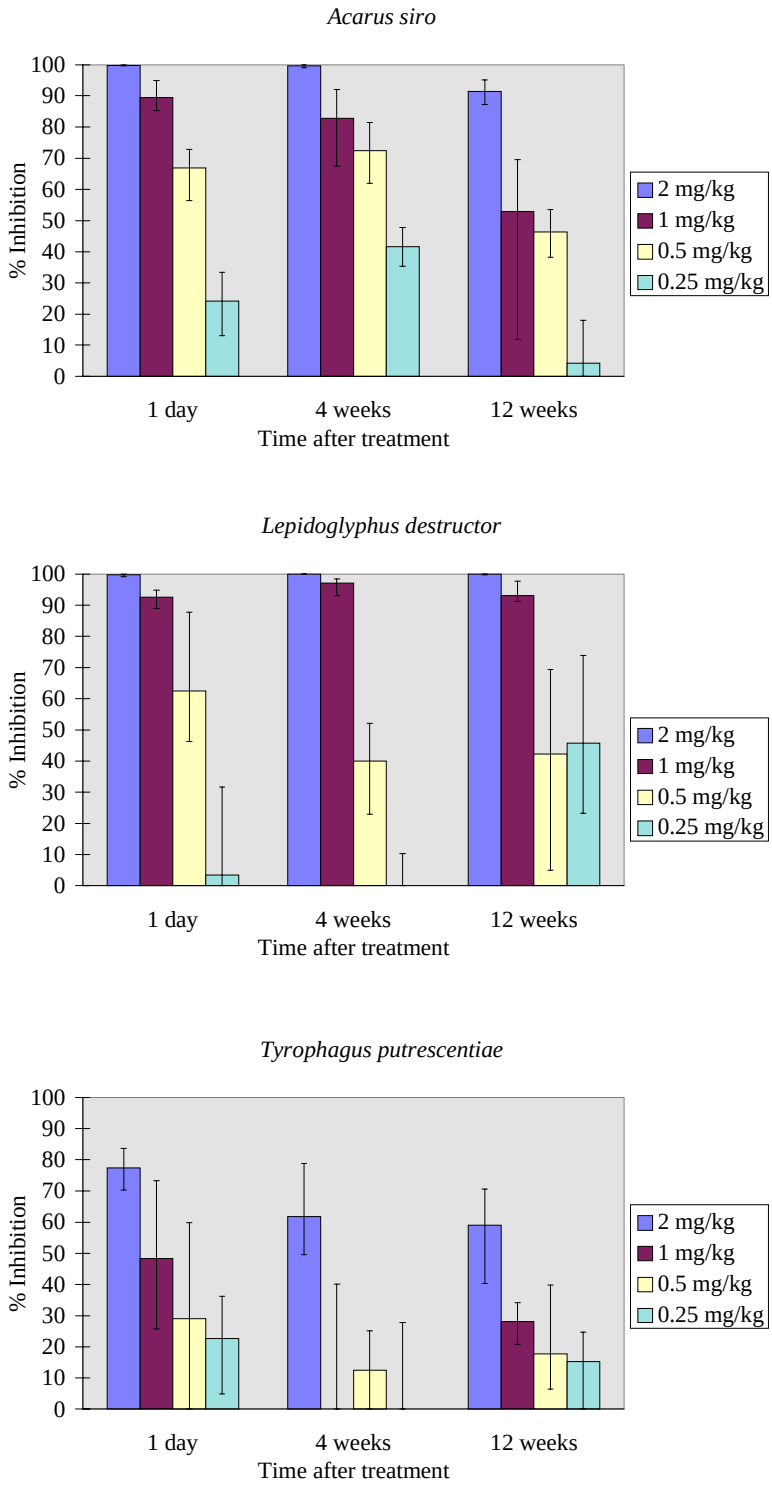
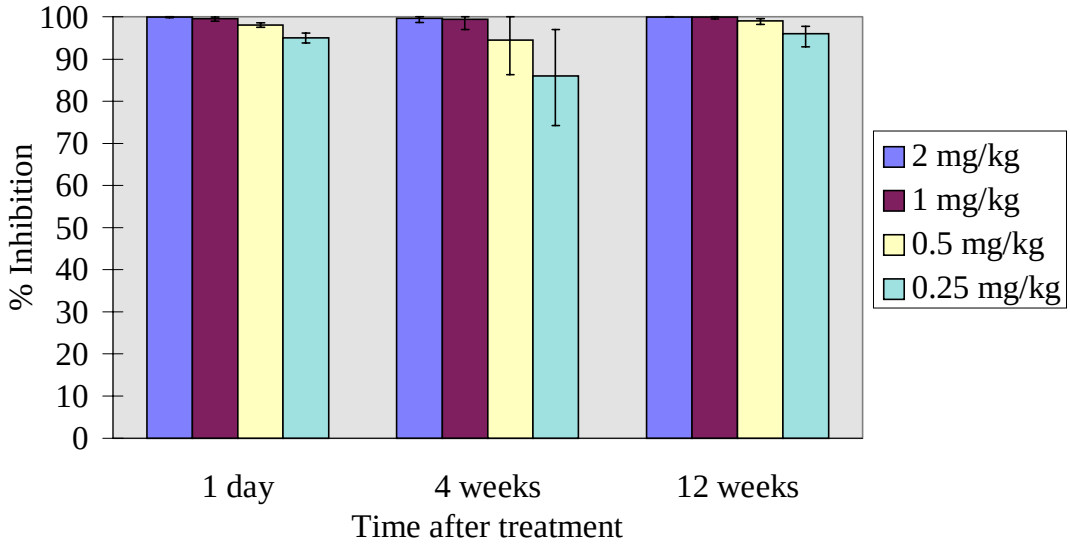


Table 18 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Bifenthrin at different times after treatment on wheat stored at 10oC and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg		2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg		2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
A. siro susceptible	1020.8 (632 - 1360)	99.9 (99.8 - 100)	99.6 (99 - 99.9)	98.1 (97.5 - 98.6)	95 (93.8 - 96.2)	65.8 (32 - 90)	99.7 (98.5 - 100)	99.4 (97 - 100)	94.5 (86.3 - 100)	86 (74.2 - 97)	1099.2 (760 - 1424)	99.9 (99.9 - 100)	99.9 (99.5 - 100)	99.1 (98.2 - 99.6)	96 (92.9 - 97.7)
A. siro resistant	708.8 (480 - 1144)	99.97 (99.9 - 100)	99 (97.5 - 99.7)	90.9 (86.5 - 99.7)	86.2 (76.3 - 97.7)	385.6 (288 - 496)	99.7 (99.2 - 100)	95 (92 - 97.7)	82.1 (68.1 - 92)	24.5 (0 - 44)	651.2 (408 - 848)	99.8 (99.4 - 100)	98.8 (97.1 - 99.9)	92.1 (90 - 94.9)	60.4 (36.1 - 80.3)
L. destructor susceptible	20.2 (15 - 29)	100	99 (95 - 100)	92.1 (75.2 - 100)	69.3 (50.5 - 80.2)	158.4 (80 - 232)	100	99.7 (99.4 - 100)	98 (94.9 - 100)	85.1 (77.9 - 91.2)	185.6 (144 - 256)	100	99.8 (98.9 - 100)	99.5 (98.9 - 100)	87.3 (79 - 91.9)
L. destructor resistant	60.4 (34 - 94)	100	100	99 (96.7 - 100)	88.7 (73.5 - 98.3)	403.2 (296 - 536)	100	99.9 (99.8 - 100)	98.6 (98 - 99.3)	92.8 (89.1 - 95)	286.4 (176 - 416)	99.9 (99.7 - 100)	99.7 (99 - 100)	99.3 (99 - 99.7)	93.9 (92.3 - 95.5)

Figure 14 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Bifenthrin on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

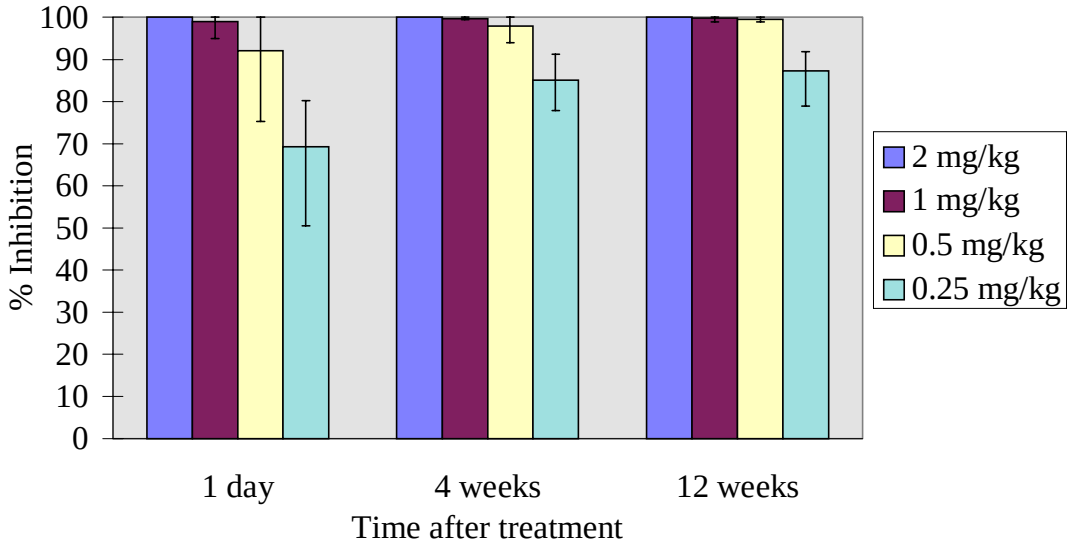


Figure 15 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Bifenthrin on wheat stored at 10°C and 75% rh (n=5)

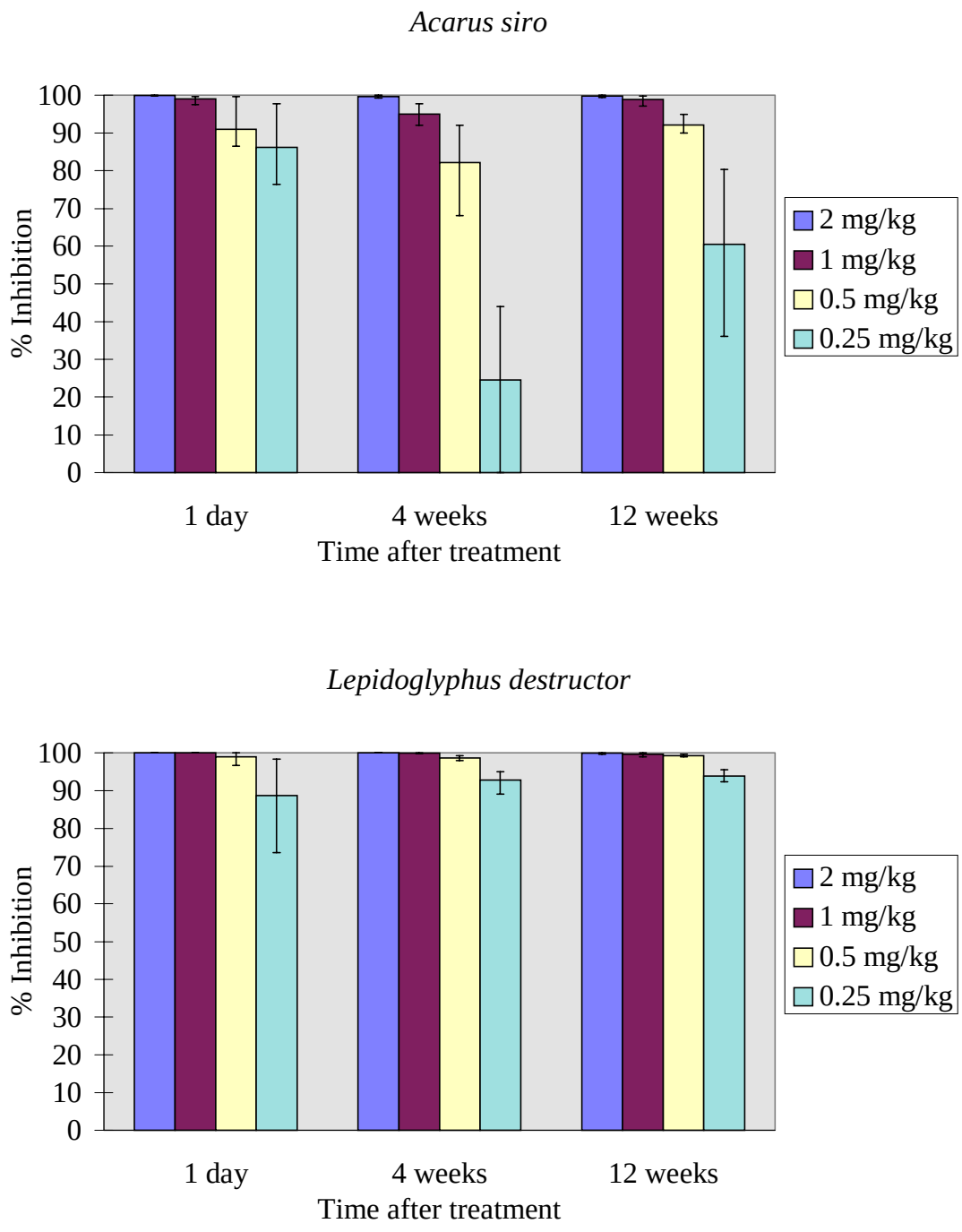


Table 19 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Bifenthrin at different times after treatment on wheat stored at 25oC and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
O. surinamensis susceptible	100	100	100	100	100	100	100	100	100	100	100	99.2
O. surinamensis resistant	100	100	99.2	92	99.2	100	90	62.8	100	98.4	94.4	62.9
S. granarius susceptible	93.5	83.7	59.4	18.5	92	68.8	40.8	4	94.3	49.6	21.2	6.4
S. granarius resistant	59.2	5.5	0	0	30.6	0	0	0	9.8	0	0	0
R. dominica susceptible	100	100	90.4	100	100	99.2	95.1	69.9	100	91.2	72.8	56.8
R. dominica resistant	100	100	100	100	100	100	100	98.2	100	100	100	100

Table 20 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Bifenthrin at different times after treatment on wheat stored at 10oC and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
O. surinamensis susceptible	78.7	72.4	55.3	47.4	100	100	93.4	83.1	92.2	100	81.9	76.7
O. surinamensis resistant	56.4	54.4	16	22.3	82.3	66.1	35.4	23.6	86.3	100	75.8	57.3
S. granarius susceptible	14.1	12.8	0.8	1.6	46.4	16.8	12	8	18.2	20.8	0.8	1.6
S. granarius resistant	6.5	9.7	0.8	1.6	18.5	6.5	13.9	1.6	7.8	4.8	8.7	8
R. dominica susceptible	97.5	85.6	79.1	81.6	100	86.9	73.8	40.2	99.2	100	87.8	97.5
R. dominica resistant	97.5	91.7	78.7	94.2	89.6	92	76.7	61.6	97.5	100	89.4	99.2

Table 21 : Mean % population inhibition and ranges of each beetle species exposed to 4 doses of Bifenthrin at different times after treatment on wheat stored at 25oC and 75% rh

Figures in square brackets are the mean % of the F1 adults that were dead (n=5*)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg		2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg		2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
O. surinamensis susceptible	118 [4.1] (84 - 136)	100	100	100	100	118.4 [17] (105 - 134)	100	100	100	100	5.6 [0] (1 - 9)	100	100	100	100
O. surinamensis resistant	116.4 [6.3] (98 - 140)	100	100	100	100	51.4 [26.6] (44 - 59)	100	100	100	99.6 [0*] (98.1 - 100)	16.4 [7.7] (6 - 27)	100	100	100	98.8 [0*] (93.9 - 100)
S. granarius susceptible	293.2 [0] (164 - 406)	99.7 [66.7*] (99 - 100)	96.5 [8.9] (94.5 - 99)	69 [3.7] (56 - 85.7)	8.1 [0.2] (0 - 37.6)	264.2 [0.5] (179 - 345)	97.4 [84.8] (94.7 - 98.5)	77.2 [44.7] (61.8 - 87.5)	38.4 [4.8] (21.7 - 62.1)	0 [0.4] (0 - 20.5)	294.6 [0.2] (217 - 441)	95.7 [47.2] (92.9 - 98)	59.9 [12.7] (48.1 - 69.1)	15.5 [1] (0 - 50.4)	0 [0.2]
S. granarius resistant	252.2 [0.2] (178 - 335)	92.5 [0] (90.1 - 93.7)	51.3 [0.2] (25.5 - 65.1)	2.1 [0.2] (0 - 23.5)	17.1 [0] (0 - 41.3)	221.8 [0.1] (60 - 348)	89.4 [0] (87.4 - 91.9)	10.2 [0.3] (0 - 29.7)	0 [0.5]	0 [0.4] (0 - 18.8)	135.6 [0] (35 - 180)	10.6 [0] (0 - 41.7)	0 [0.2]	0 [0.1]	0 [0.1]
R. dominica susceptible	481.8 [0.3] (393 - 578)	100	100	100	100	498.2 [0.1] (353 - 716)	100	100	100	100	290.2 [0.2] (120 - 373)	100	99.9 [100*] (99.9 - 100)	100	100
R. dominica resistant	375.8 [0.5] (297 - 456)	99.8 [100*] (99.7 - 100)	100	100	99.9 [100*] (99.7 - 100)	557 [0.3] (453 - 671)	100	100	100	99.9 [100*] (99.8 - 100)	87 [1.1] (65 - 97)	100	100	100	100

* - n = < 5 where 100% inhibition occurred in one or more replicates

Figure 16 : Mean % population inhibition (and ranges) of susceptible strains of insects exposed to 4 doses of Bifenthrin on wheat stored at 25ºC and 75% rh (n=5)

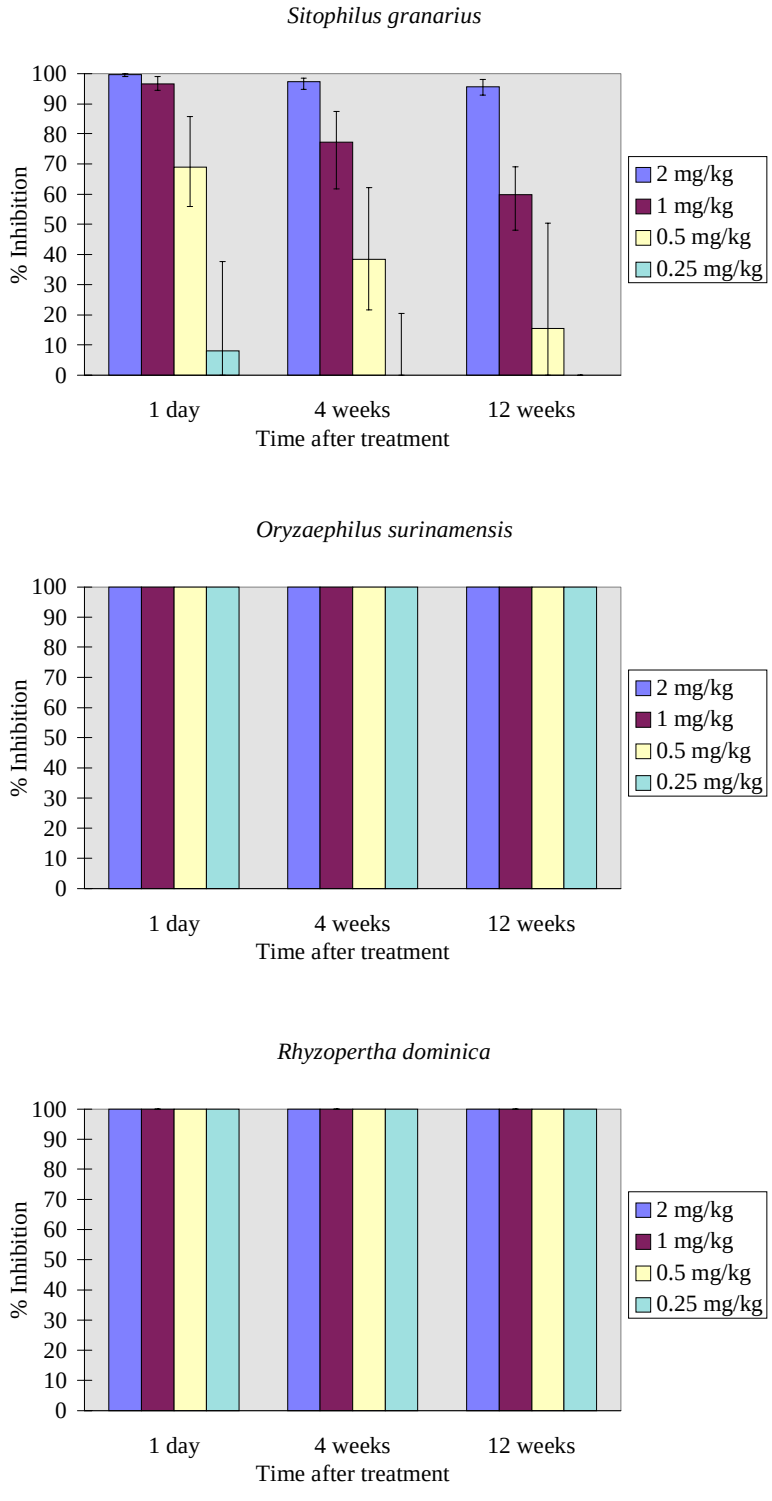


Figure 17 : Mean % population inhibition (and ranges) of OP resistant strains of insects exposed to 4 doses of Bifenthrin on wheat stored at 25ºC and 75% rh (n=5)

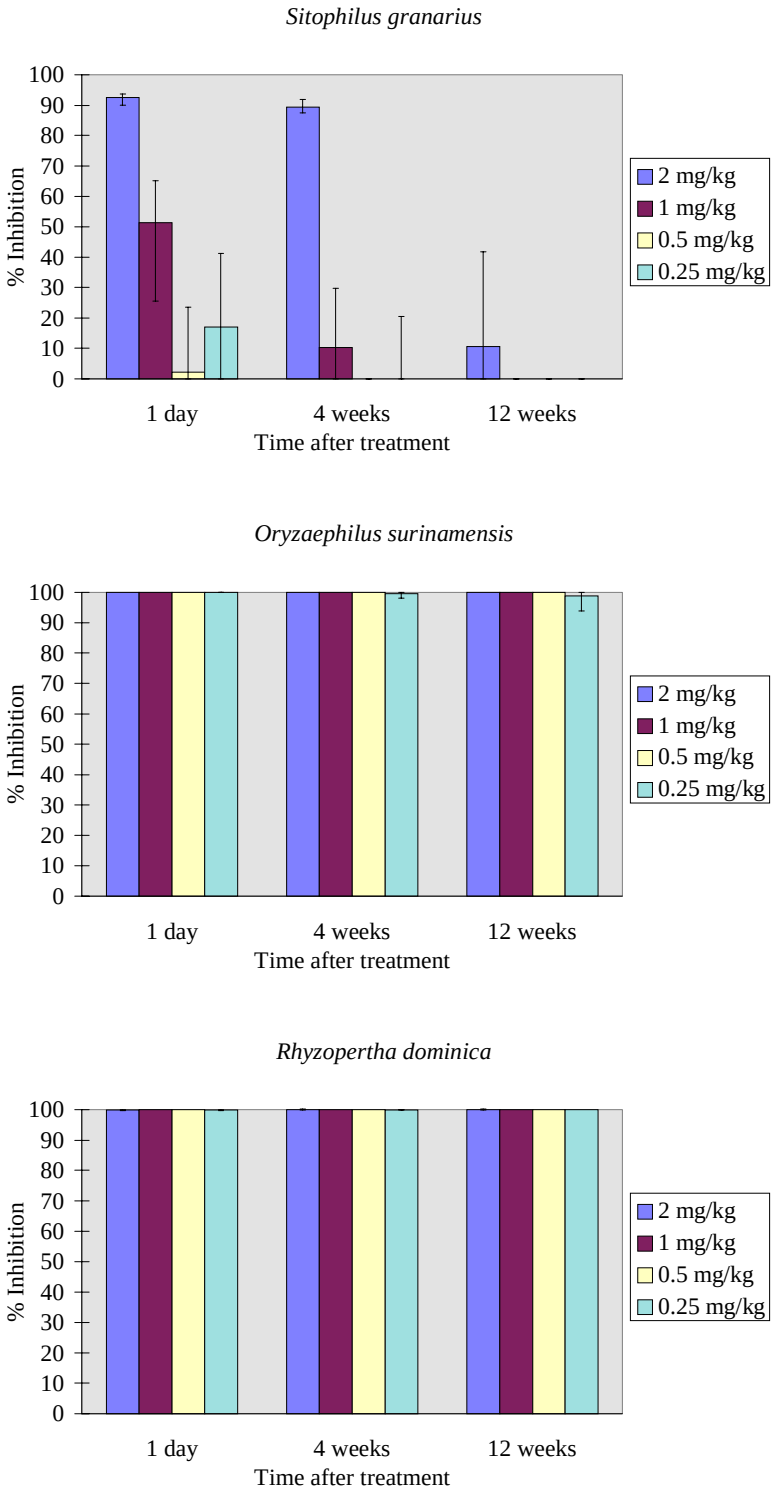


Table 22 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Flufenoxuron at different times after treatment on wheat stored at 25°C and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg
A. siro susceptible	1281.6 (1168 - 1448)	92.4 90.5 - 95.2)	52.3 (36.3 - 69.4)	31.2 (23.8 - 39.5)	10.7 (0 - 22)	1334.4 (1240 - 1464)	78.5 (70 - 85)	21.7 (0 - 45.4)	0 (0 - 1.1)	0.2 (0 - 13.1)	1148.8 (1096 - 1192)	70.6 (61.7 - 83.3)	24.4 (0 - 46.4)	0	0 (0 - 1.8)
A. siro resistant	1497.6 (1256 - 1960)	88.4 (83.4 - 96.9)	50.2 (39.6 - 56.2)	23.2 (3.8 - 37.5)	0 (0 - 11.3)	1400 (1048 - 1808)	71.1 (54.9 - 82.5)	0 (0 - 7.4)	0 (0 - 4.6)	0	979.2 (664 - 1208)	59.8 (41.2 - 77.9)	32.8 (0 - 59.2)	0 (0 - 3.6)	0
L. destructor susceptible	392 (248 - 592)	98.5 (94.4 - 99.7)	99 (96.7 - 100)	99.2 (99 - 99.5)	85.5 (78.6 - 93.9)	235.2 (160 - 336)	99.8 (99.6 - 100)	99.7 (99.1 - 100)	99.5 (99.1 - 100)	77.2 (69 - 84.3)	65 (28 - 101)	99.7 (98.5 - 100)	91.4 (83.1 - 98.5)	96.3 (95.4 - 98.5)	67.1 (46.2 - 78.5)
L. destructor resistant	636.8 (568 - 688)	97.6 (96.7 - 98.3)	97.7 (96.4 - 98.6)	96.9 (95.9 - 98.1)	36.7 (15.8 - 63.7)	163.2 (120 - 216)	96 (88.4 - 100)	98.7 (96.3 - 99.4)	97.5 (95.7 - 98.8)	21.6 (0 - 60.8)	10.6 (2 - 22)	100	0 (0 - 52.8)	88.7 (81.1 - 100)	0
T. putrescentiae susceptible	1371.2 (1032 - 1736)	65.9 (45.2 - 83.7	47.5 (30.6 - 5.1)	14.1 (8.4 - 20.1)	0 (0 - 4.9)	1006.4 (848 - 1232)	40.2 (28.5 - 57.9)	5.2 (0 - 28.5)	0 (0 - 32.4)	19.4 (5.4 - 38.8)	809.6 (592 - 1032)	46.8 (20 - 65.4)	13.2 (0.2 - 31.8)	17 (0 - 67.4)	0 (0 - 17)
T. putrescentiae resistant	1014.4 (848 - 1176)	92.4 (90.6 - 94)	49.7 (40.9 - 59)	24.6 (11.7 - 45.6)	5.5 (0 - 19.6)	665.6 (584 - 760)	48.3 (38.7 - 57.9)	18 (2.6 - 31.5)	0 (0 - 13.5)	0 (0 - 7.5)	524.8 (408 - 688)	58.5 (28.4 - 75.6)	0 (0 - 19.2)	21.3 (5.5 - 32.9)	0 (0 - 13.1)

Figure 18 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Flufenoxuron on wheat stored at 25°C and 75% rh (n=5)

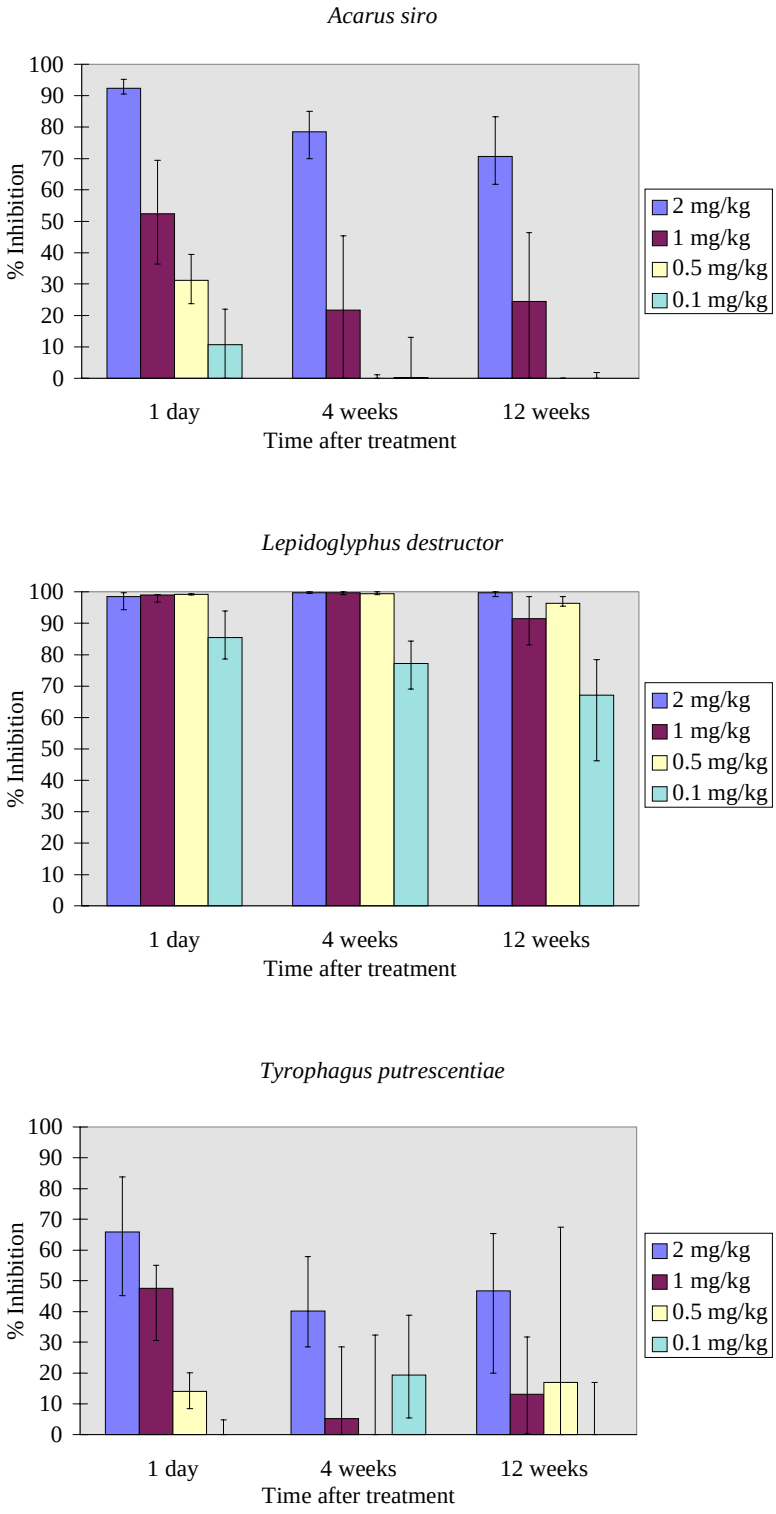


Figure 19 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Flufenoxuron on wheat stored at 25ºC and 75% rh (n=5)

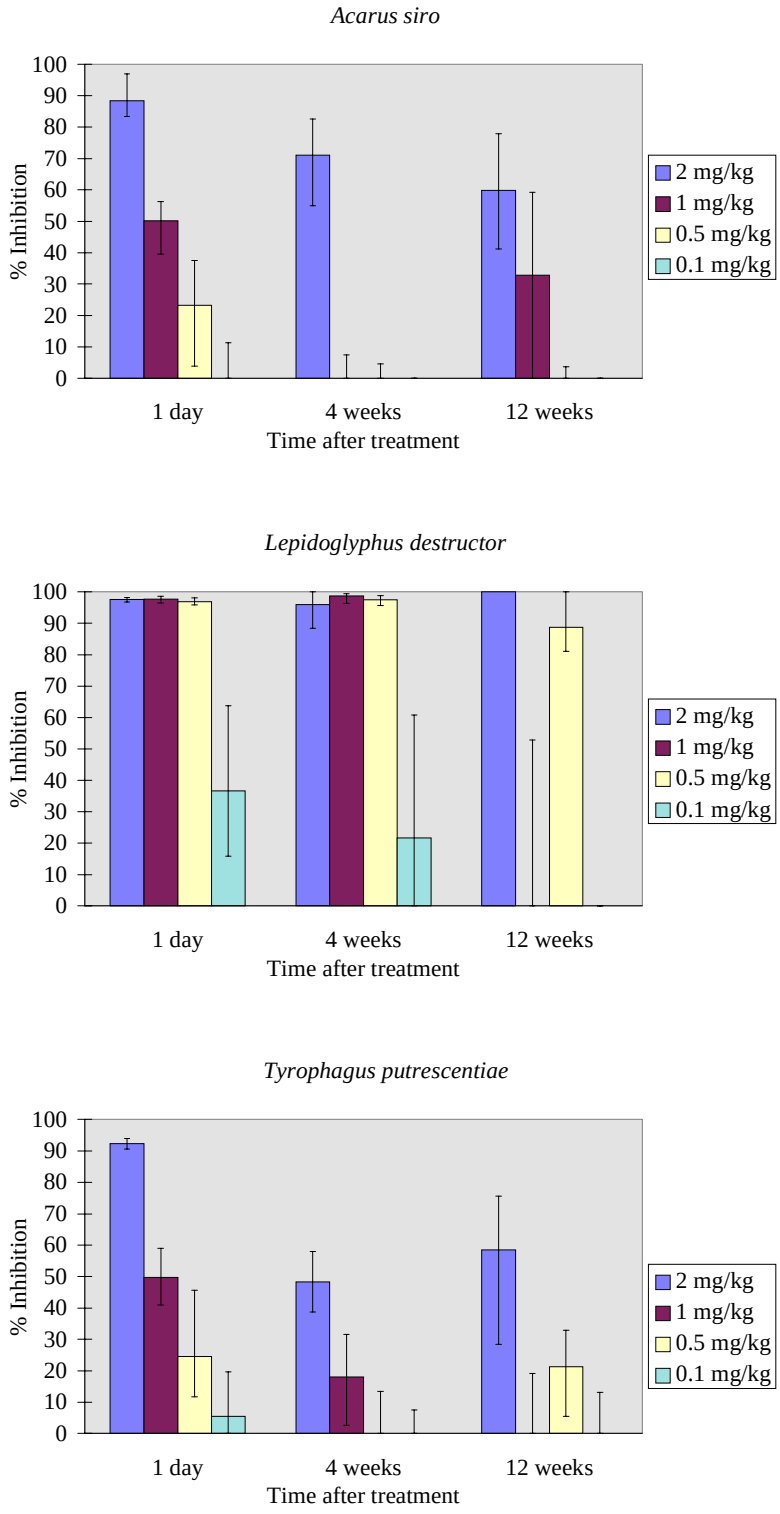
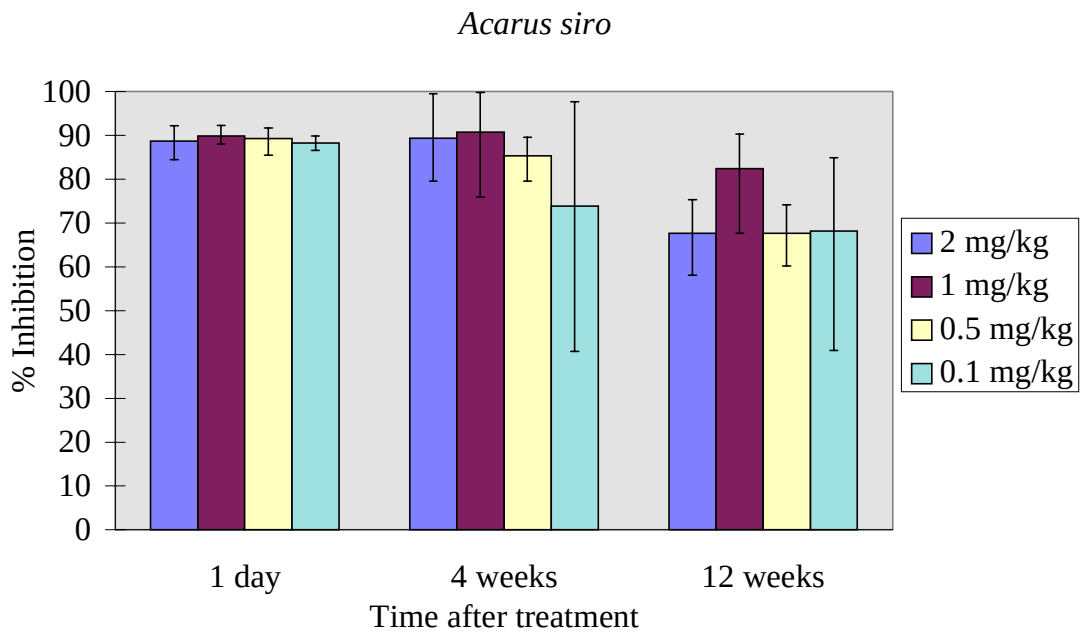


Table 23 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Flufenoxuron at different times after treatment on wheat stored at 10^{o}C and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg
A. siro susceptible	731.2 (624 - 856)	88.7 (84.5 - 92.2)	89.9 (88 - 92.3)	89.3 (85.5 - 91.7)	88.3 (86.6 - 89.9)	432 (224 - 720)	89.4 (79.6 - 99.5)	90.8 (75.9 - 99.8)	85.3 (79.6 - 89.6)	73.9 (40.7 - 97.7)	93 (64 - 128)	67.7 (58.1 - 75.3)	82.4 (67.7 - 90.3)	67.7 (60.2 - 74.2)	68.2 (40.9 - 84.9)
A. siro resistant	881.6 (632 - 1048)	76.8 (64.6 - 84.6)	77.3 (56.4 - 90.9)	73.3 (61 - 80)	51 (7.4 - 70.1)	78.2 (51 - 93)	91.6 (84.6 - 94.9)	90 (85.9 - 93.6)	84.7 (75.7 - 89.8)	84.4 (78.3 - 91)	61.4 (42 - 82)	67.4 (38.1 - 83.7)	54.7 (33.2 - 70.7)	52.1 (31.6 - 59.3)	24.8 (0 - 73.9)
L. destructor susceptible	478.4 (304 - 736)	99.5 (98.3 - 100)	99 (97.9 - 100)	97.3 (95.6 - 99.2)	93.4 (81.6 - 97.9)	24.4 (15 - 39)	91.8 (79.5 - 100)	94.3 (87.7 - 100)	97.5 (95.9 - 100)	94.3 (87.7 - 100)	72.8 (56 - 105)	97.8 (95.9 - 100)	97.5 (95.9 - 100)	97.5 (95.9 - 100)	94.8 (93.1 - 95.9)
L. destructor resistant	438.4 (352 - 568)	97.1 (95.4 - 98.6)	95 (90 - 97.7)	95.1 (92.2 - 97.9)	92.2 (87.7 - 96.8)	79.8 (41 - 104)	93.5 (91.2 - 96.2)	91 (87.5 - 95)	92 (87.5 - 93.7)	91.5 (86.2 - 95)	36.4 (18 - 52)	92.9 (91.8 - 97.3)	98.9 (94.5 - 100)	91.8 (83.5 - 94.5)	96.7 (89 - 100)

Figure 20 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Flufenoxuron on wheat stored at 10°C and 75% rh (n=5)



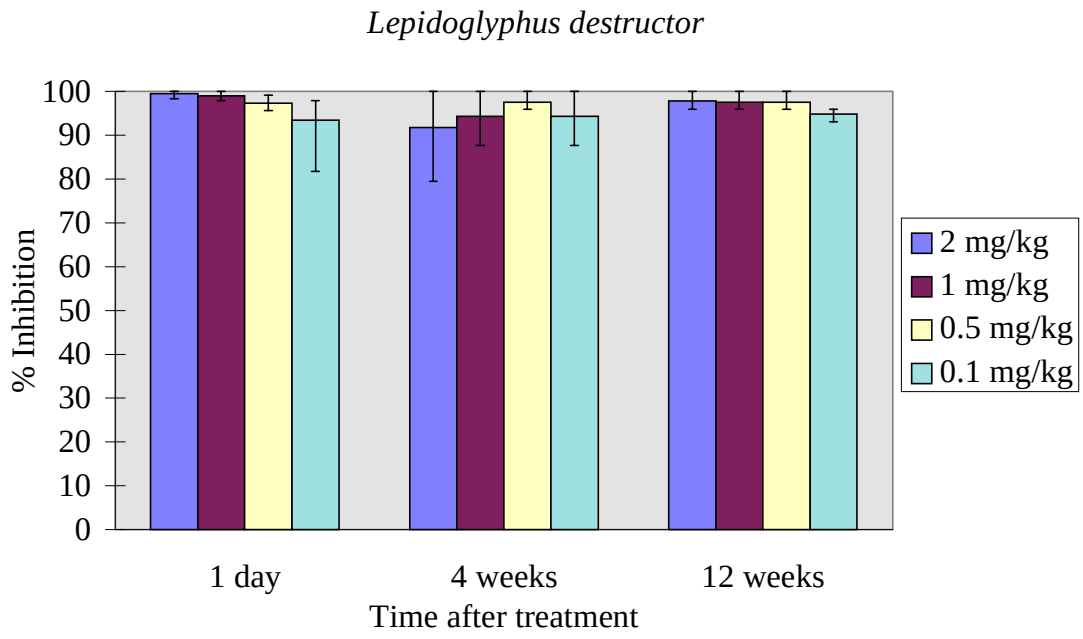
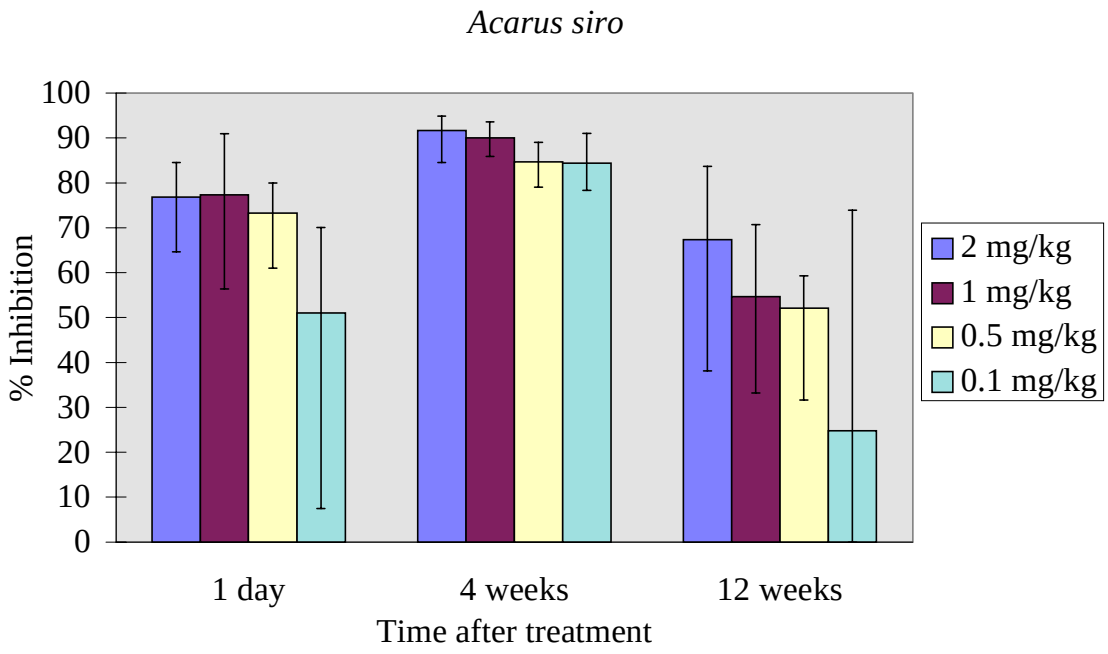


Figure 21 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Flufenoxuron on wheat stored at 10°C and 75% rh (n=5)



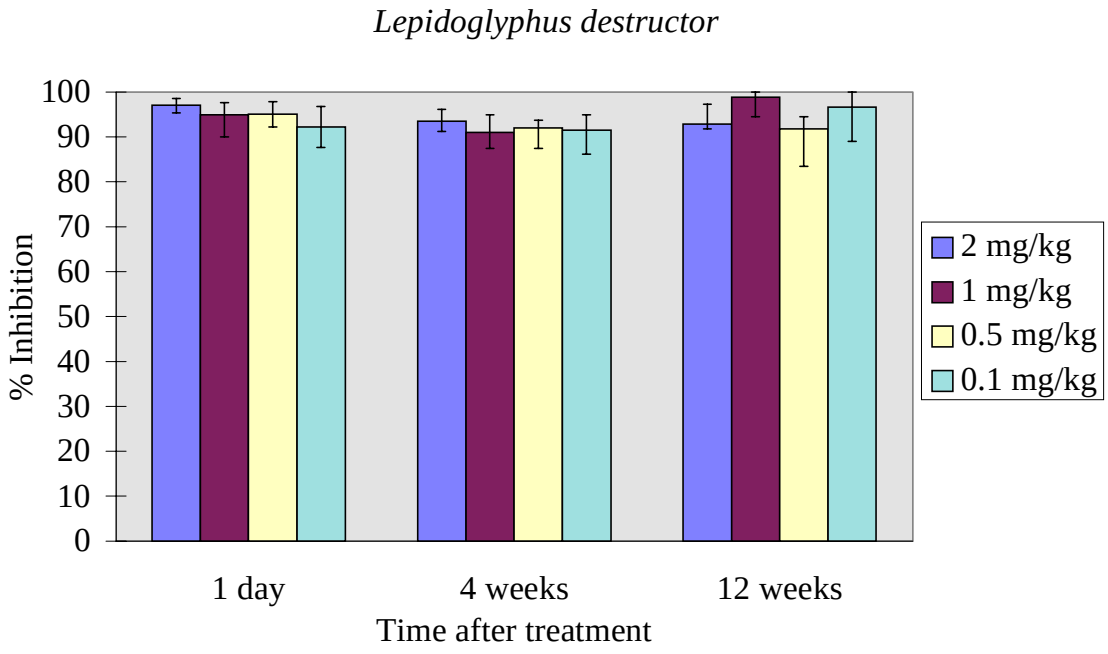


Table 24 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Flufenoxuron at different times after treatment on wheat stored at 25°C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg
O. surinamensis susceptible	2.5	1.6	0	0.8	0	0	0	2.4	0	0	0.1	0
O. surinamensis resistant	0	0	0	0	4	2.4	0	2.4	1.6	0.8	1.6	0
S. granarius susceptible	0	0.8	0	0	0.8	1.6	0.8	0.8	0	0	0	0
S. granarius resistant	0.8	1.6	0.8	0.8	0	0.8	0	0.8	0	0	0.8	0
R. dominica susceptible	1.6	0.8	0.8	0	0	0.8	0	0.8	2.4	0	1.6	0
R. dominica resistant	0	1.6	0.8	2.4	0.8	0	0	0.8	0	0.8	0.8	0

Table 25 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Flufenoxuron at different times after treatment on wheat stored at 10^{o}C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg
O. surinamensis susceptible	0.8	4.1	3.2	0	0	0	0	0	9.8	13.8	11.2	2.4
O. surinamensis resistant	0	0	0	1.6	0	0	0	0	0	2.4	0	0
S. granarius susceptible	0	0	0	0	0	0	0	0	0.8	0	0	0
S. granarius resistant	0	0	0	0	0	0	0	0	0	0	0	0
R. dominica susceptible	0	0	0	0.8	0	0	0	0.8	1.6	0	0	0.8
R. dominica resistant	0	0	1.6	0	0	0	0	0.8	0	0.8	0	1.6

Table 26 : Mean % population inhibition and ranges of each beetle species exposed to 4 doses of Flufenoxuron at different times after treatment on wheat stored at 25^{o}C and 75% rh

Figures in square brackets are the mean % of the F1 adults that were dead (n=5*)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg	nos (range)	2 mg/kg	1 mg/kg	0.5 mg/kg	0.1 mg/kg
O. surinamensis susceptible	97.2 [5.5] (64 - 126)	100	100	97.1 [56.3 *] (94.9 - 100)	26.1 [3.1] (0.2 - 37.2)	76.6 [2.5] (58 - 99)	98.2 [90] (97.4 - 98.7)	98.2 [75 *] (96.1 - 100)	92.2 [27.9] (89.6 - 94.8)	47.5 [0.6] (28.2 - 70)	44 [3.4] (24 - 69)	99.1 [100 *] (97.7 - 100)	100	100	98.2 [0 *] (95.5 - 100)
O. surinamensis resistant	73.8 [11.3] (60 - 92)	98.6 [66.7 *] (97.3 - 100)	98.4 [87.5 *] (97.3 - 100)	95.4 [56.4] (90.5 - 98.6)	7.1 [12.2] (0 - 55.6)	94.8 [18.2] (62 - 125)	99.8 [100 *] (98.9 - 100)	99.8 [100 *] (98.9 - 100)	96.2 [41.4 *] (92.6 - 100)	33.5 [5.6] (12.4 - 62)	39.6 [6.4] (30 - 45)	100	100	99.5 [100 *] (97.5 - 100)	97.5 [75 *] (94.9 - 100)
S. granarius susceptible	284.8 [0.2] (205 - 389)	0.6 [0.4] (0 - 38.2)	0 [0.2]	1 [0] (0 - 44.2)	29.8 [0.1] (0 - 81)	277 [0.1] (146 - 433)	19.8 [0.2] (0 - 48)	0 [0.2] (0 - 54.5)	0 [0.2] (0 - 52)	11.2 [0.1] (0 - 38.3)	361.8 [0.2] (246 - 439)	25.5 [0] (13.5 - 42)	37.9 [0.1] (22.9 - 56.3)	30.4 [0.2] (6.9 - 44.7)	48.9 [0.5] (22.9 - 72.1)
S. granarius resistant	296 [0.2] (227 - 352)	52.5 [0] (28 - 79.1)	37.8 [0.1] (27.4 - 73.3)	17.3 [0.2] (0 - 36.1)	5.5 [0] (0 - 57.1)	240.6 [0.1] (142 - 341)	48 [0] (22.3 - 74.2)	17.5 [0.2] (0 - 60.5)	0 [0] (0 - 78)	7.9 [0.1] (0 - 29.8)	312.4 [0.2] (217 - 382)	40.7 [0] (14.2 - 67.7)	18.6 [0.2] (0 - 64.8)	7.9 [0.4] (0 - 30.2)	36 [0.4] (23.2 - 43.3)
R. dominica susceptible	593.2 [0.6] (559 - 656)	91.1 [7.2] (89.4 - 92.8)	92.8 [4.7] (87.7 - 97.1)	91.7 [0.9] (86.3 - 95.3)	69.9 [0.3] (58.7 - 79.8)	503.6 [0.1] (422 - 576)	95.4 [2.7] (94 - 97.8)	95.6 [0.6] (93.6 - 97.6)	89.9 [0.6] (85.9 - 94.4)	69.8 [0.6] (58.7 - 86.3)	590 [0.4] (524 - 657)	92.6 [4.6] (91 - 94.9)	94.9 [2.4] (94.2 - 95.9)	89.3 [0.6] (87.6 - 90.7)	65.4 [0.7] (59.3 - 73.4)
R. dominica resistant	448.4 [0.2] (378 - 538)	90.1 [3.7] (89.3 - 91.1)	88.4 [2.1] (87.3 - 90.2)	86.9 [0.2] (78.1 - 92.9)	56.8 [0.5] (47.4 - 72.8)	364.2 [0.7] (277 - 442)	95.3 [3.6] (94.5 - 96.7)	93.9 [0] (92.6 - 96.2)	91.5 [4.5] (85.7 - 94.8)	62.2 [1.3] (43.2 - 71.4)	480.6 [0.6] (346 - 553)	90.8 [6.4] (86.3 - 92.9)	90 [4.5] (87.3 - 94)	85.3 [2.3] (79.4 - 92.1)	26.3 [0.9] (3 - 37.6)

* - n = < 5 where 100% inhibition occurred in one or more replicates

Figure 22 : Mean % population inhibition (and ranges) of susceptible strains of insects exposed to 4 doses of Flufenoxuron on wheat stored at 25ºC and 75% rh (n=5)

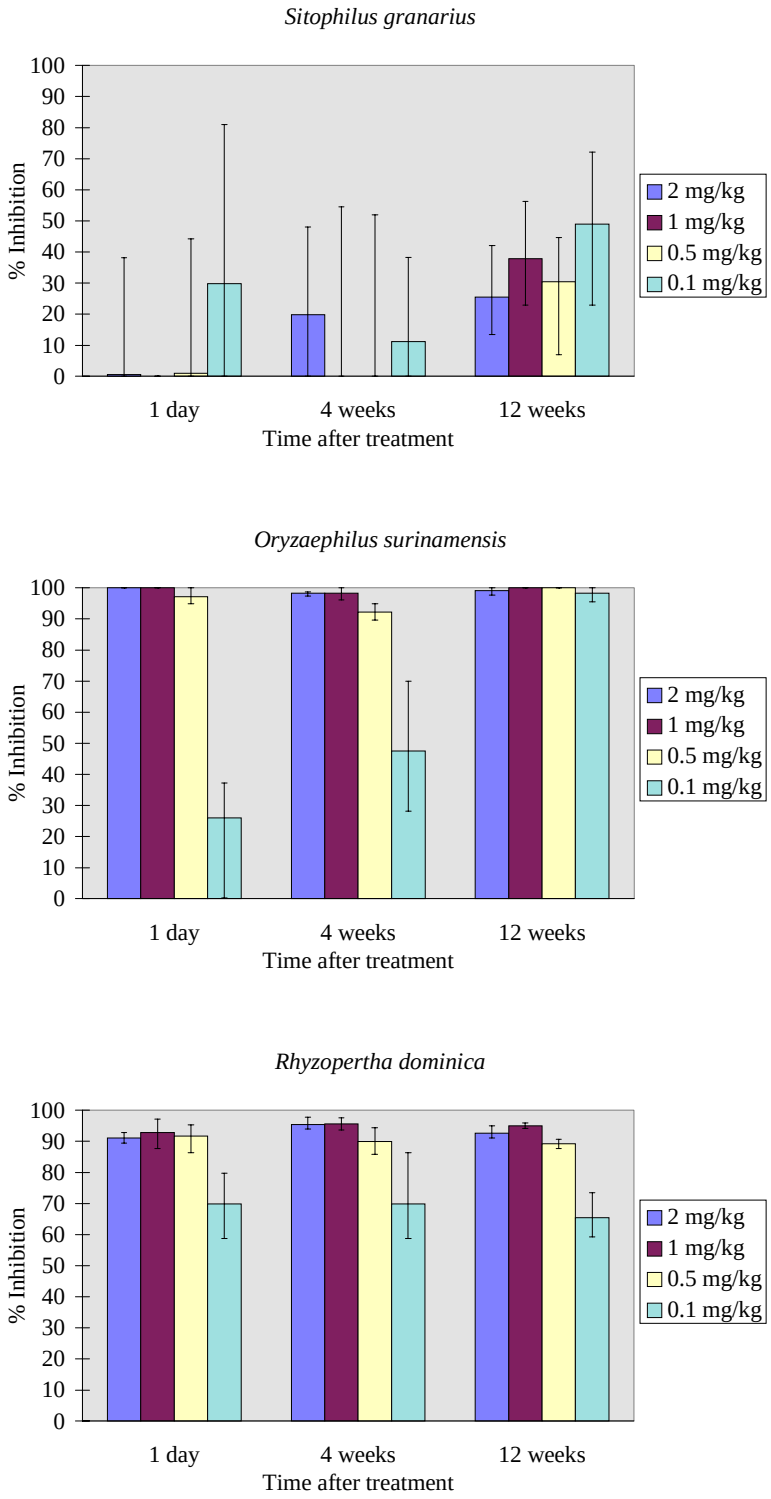


Figure 23 : Mean % population inhibition (and ranges) of OP resistant strains of insects exposed to 4 doses of Flufenoxuron on wheat stored at 25ºC and 75% rh (n=5)

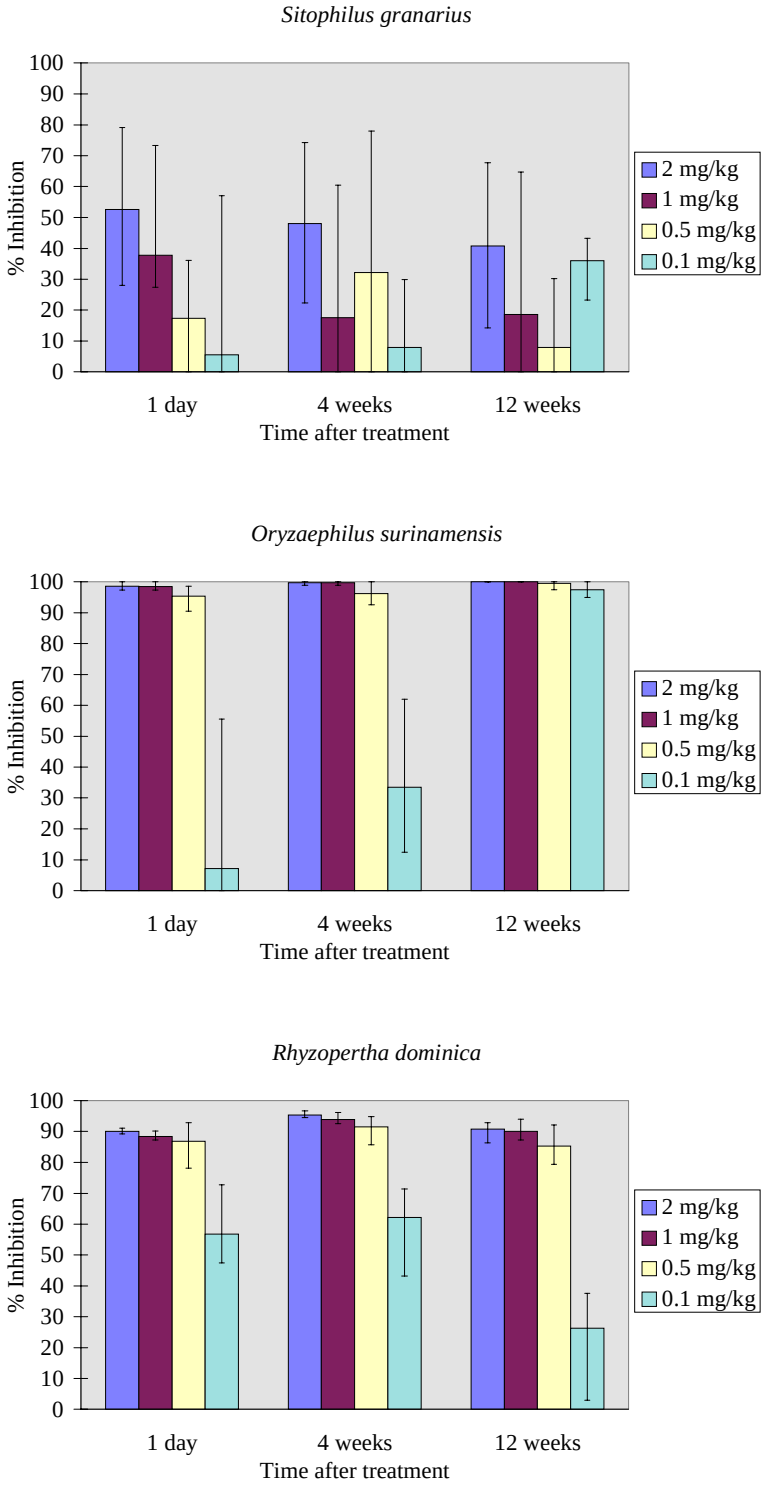


Table 27 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Sodium polyborate at different times after treatment on wheat stored at 25^{o}C and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
A. siro susceptible	1948.8 (1520 - 2208)	99.9 (99.8 - 100)	80 (73.3 - 86.9)	0.7 (0 - 14.6)	0 (0 - 14.6)	760 (528 - 1048)	69.5 (55.8 - 87)	40 (12.6 - 55.8)	21.1 (3.2 - 31.6)	14.3 (0 - 30.5)	987.2 (752 - 1128)	65.3 (50.6 - 78.1)	24.8 (0 - 44.1)	21.1 (0 - 32.7)	12 (0 - 36)
A. siro resistant	1358.4 (920 - 1840)	100	99.9 (99.9 - 100)	96.2 (93.9 - 98.7)	16.4 (0 - 49.9)	2236.8 (1840 - 2544)	99.3 (98.8 - 99.7)	60.2 (48.5 - 71)	20.1 (3.4 - 32)	6 (0 - 29.9)	609.6 (488 - 720)	97.1 (94.9 - 99.5)	59.5 (50.1 - 71.1)	30.7 (14.7 - 43.6)	4.7 (0 - 13.4)
L. destructor susceptible	785.6 (504 - 1040)	100	100	99.9 (99.9 - 100)	90.6 (80.7 - 97.6)	316.8 (272 - 392)	100	100	71.4 (67.8 - 75.1)	1 (0 - 24.2)	51 (30 - 79)	100	47.8 (21.6 - 62.7)	0	0
L. destructor resistant	374.4 (208 - 496)	100	100	99.8 (99.5 - 100)	66.6 (54.3 - 73)	288 (224 - 408)	100	99.7 (99 - 100)	46 (8.3 - 83)	13.9 (0 - 33.3)	326.4 (208 - 432)	99.9 (99.7 - 100)	93.8 (91.1 - 98.2)	36.3 (16.7 - 53.4)	22.1 (0 - 53.4)
T. putrescentiae susceptible	883.2 (440 - 1480)	99.8 (99.7 - 100)	99.9 (99.9 - 100)	98.4 (97.7 - 99.7)	0	988.8 (856 - 1184)	99.9 (99.7 - 100)	91.6 (90.5 - 92.8)	58.7 (43.4 - 70.1)	26.5 (6.1 - 41.7)	507.2 (320 - 728)	92.5 (71.6 - 98.6)	62.6 (62.1 - 83.4)	0 (0 - 38.5)	0
T. putrescentiae resistant	1051.2 (576 - 2016)	100	100	99.9 (99.8 - 100)	45.2 (11 - 71.1)	750.4 (496 - 888)	100	92.7 (90 - 92.7)	28.6 (10.4 - 57.4)	0	348.8 (216 - 488)	98.1 (96.6 - 98.9)	73.1 (61 - 81.9)	21.1 (8.3 - 35.8)	0 (0 - 15.1)

Figure 24 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Sodium polyborate on wheat stored at 25ºC and 75% rh (n=5)

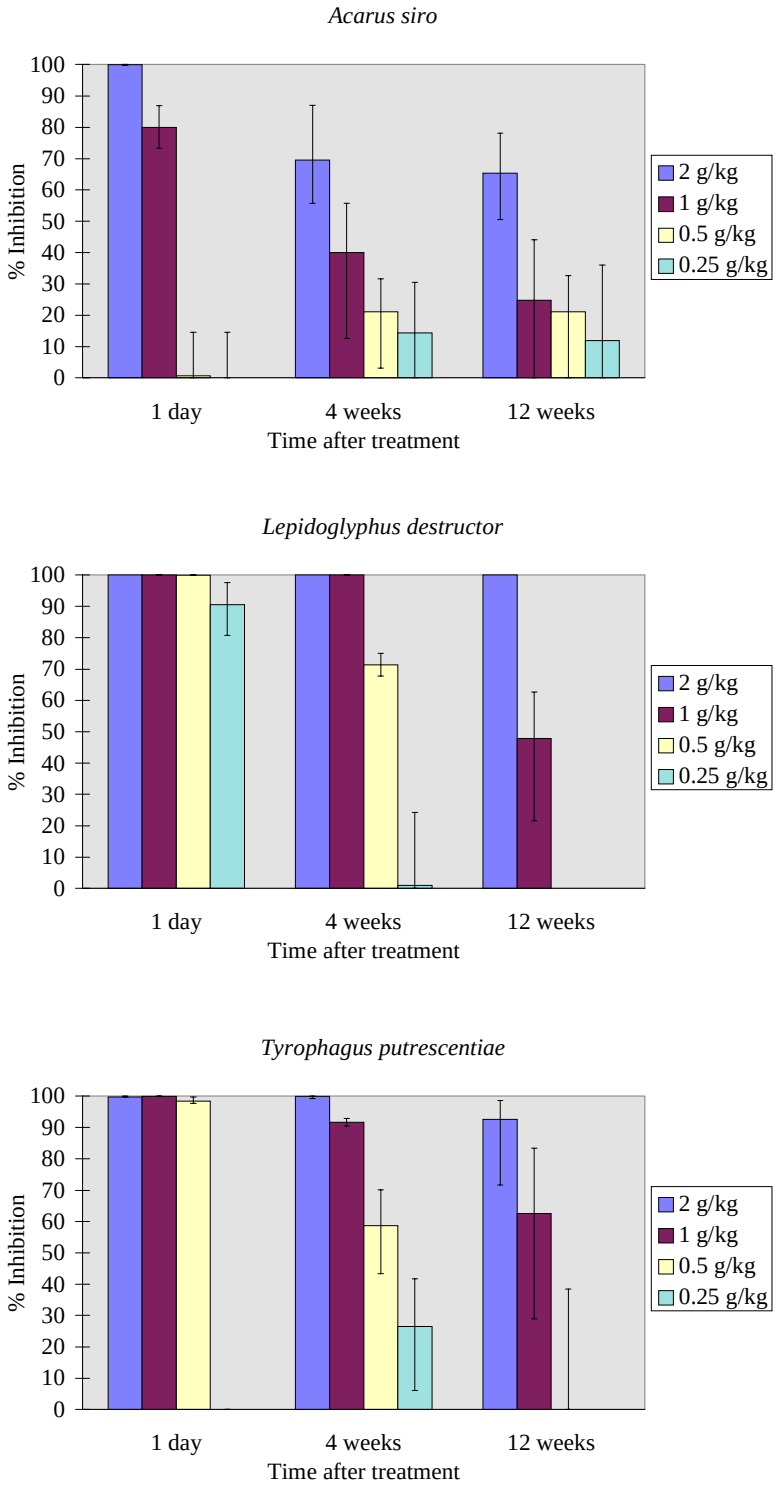


Figure 25 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Sodium polyborate on wheat stored at 25ºC and 75% rh (n=5)

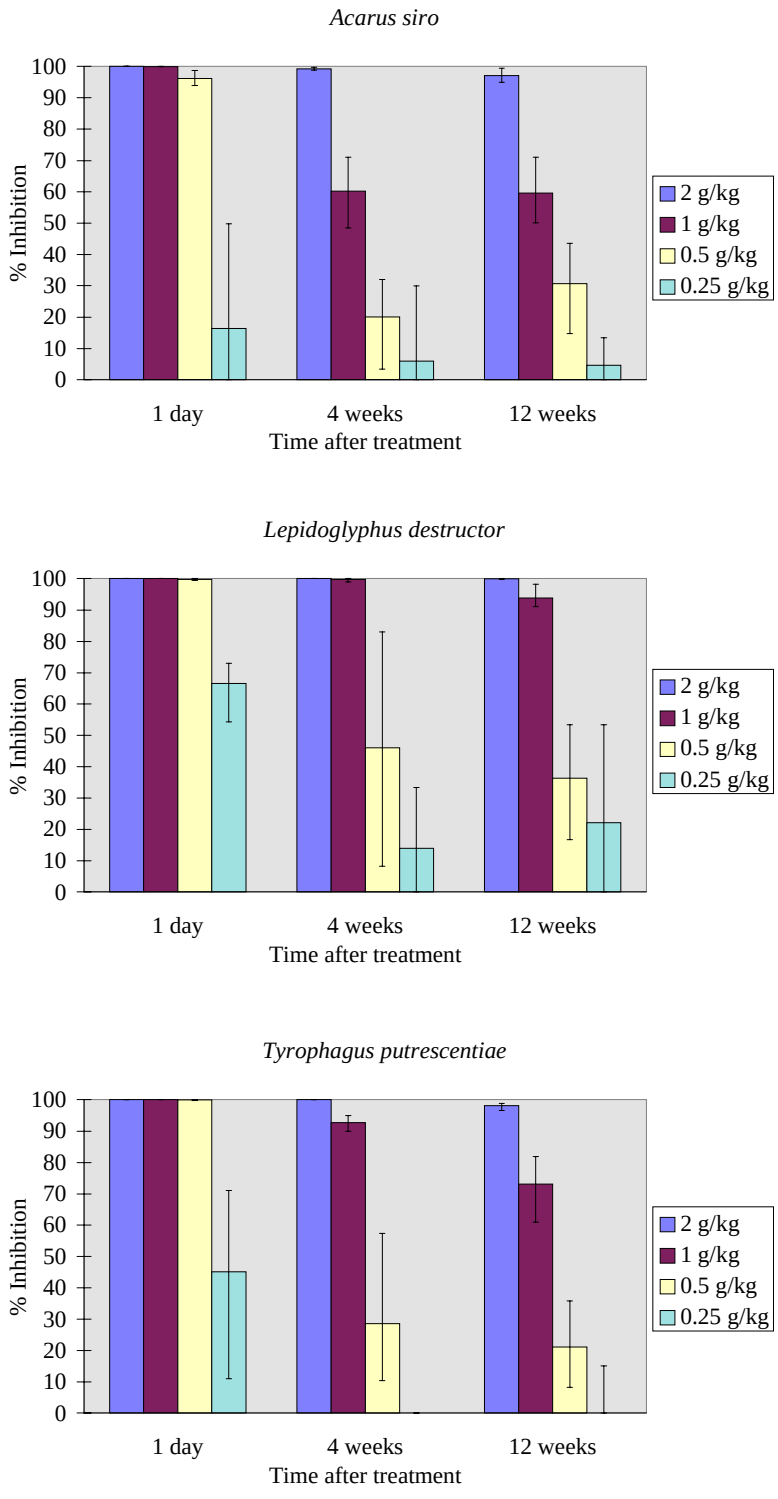


Table 28 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Sodium polyborate at different times after treatment on wheat stored at 10^{o}C and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg		2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
A. siro susceptible	713.6 (640 - 752)	99.9 (99.7 - 100)	95.5 (94.1 - 96.8)	23.1 (0 - 69.7)	22.9 (0 - 51.8)	499.4 (392 - 632)	98.6 (97.4 - 100)	32.7 (2.2 - 66.3)	4.8 (0 - 31.1)	20.2 (7.1 - 32.7)	208 (200 - 224)	92.2 (88.5 - 96.2)	67.9 (60.6 - 79.3)	53.1 (16.8 - 79.3)	9 (3.8 - 11.5)
A. siro resistant	713.6 (488 - 1104)	100	99.9 (99.8 - 100)	100	93.9 (88.5 - 98.3)	384 (216 - 504)	100	100	81.4 (75 - 89.3)	20.2 (0 - 75.3)	109 (110 - 124)	100	93.2 (84.4 - 99.1)	54.3 (43.1 - 66.1)	21.3 (5.5 - 43.1)
L. destructor susceptible	364.8 (264 - 640)	100	100	100	100	35 (27 - 47)	100	100	100	77.7 (57.1 - 91.4)	13.8 (2 - 27)	100	100	100	7.2 (0 - 63.8)
L. destructor resistant	140.2 (109 - 168)	100	100	100	98.1 (94.2 - 100)	49.6 (28 - 75)	100	100	98.8 (94 - 100)	70.2 (49.6 - 85.9)	33.6 (17 - 50)	100	100	85.7 (79.2 - 88.1)	53 (31.5 - 73.2)

Figure 26 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Sodium polyborate on wheat stored at 10°C and 75% rh (n=5)

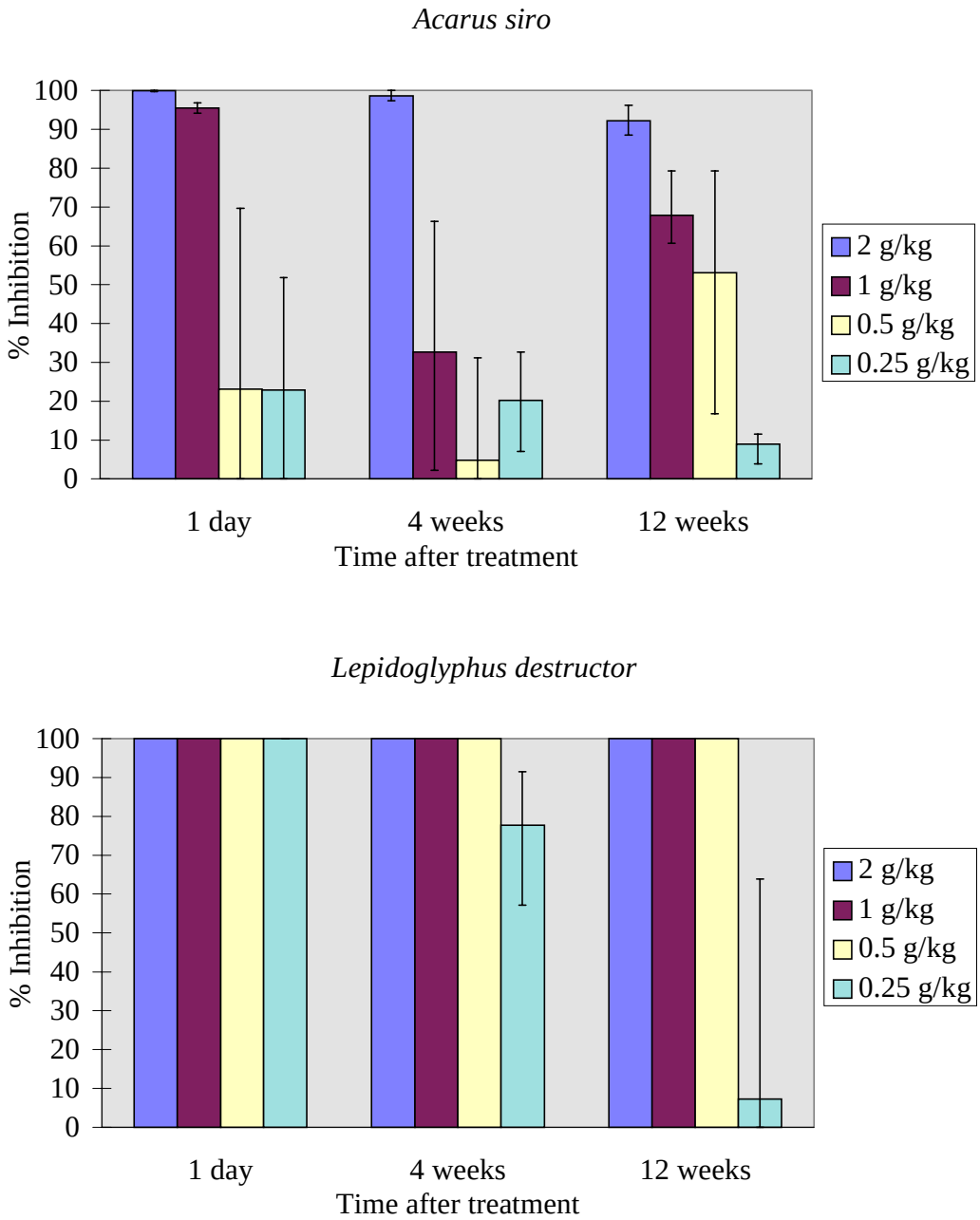
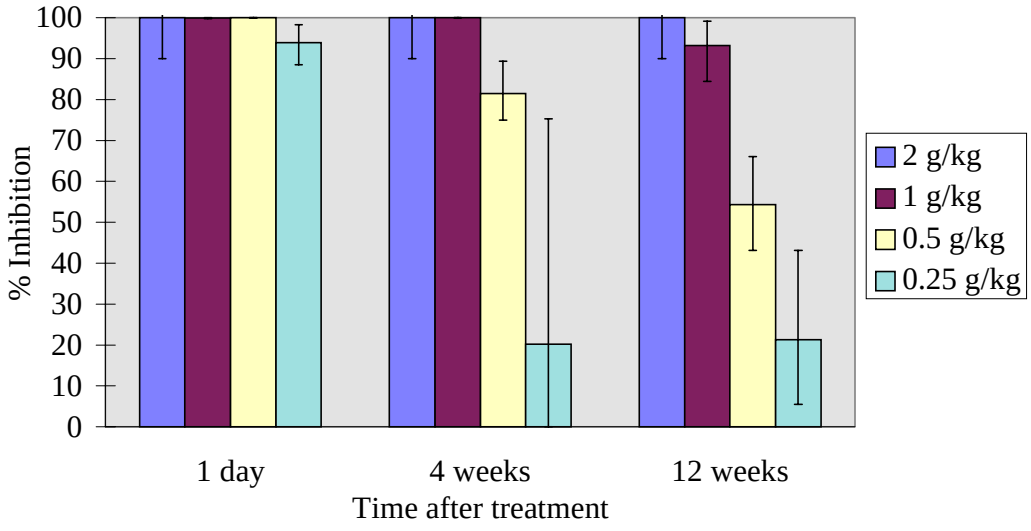


Figure 27 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Sodium polyborate on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

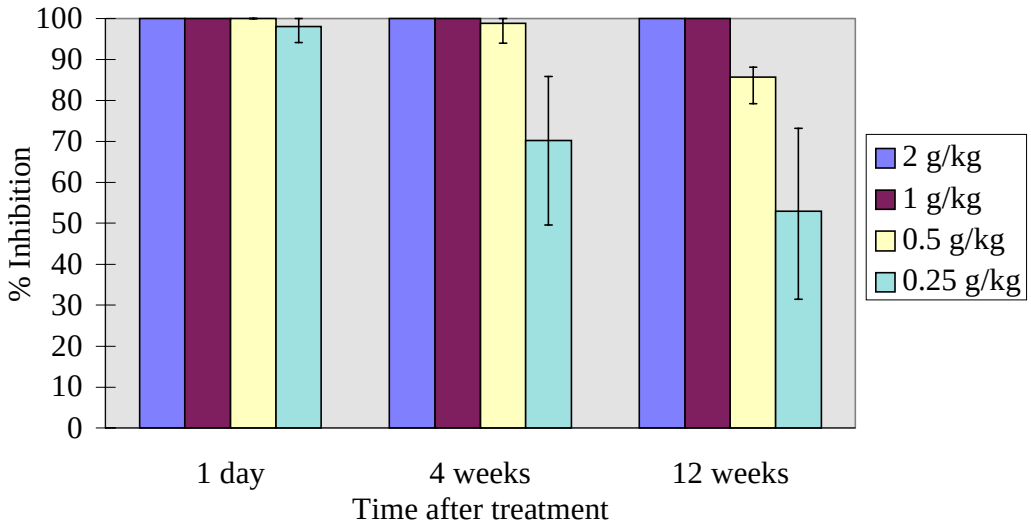


Table 29 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Sodium polyborate at different times after treatment on wheat stored at 25°C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
O. surinamensis susceptible	100	30.5	1.6	0.8	87.9	4	0.8	2.4	50.4	7.3	0.8	0
O. surinamensis resistant	74.4	7.2	4	0.8	14.5	0	1.6	0	4.8	0.9	0	0
S. granarius susceptible	0.8	0	0	0	0.8	0	0	0	0	0	0	1.6
S. granarius resistant	0	0	0	0	0	0	0.8	0	0	0	0	0
R. dominica susceptible	8	3.2	2.4	0.8	3.3	0	0.8	0	0.8	2.4	0	0
R. dominica resistant	7.2	0	0.8	1.6	6.6	0.8	1.6	0.8	0	0	0	0

Table 30 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Sodium polyborate at different times after treatment on wheat stored at 10^{o}C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
O. surinamensis susceptible	0	1.6	0	0	0.8	0	0	0	0	1.6	0	0
O. surinamensis resistant	0	0	0	0	0	0	1.6	0	0.8	1.6	1.6	0
S. granarius susceptible	0	0	0	0	0	0	0	0	0	0	0	0
S. granarius resistant	0	0	0	0	0	0	0.8	0	0	0	0	0
R. dominica susceptible	0	0	0.8	1.6	0	2.4	0.8	4	0	0	0	0
R. dominica resistant	3.1	2.4	0.8	2.4	1.6	0	0.8	2.4	1.7	0	0	3.2

Table 31 : Mean % population inhibition and ranges of each beetle species exposed to 4 doses of Sodium polyborate at different times after treatment on wheat stored at 25°C and 75% rh

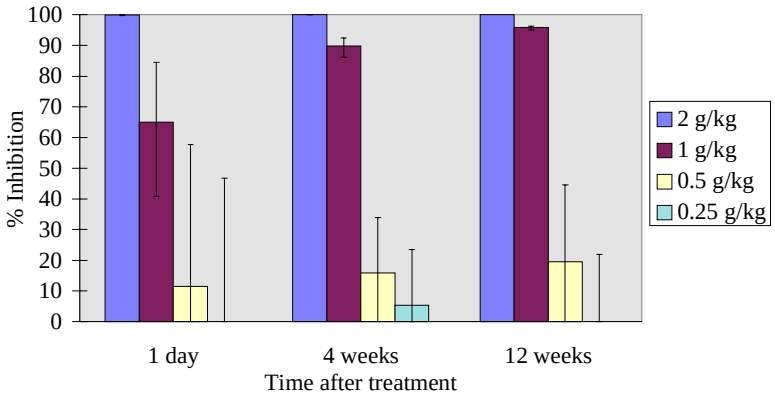
Figures in square brackets are the mean % of the F1 adults that were dead (n=5*)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	nos (range)	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg	nos (range)	2 g/kg	1 g/kg	0.5 g/kg	0.25 g/kg
O. surinamensis susceptible	97.4 [4.7] (68 - 126)	100	99 [0 *] (98 - 100)	85.8 [3.7] (74.3 - 95.9)	41.8 [3.5] (0 - 74.3)	92.8 [4.3] (60 - 116)	99.8 [100*] (98.9 - 100)	97.4 [6.25*] (95.7 - 100)	70.7 [5.1] (47.2 - 86)	17.7 [4.7] (0 - 68.8)	77 [2.6] (45 - 93)	100	96.9 [0*] (93.5 - 100)	67.3 [3.2] (37.7 - 81.8)	12.5 [4.1] (0 - 48.1)
O. surinamensis resistant	69.4 [9.2] (58 - 85)	100	98.8 [16.7 *] (97.1 - 100)	81 [3.3] (71.2 - 85.6)	5.5 [3.9] (0 - 45.2)	53 [32.5] (25 - 79)	99.2 [100*] (98.1 - 100)	99.6 [100*] (98.1 - 100)	77 [1.4] (47.2 - 90.6)	0 [2.2] (0 - 30.2)	77.4 [12] (64 - 87)	99.7 [0] (98.7 - 100)	96.6 [0] (92.2 - 98.7)	65.4 [2.5] (28.9 - 84.5)	0 [2.6] (0 - 9.6)
S. granarius susceptible	289.4 [0.2] (244 - 341)	99.9 [0*] (99.7 - 100)	65 [3.8] (40.9 - 84.5)	11.5 [0.2] (0 - 57.8)	0 [0.1] (0 - 46.8)	316.2 [0.1] (266 - 399)	100	89.8 [1.3] (86.1 - 92.4)	16 [0.1] (0 - 33.9)	5.4 [0.1] (0 - 23.5)	299.8 [0.2] (126 - 467)	100	95.9 [3.4] (95 - 96.3)	19.5 [0.1] (0 - 44.6)	0 [0.2] (0 - 21.9)
S. granarius resistant	235.4 [0.4] (155 - 336)	100	82.9 [1.6] (79.6 - 88.5)	14 [0] (0 - 32)	0 [0.4] (0 - 24.4)	270.8 [0.2] (203 - 322)	100	95.9 [1.2] (93.7 - 97.4)	58.9 [0.8] (49.4 - 77.8)	14.5 [0.2] (0 - 58.6)	262.4 [0.1] (185 - 373)	100	98.5 [0] (97.7 - 99.6)	62.1 [0.2] (53.9 - 71.8)	2.9 [0.1] (0 - 35.2)
R. dominica susceptible	561.6 [0.6] (484 - 616)	100	99.6 [16.7] (99.5 - 99.6)	95.7 [12.9] (91.5 - 98.8)	60.5 [1.7] (46.9 - 70.8)	506 [0.8] (400 - 584)	99.8 [100] (99.6 - 99.8)	99.2 [21.3] (99 - 99.6)	95.7 [12.7] (94.9 - 96.8)	43.4 [0.6] (35.4 - 50.2)	520 [0.5] (346 - 692)	99.5 [100] (99.2 - 99.6)	99.9 [0 *] (99.6 - 100)	96 [13.4] (94.8 - 97.5)	62.2 [1.1] (51.7 - 75.4)
R. dominica resistant	520.8 [0.3] (402 - 585)	100	98.7 [34.7] (97.7 - 99.2)	87.7 [9.8] (84.4 - 90.6)	66.6 [1] (55.5 - 81.4)	504.6 [0.4] (447 - 552)	99.7 [100*] (99.4 - 100)	98.9 [47] (97.6 - 99.8)	92.4 [12.7] (87.5 - 96.4)	67.3 [1.9] (46.9 - 81.2)	301.2 [0.5] (214 - 400)	99.5 [100*] (98.7 - 100)	100	98.1 [29.5] (96.3 - 99.3)	73.4 [2.1] (58.5 - 88.4)

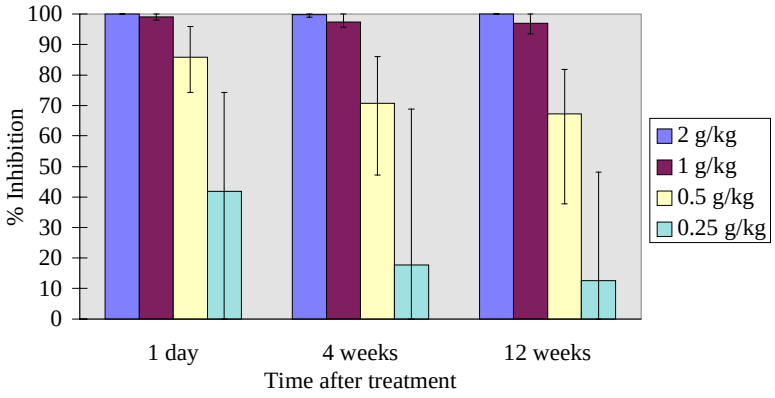
* - n = < 5 where 100% inhibition occurred in one or more replicates

Figure 28 : Mean % population inhibition (and ranges) of susceptible strains of insects exposed to 4 doses of Sodium polyborate on wheat stored at 25ºC and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

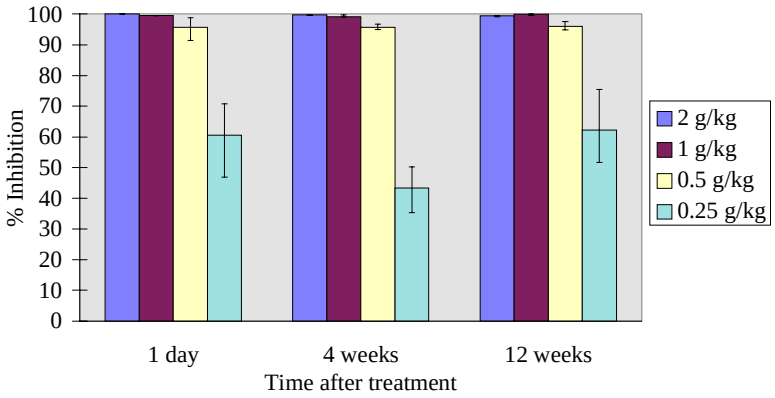


Figure 29 : Mean % population inhibition (and ranges) of OP resistant strains of insects exposed to 4 doses of Sodium polyborate on wheat stored at 25ºC and 75% rh (n=5)

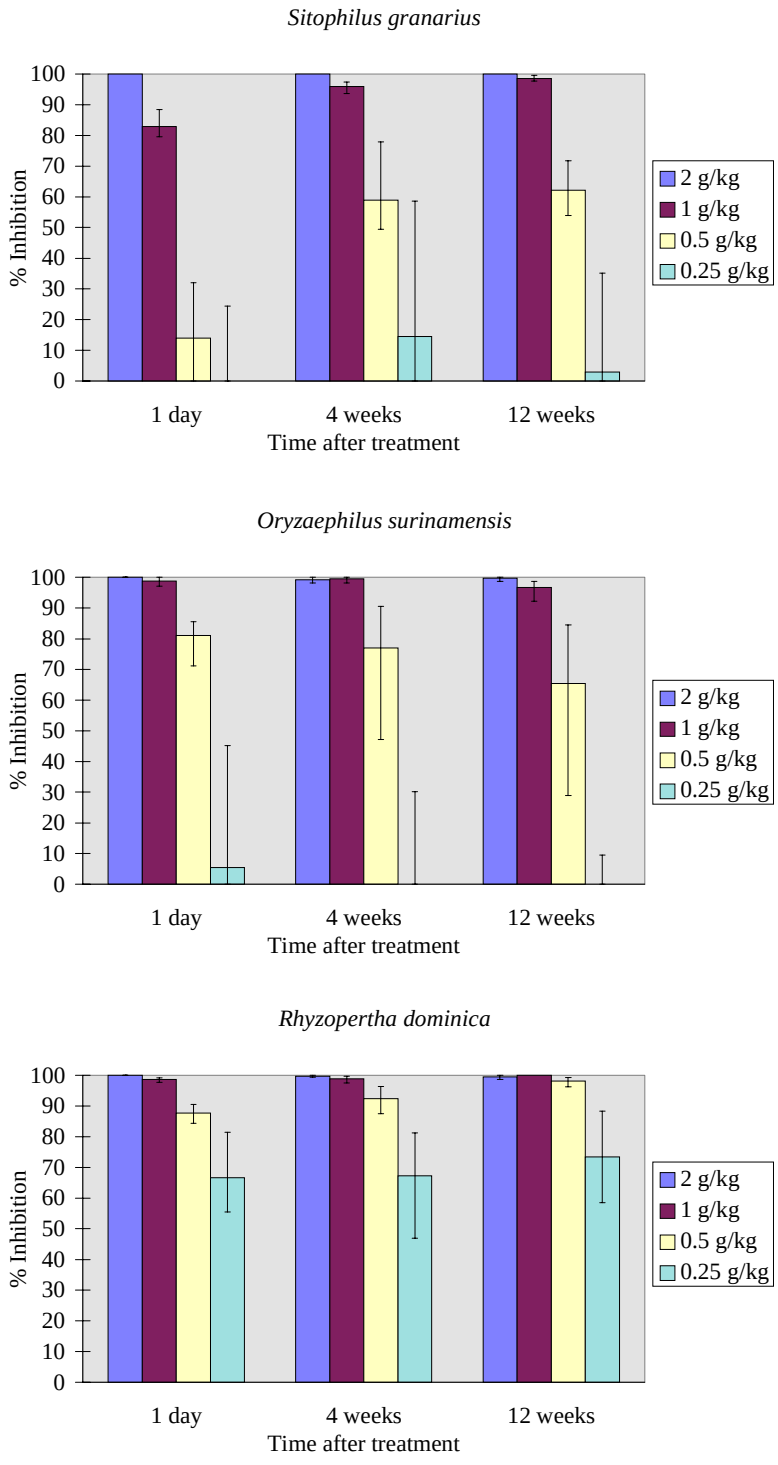
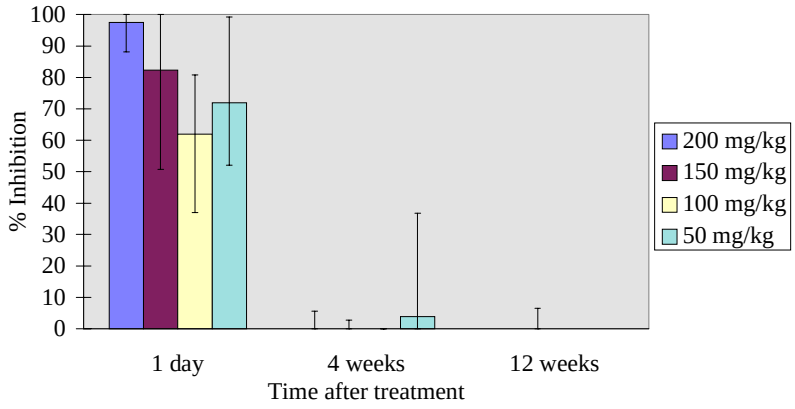


Table 32 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Benzyl benzoate at different times after treatment on wheat stored at 25^{o}C and 75% rh (n=5)

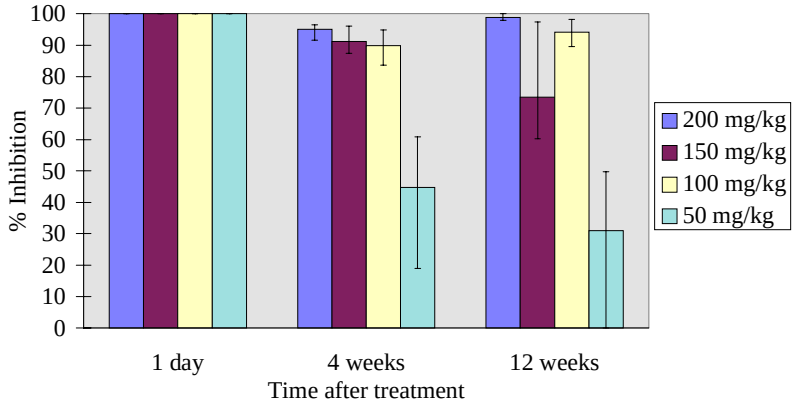
Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg		200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg		200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg
A. siro susceptible	584 (448 - 672)	97.6 (88.2 - 100)	82.3 (50.7 - 100)	61.9 (37 - 80.8)	71.9 (52.1 - 99.3)	1152 (824 - 1856)	0 (0 - 5.6)	0 (0 - 2.8)	0	3.9 (0 - 36.8)	1062.4 (720 - 1296)	0	0 (0 - 6.6)	0	0
A. siro resistant	1452.8 (712 - 2752)	100	61.5 (0 - 100)	55.1 (0 - 99.7)	42.6 (0 - 96.8)	1254.4 (1032 - 1480)	38.3 (33.7 - 43.2)	3.4 (0 - 24.1)	0 (0 - 8.8)	0 (0 - 13.3)	1203.2 (920 - 1512)	0 (0 - 20.9)	0 (0 - 19.5)	8 (0 - 20.9)	0 (0 - 5.6)
L. destructor susceptible	609.6 (536 - 776)	100	100	100	100	286.4 (208 - 448)	95 (91.6 - 96.5)	91.2 (87.4 - 96.1)	89.9 (83.6 - 94.8)	44.7 (19 - 60.9)	382.4 (264 - 488)	98.8 (97.9 - 100)	73.5 (60.3 - 97.4)	94.2 (89.5 - 98.2)	31 (0 - 49.8)
L. destructor resistant	592 (368 - 776)	100	100	99.7 (98.8 - 100)	100	435.2 (288 - 664)	94.3 (91.5 - 96.3)	54.4 (39.3 - 68.8)	62.1 (55.9 - 65.1)	36.8 (15.4 - 68.8)	265.6 (208 - 320)	99.6 (98.5 - 100)	69.3 (60.8 - 75.9)	93 (88 - 98.5)	7.8 (0 - 45.8)
T. putrescentiae susceptible	832 (616 - 1072)	99.1 (95.7 - 100)	66.4 (32.7 - 95.3)	64 (30.8 - 82.7)	2.1 (0 - 91.3)	840 (624 - 1096)	31.8 (10.5 - 52.4)	0	0	0	918.4 (760 - 1000)	29.1 (14.6 - 38.2)	0 (0 - 5.9)	0 (0 - 11.1)	0
T. putrescentiae resistant	817.6 (592 - 1000)	99.8 (98.8 - 100)	99.9 (99.9 - 100)	9.8 (0 - 87.3)	77.2 (50.1 - 93.4)	841.6 (640 - 1032)	35.7 (28.7 - 45.8)	0	0	0	409.6 (256 - 696)	13.7 (0 - 31.6)	0	20.7 (0.4 - 41.4)	0

Figure 30 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Benzyl benzoate on wheat stored at 25°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae

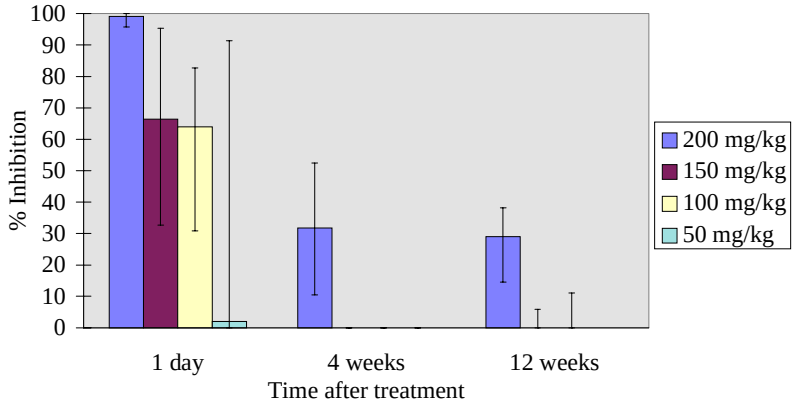
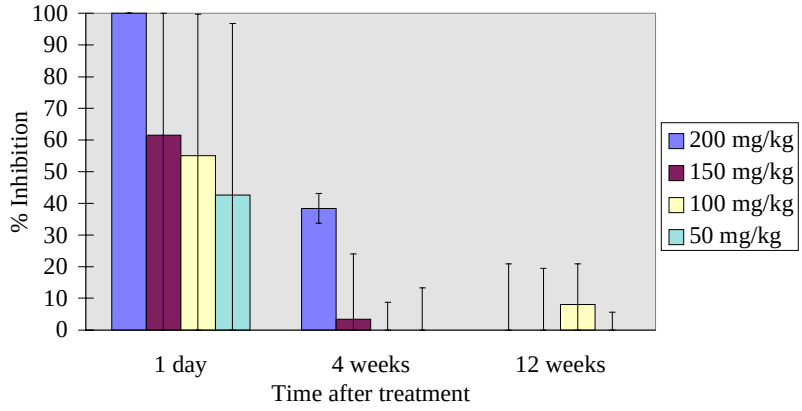
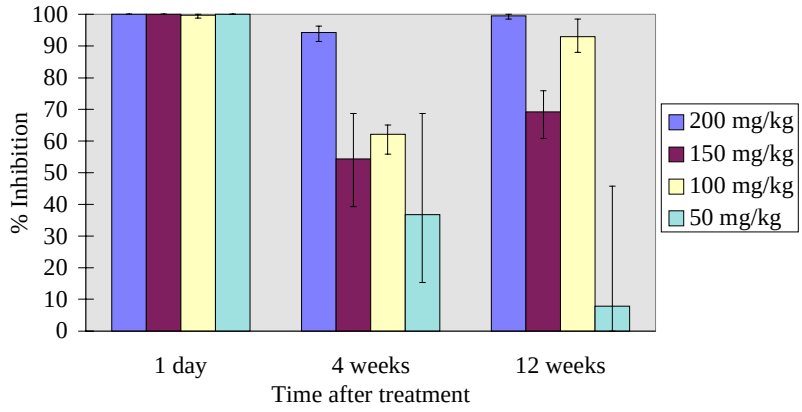


Figure 31 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Benzyl benzoate on wheat stored at 25ºC and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae

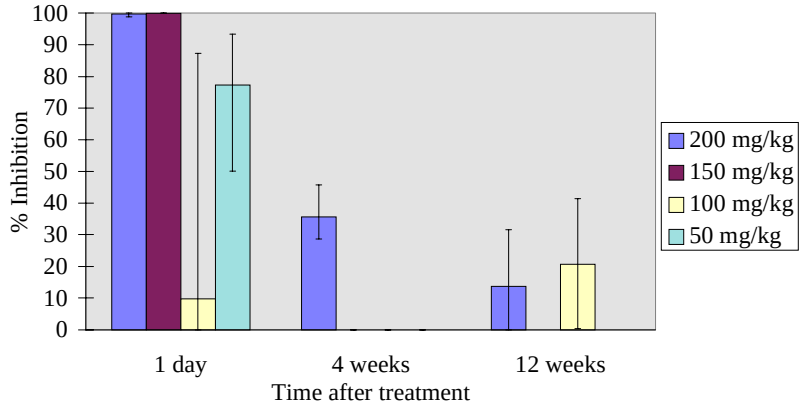
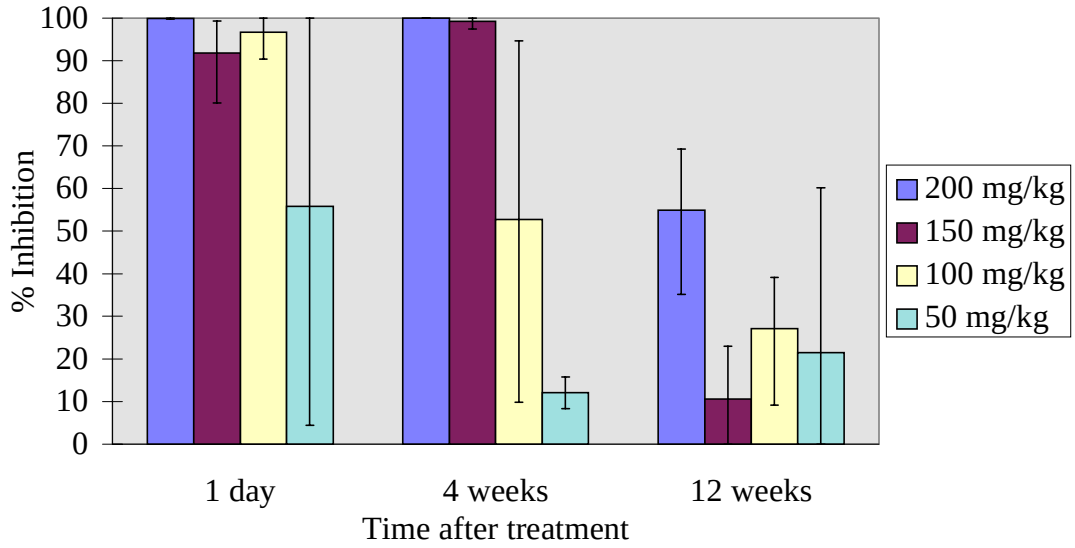


Table 33 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Benzyl benzoate at different times after treatment on wheat stored at 10^{o}C and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg	nos (range)	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg	nos (range)	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg
A. siro susceptible	401.6 (352 - 504)	99.9 (99.8 - 100)	91.8 (80.1 - 99.3)	96.6 (90.3 - 100)	55.8 (4.4 - 100)	540.8 (392 - 784)	100	99.2 (97.4 - 100)	52.7 (9.8 - 94.6)	12.1 (8.3 - 15.7)	985.6 (792 - 1152)	54.9 (35.1 - 69.2	10.6 (0 - 22.9)	27.1 (9.1 - 39.1	21.4 (0 - 60.2)
A. siro resistant	235.2 (184 - 328)	100	100	98.4 (91.9 - 100)	39.3 (0 - 100)	548.8 (408 - 696)	96.4 (90.3 - 98.5)	96.2 (93.3 - 98.7)	76.1 (66.5 - 85.4)	31.2 (15.5 - 43.1)	984 (752 - 1336)	40.8 (17.1 - 62.6	13.7 (2.4 - 25.2)	9.3 (0 - 23.6)	0 (0 - 1.6)
L. destructor susceptible	9.8 (2 - 25)	100	100	100	100	48.4 (5 - 168)	99.6 (97.9 - 100)	97.1 (87.6 - 100)	98.8 (93.8 - 100)	67.4 (54.5 - 79.3)	69.4 (38 - 113)	85.3 (79.8 - 91.4)	72.6 (66.9 - 79.8)	54.2 (39.5 - 62.5)	53 (40.9 - 64)
L. destructor resistant	17.4 (7 - 35)	100	100	100	100	31.2 (10 - 57)	99.4 (96.8 - 100)	100	92.9 (67.9 - 100)	0 (0 - 19.9)	90.6 (19 - 138)	23.6 (0 - 43.6)	65.1 (52.5 - 69.1)	54.3 (43.7 - 76.8)	36.2 (11.7 - 64.7)

Figure 32 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Benzyl benzoate on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

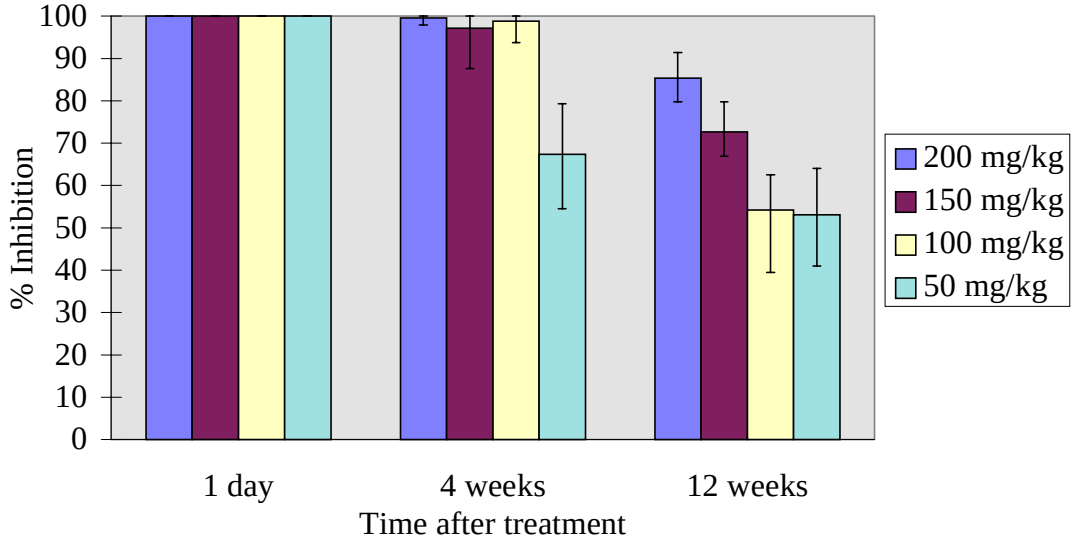
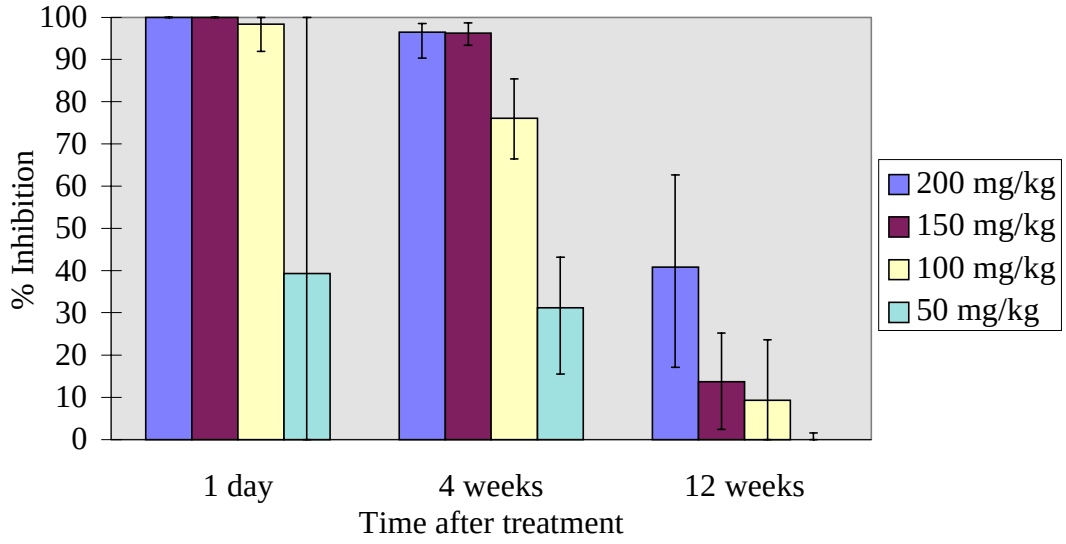


Figure 33 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Benzyl benzoate on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

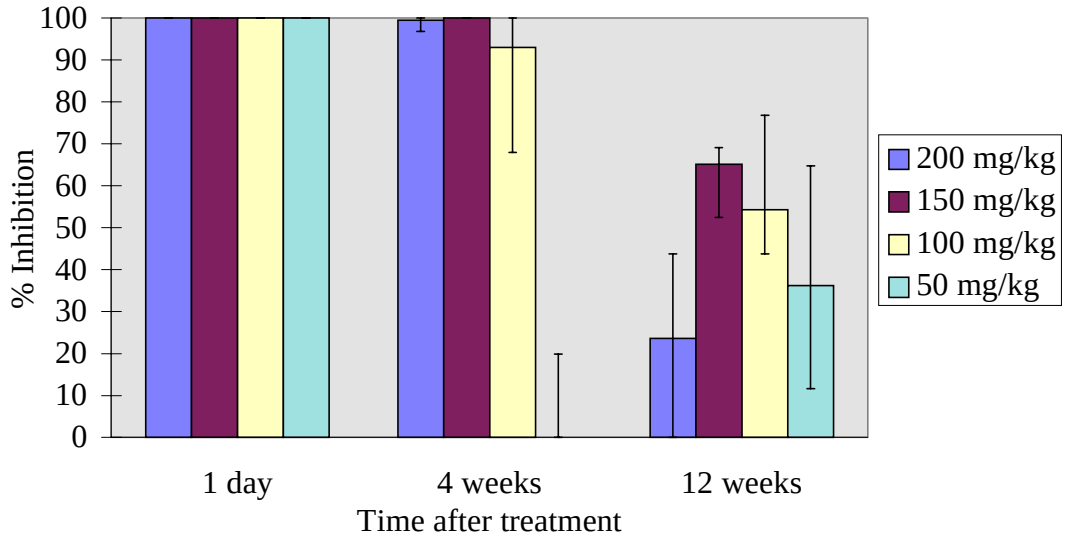


Table 34 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Benzyl benzoate at different times after treatment on wheat stored at 25°C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg
O. surinamensis susceptible	10.4	1.6	0.8	2.3	5.6	0.8	0.8	2.4	24.8	5.7	5	2.4
O. surinamensis resistant	1.6	1.6	1.6	0	0	0	0	0.8	0	0	0.8	0
S. granarius susceptible	0	0	1.6	0	0	0	0	0.8	1.6	1.6	0.8	1.6
S. granarius resistant	0	0	0.8	0	0.8	0	0	0.8	0	0	0	0
R. dominica susceptible	0	0.8	1.6	0	0	0	0	2.4	2.4	0.9	0	0
R. dominica resistant	0.8	1.6	0.8	0.8	0	0	0	0	0	0	0	3.2

Table 35 : Mean mortality of each beetle species exposed for 7 days to 4 doses of Benzyl benzoate at different times after treatment on wheat stored at 10^{o}C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg	200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg
O. surinamensis susceptible	99.2	97.6	78.1	50.7	47.4	27.4	1.8	0.1	17.7	4	8.6	0
O. surinamensis resistant	94.4	90.4	68.6	54.4	5.6	0.8	0.8	0	4.1	0.8	2.4	0.8
S. granarius susceptible	0	0.8	0.8	0	0	0.8	0	0	0	0	0	0.8
S. granarius resistant	0	4	0	8	0.8	0	0	0.8	0.8	0.8	4	0.8
R. dominica susceptible	45.3	13.9	12.8	2.4	0.8	0.8	0	0.8	3.2	0	0.8	0
R. dominica resistant	17.6	22.4	4	0.8	0	0	0	1.6	0	0	1.5	2.4

Table 36 : Mean % population inhibition and ranges of each beetle species exposed to 4 doses of Benzyl benzoate at different times after treatment on wheat stored at 25^{o}C and 75% rh

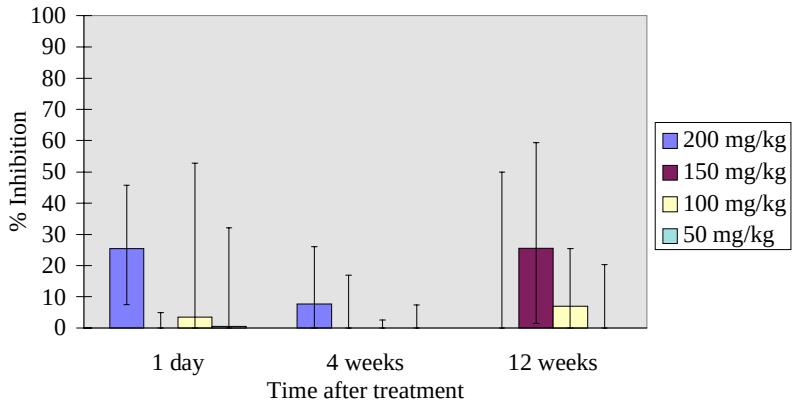
Figures in square brackets are the mean % of the F1 adults that were dead (n=5*)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)				Mean control nos (range)	Mean % Inhibition (ranges)			
		200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg		200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg		200 mg/kg	150 mg/kg	100 mg/kg	50 mg/kg
O. surinamensis susceptible	89.2 [5] (71 - 107)	96.2 [0] (92.2 - 98.9)	86.8 [2.1] (78.7 - 92.2)	45.7 [1.7] (35 - 59.6)	0 [4.6] (0 - 26)	118 [5.8] (96 - 160)	48.6 [4.3] (32.2 - 61.9)	41.2 [6.3] (23.7 - 67.8)	38.5 [12.7] (11.9 - 54.2)	17.1 [8.6] (0 - 59.3)	92.6 [4.7] (57 - 139)	92.4 [5*] (78.4 - 100)	40.6 [7.2] (23.3 - 56.8)	67.6 [2.7] (33 - 87)	0 [4.9] (0 - 61.1)
O. surinamensis resistant	79.2 [15.6] (66 - 86)	84.3 [7.3] (59.6 - 94.9)	76.8 [3.7] (54.5 - 89.9)	42.2 [5.2] (38.1 - 52)	3.8 [4.3] (0 - 41.9)	128 [11.1] (121 - 136)	50.2 [12.9] (33.6 - 64.1)	36.6 [4.3] (10.9 - 53.1)	40.8 [20] (9.4 - 57)	8.1 [7.5] (0 - 25)	84.2 [11.8] (48 - 143)	79.6 [7] (70.3 - 85.7)	36.3 [5.1] (22.8 - 71.5)	59.9 [5] (52.5 - 72.7	0 [11.3] (0 - 24)
S. granarius susceptible	265 [0.1] (178 - 363)	25.4 [0.2] (7.5 - 45.7)	0 [0.1] (0 - 4.9)	3.5 [0.1] (0 - 52.8)	0.5 [0.4] (0 - 32.1)	326 [0] (159 - 427)	7.7 [0] (0 - 26.1)	0 [0.3] (0 - 16.9)	0 [0.1] (0 - 2.5)	0 [0.1] (0 - 7.4)	329.8 [0] (249 - 420)	0 [0.04] (0 - 50)	25.6 [0.2] (1.5 - 59.4)	7 [0.1] (0 - 25.4)	0 [0.1] (0 - 20.3)
S. granarius resistant	271.8 [0.1] (198 - 329)	0 [0] (0 - 41.1)	0 [0.1] (0 - 33.8)	2.1 [0.2] (0 - 20.5)	0 [0.1] (0 - 25.3)	298 [0.2] (222 - 410)	0.6 [0.2] (0 - 21.5)	6.9 [0.1] (0 - 50.3)	10.7 [0.4] (1.3 - 25.5)	28.3 [0.1] (4.4 - 74.5)	259.8 [0] (180 - 336)	0 [0.2]	0 [0.1]	0 [0.1] (0 - 13.4)	0 [0] (0 - 11.9)
R. dominica susceptible	449.6 [0.4] (364 - 534)	30 [0.2] (1.5 - 58.2)	5.3 [0.7] (0 - 49.7)	0 [0.4] (0 - 11)	0 [0.3]	555.6 [0.2] (417 - 837)	40 [0.5] (28.2 - 49.4)	34.2 [0.7] (27.8 - 44.4)	9.8 [0.2] (2.4 - 17.2)	18.8 [0.3] (11.8 - 26.6)	473.6 [0.8] (400 - 567)	1.9 [0.9] (0 - 18.7)	0 [0.8] (0 - 6.5)	0 [0.8] (0 - 9)	0 [0.9] (0 - 29.1)
R. dominica resistant	427.6 [0.2] (335 - 504)	42.2 [1.4] (21.4 - 62.1)	41.8 [0.8] (28 - 58.4)	15.7 [1.1] (0 - 32.4)	16.1 [0.8] (7.4 - 27)	466 [0.3] (398 - 536)	0 [0.8] (0 - 12)	0 [0.7] (0 - 34.5)	0 [0.2] (0 - 7.1)	0 [0.5] (0 - 15)	502.8 [1.4] (386 - 585)	14.4 [1.4] (7.1 - 28.8)	8.4 [1] (0 - 44.3)	16.7 [0.7] (1.2 - 58)	7.9 [0.9] (0 - 36.2)

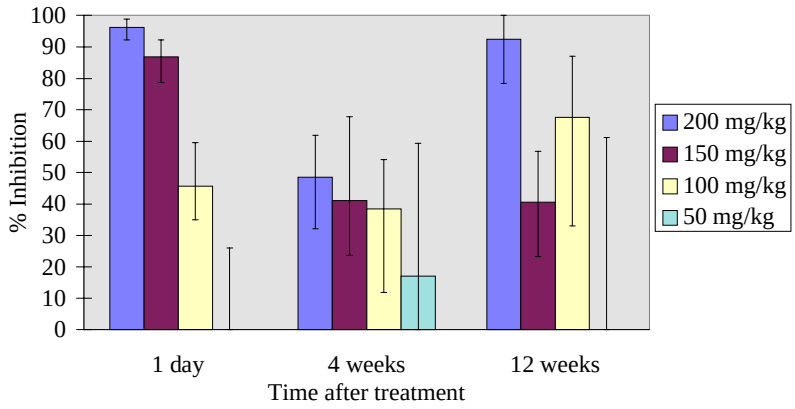
* - n = < 5 where 100% inhibition occurred in one or more replicates

Figure 34 : Mean % population inhibition (and ranges) of susceptible strains of insects exposed to 4 doses of Benzyl benzoate on wheat stored at 25°C and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

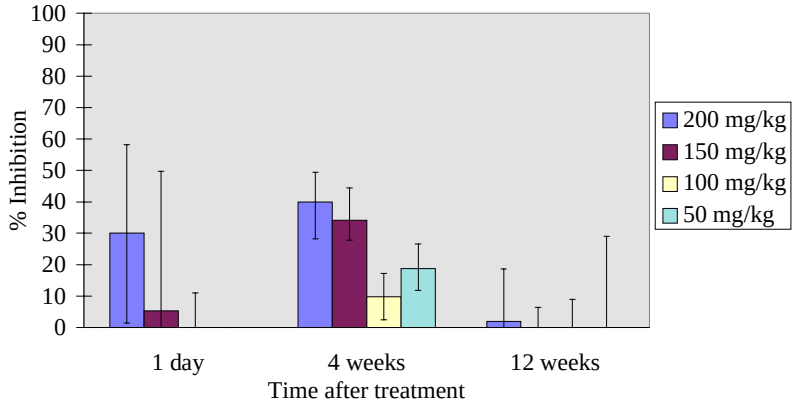
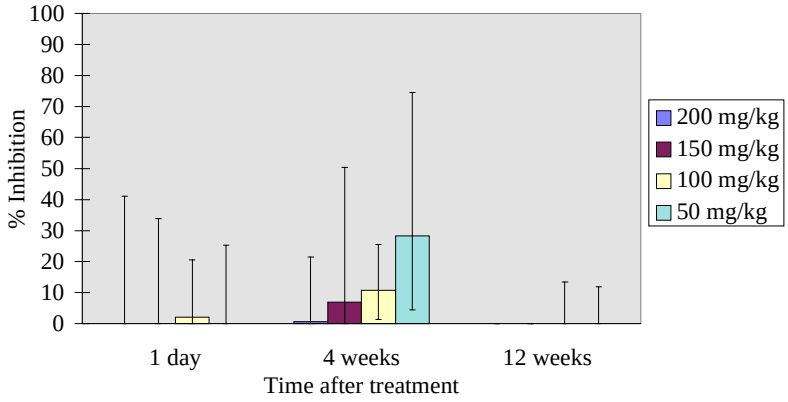
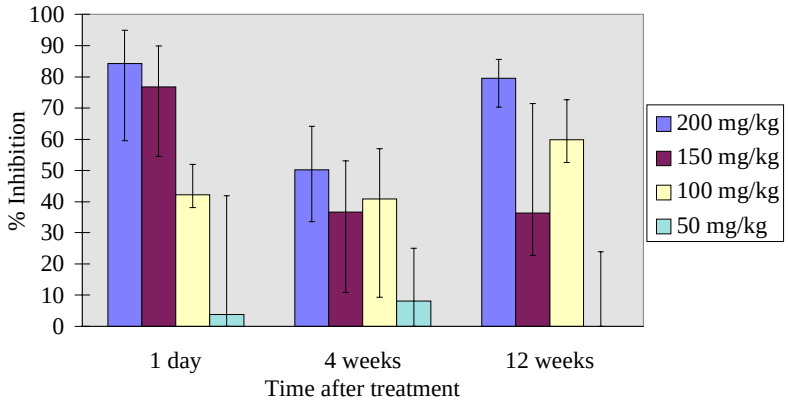


Figure 35 : Mean % population inhibition (and ranges) of OP resistant strains of insects exposed to 4 doses of Benzyl benzoate on wheat stored at 25°C and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

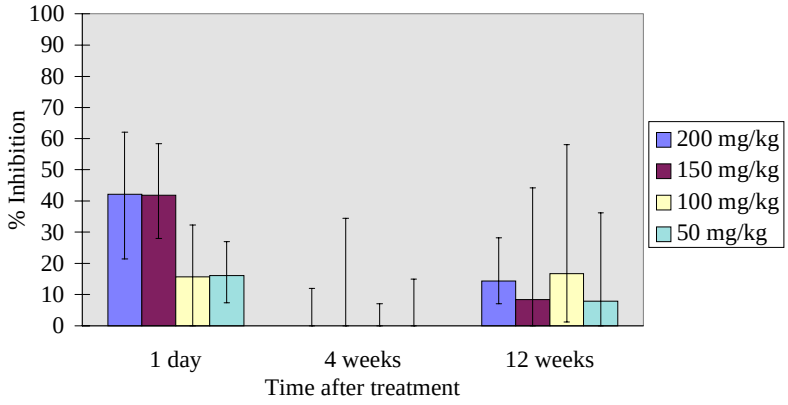
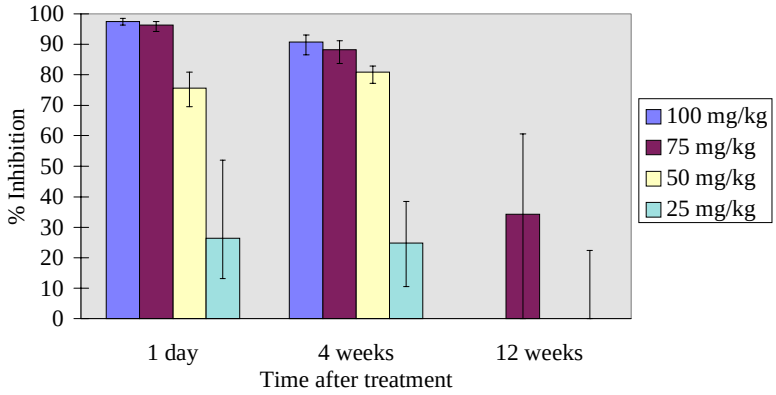


Table 37 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of azadirachtin at different times after treatment on wheat stored at 25°C and 75% rh (n=5)

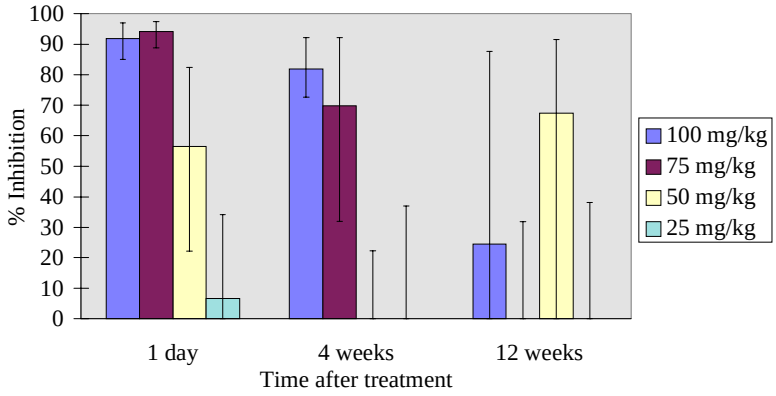
Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg
A. siro susceptible	1049.6 (976 - 1104)	97.5 (96.3 - 98.4)	96.3 (94.2 - 97.5)	75.6 (69.5 - 80.9)	26.4 (13.1 - 52)	832 (656 - 992)	90.8 (86.5 - 93)	88.2 (83.7 - 91.2)	80.9 (77.2 - 82.9)	24.8 (10.6 - 38.5)	753.6 (512 - 1040)	0	34.2 (0 - 60.7)	0	0 (0 - 22.5)
A. siro resistant	824 (720 - 944)	95.1 (93.3 - 98.1)	95.1 (94.1 - 96.1)	90.5 (88.8 - 92.1)	37.5 (31.1 - 44.7)	1070.4 (744 - 1272)	83.4 (77.6 - 90.3)	77.4 (63.4 - 87.3)	16.4 (0 - 62.6)	0 (0 - 13.3)	401.6 (352 - 472)	0 (0 - 20.3)	70 (50.2 - 85.3)	0 (0 - 14.3)	18.3 (0 - 58.2)
L. destructor susceptible	267.2 (160 - 336)	91.8 (85 - 97)	94.1 (88.8 - 97.4)	56.5 (22.2 - 82.4)	6.6 (0 - 34.1)	164.8 (128 - 248)	81.9 (72.7 - 92.1)	69.8 (32 - 92.1)	0 (0 - 22.3)	0 (0 - 36.9)	129.2 (30 - 208)	24.5 (0 - 87.6)	0 (0 - 31.9)	67.5 (0 - 91.5)	0 (0 - 38.1)
L. destructor resistant	160 (104 - 216)	93.1 (89.4 - 96.3)	88.8 (85.6 - 92.5)	73.3 (55 - 81.3)	36 (0 - 50)	95.8 (82 - 109)	54.7 (30.1 - 78.1)	62.2 (48.9 - 76)	26.5 (11.3 - 46.8)	0 (0 - 6.1)	34.8 (14 - 76)	0 (0 - 33.9)	27.6 (0 - 82.7)	28.2 (0 - 74.1)	2.3 (0 - 28.2)
T. putrescentiae susceptible	860.8 (704 - 1000)	95.4 (89.5 - 98.4)	93 (87.9 - 96.3)	66 (58.2 - 72.1)	20.1 (0 - 40.5)	508.8 (440 - 600)	89 (84.7 - 91.2)	89.3 (85.7 - 92.5)	33 (18.2 - 49.7)	0 (0 - 1)	520 (424 - 608)	0 (0 - 66.2)	23.7 (16.9 - 30.8)	0 (0 - 18.5)	0 (0 - 36.9)
T. putrescentiae resistant	652.8 (560 - 760)	89.2 (86.4 - 92.3)	85 (83.1 - 87.6)	83 (80.1 - 87.6)	29.4 (15.4 - 41.2)	417.6 (344 - 592)	84.4 (77.5 - 89)	81.9 (72.9 - 90.9)	78.4 (73.9 - 84)	41.8 (31 - 55.9)	496 (400 - 600)	30.6 (9.7 - 45.2)	47.4 (33.9 - 61.3)	0 (0 - 29)	22.6 (0 - 54.8)

Figure 36 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Azadirachtin on wheat stored at 25ºC and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae

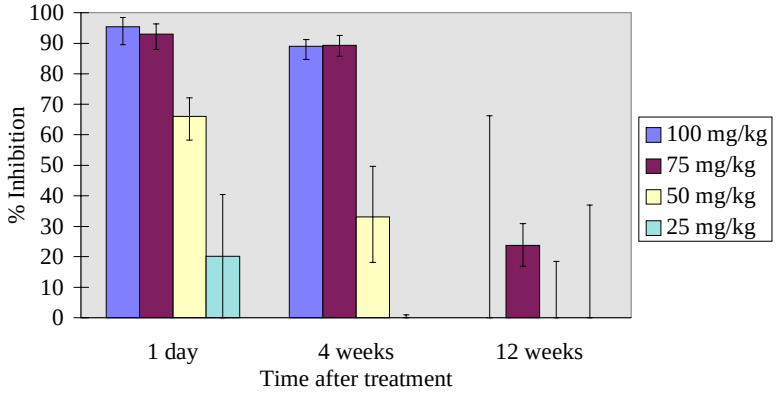
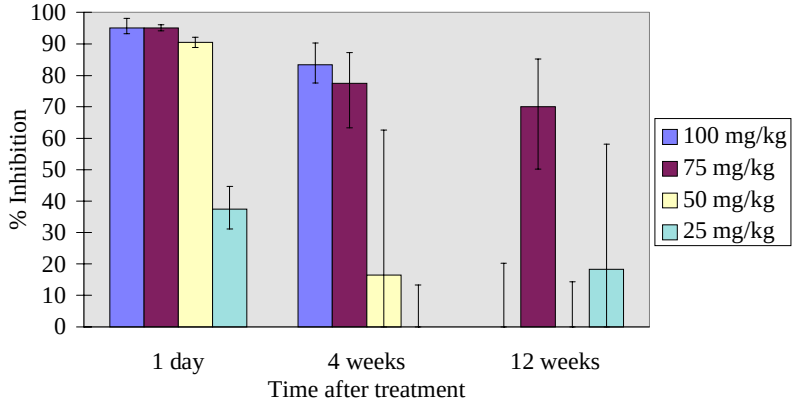
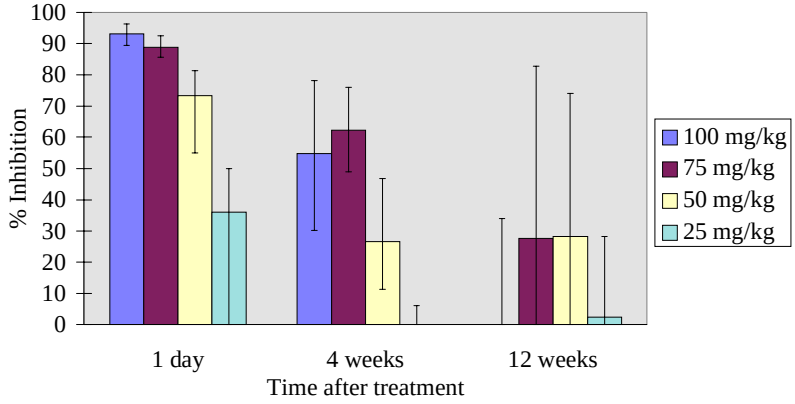


Figure 37 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Azadirachtin on wheat stored at 25°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae

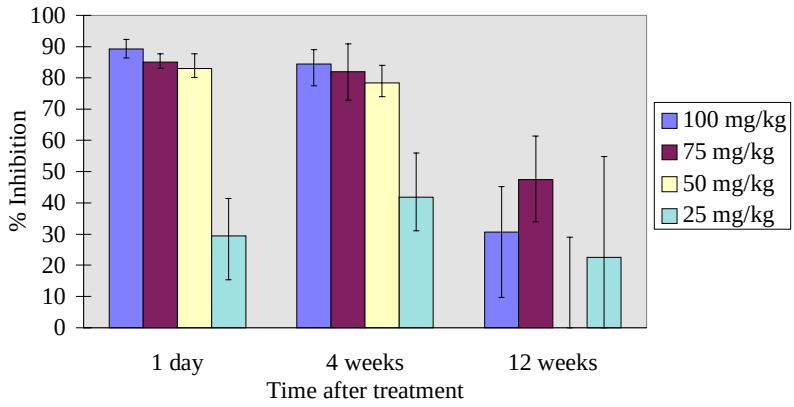
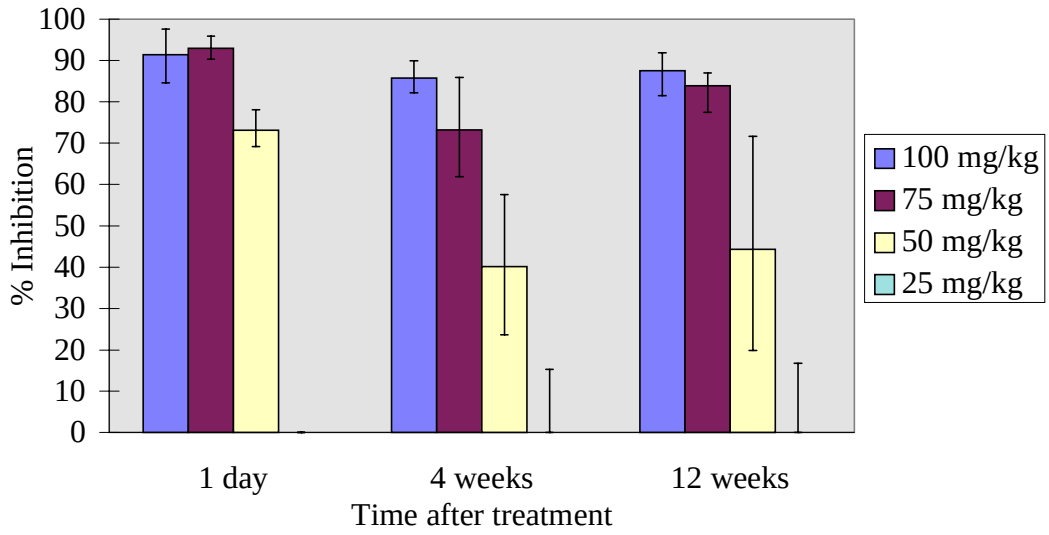


Table 38 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of azadirachtin at different times after treatment on wheat stored at 10°C and 75% rh (n=5)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg
A. siro susceptible	123.2 (72 - 184)	91.4 (84.6 - 97.6)	93 (90.3 - 95.9)	73.1 (69.2 - 78.1)	0	566.4 (376 - 864)	85.7 (82.2 - 89.9)	73.2 (61.9 - 85.9)	40.1 (23.7 - 57.6)	0 (0 - 15.3)	508.8 (408 - 624)	87.5 (81.5 - 91.9)	83.9 (77.4 - 87)	44.3 (19.8 - 71.7)	0 (0 - 16.7)
A. siro resistant	172.8 (112 - 232)	90.3 (86.1 - 93.6)	90.4 (87.3 - 94.8)	86.7 (83.8 - 89.6)	66.2 (60.1 - 73.4)	510.4 (440 - 592)	87.4 (84.5 - 89.4)	84.6 (82.8 - 85.9)	59.2 (43.6 - 74.7)	21.6 (6 - 42)	1080 (792 - 1568)	84 (81.5 - 87.4)	75.3 (72.6 - 79.3)	51.9 (45.9 - 57.8)	0.4 (0 - 13.3)
L. destructor susceptible	56.8 (6 - 184)	96.5 (93 - 100)	96.8 (91.2 - 100)	77.8 (64.8 - 98.2)	54.6 (0 - 93)	161.2 (78 - 296)	89.5 (78.9 - 98.8)	81.6 (73.9 - 94.4)	55.7 (51 - 64)	39.2 (20.6 - 56.6)	416 (248 - 544)	84.2 (61.5 - 96.4)	83.7 (67.3 - 94.9)	59.6 (46.2 - 75)	18.1 (0 - 53.8)
L. destructor resistant	36.4 (20 - 55)	95.6 (89 - 100)	89.6 (75.3 - 97.3)	58.2 (39.6 - 78)	37.9 (6.6 - 72.5)	203.2 (160 - 248)	79.5 (71.9 - 87.2)	70.6 (65.6 - 72.4)	57.4 (45.9 - 68)	12.6 (0 - 37)	409.6 (240 - 528)	75.8 (53.1 - 85.8)	53.9 (35.5 - 68.8)	12.5 (0 - 31.6)	5.5 (0 - 23.8)

Figure 38 : Mean % population inhibition (and ranges) of susceptible strains of mites exposed to 4 doses of Azadirachtin on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

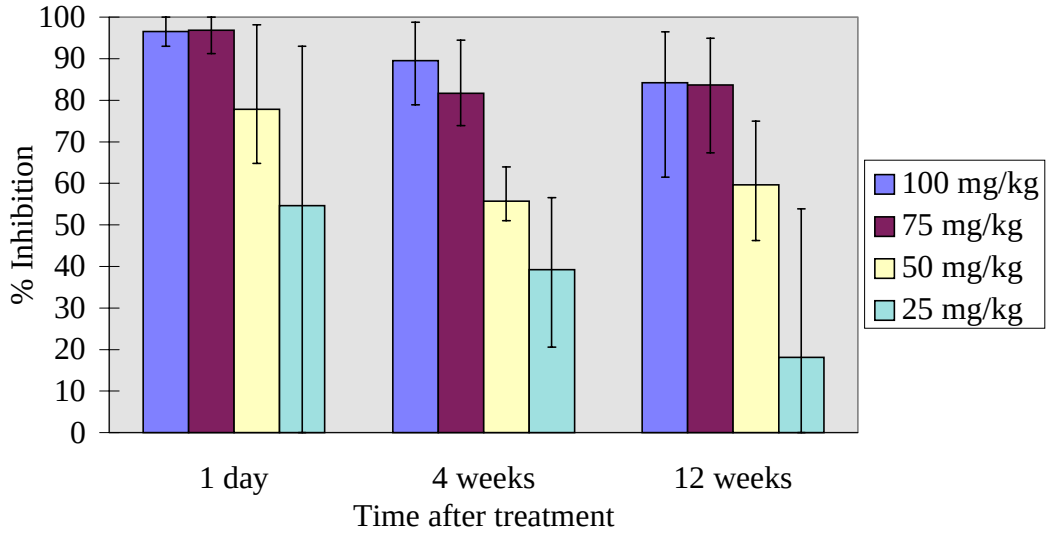
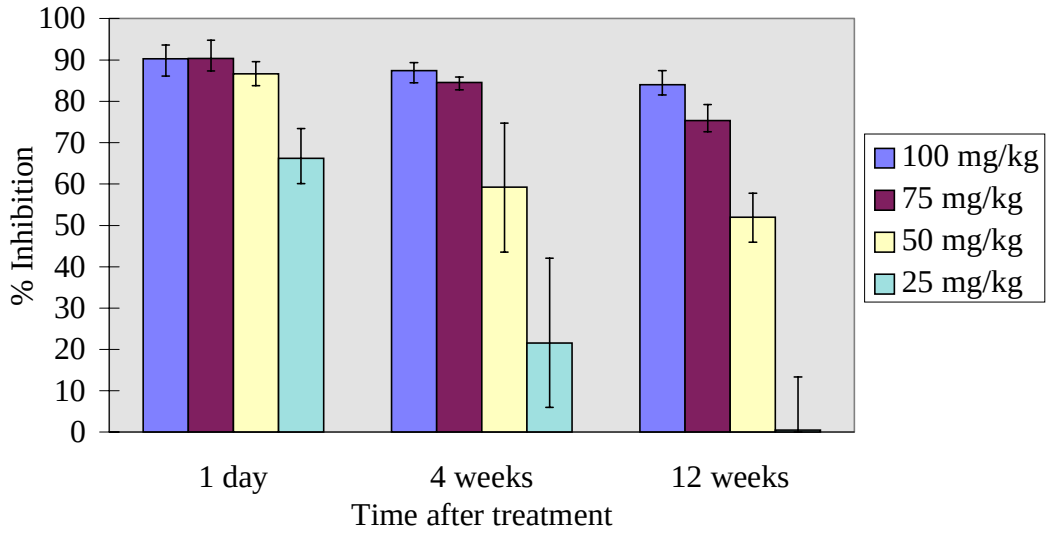


Figure 39 : Mean % population inhibition (and ranges) of OP resistant strains of mites exposed to 4 doses of Azadirachtin on wheat stored at 10°C and 75% rh (n=5)

Acarus siro



Lepidoglyphus destructor

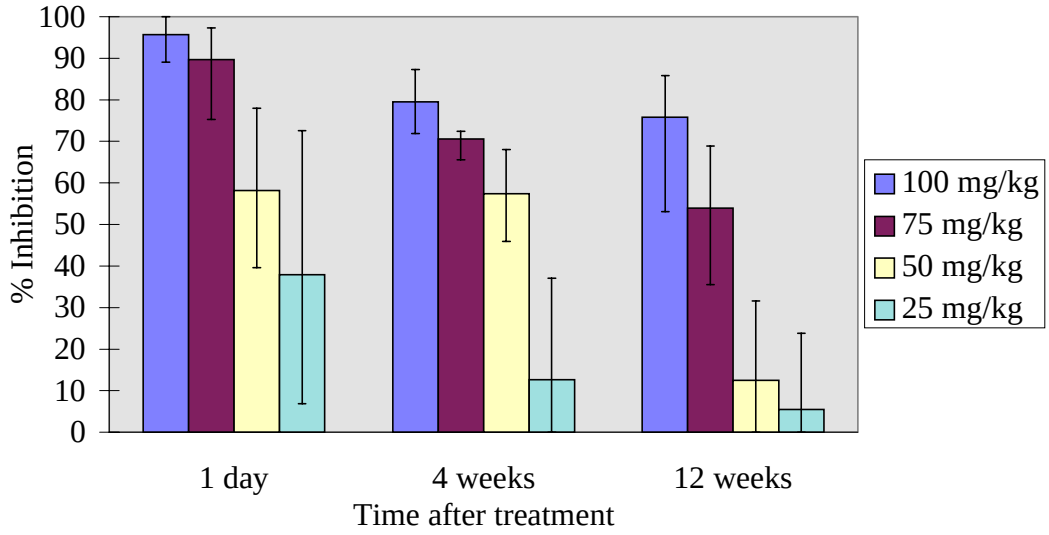


Table 39 : Mean mortality of each beetle species exposed for 7 days to 4 doses of azadirachtin at different times after treatment on wheat stored at 25°C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg
O. surinamensis susceptible	9.6	2.4	3.2	1.6	3.4	2.8	4.2	0	0	0	23.8	0
O. surinamensis resistant	1.6	0	0.8	2.4	0	0	0	0	0	0	0	0.7
S. granarius susceptible	0.8	0.8	0	0.8	0	0.8	0	0	0.8	0	0	4
S. granarius resistant	1.6	0.8	0.8	1.6	0	0	0	0	0	0	0	0
R. dominica susceptible	0.8	4	0.8	1.6	0	0	0	0	0	0	0	0
R. dominica resistant	0.8	0	0.8	3.2	0	0	0	0	0.8	0.9	0.8	0

Table 40 : Mean mortality of each beetle species exposed for 7 days to 4 doses of azadirachtin at different times after treatment on wheat stored at 10°C and 75% rh (n=5)

Species/ Strain	Time after treatment / Doses											
	One day				4 weeks				12 weeks			
	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg
O. surinamensis susceptible	6.3	5.6	1.6	2.4	0	1.5	1.6	0	9.8	3.4	8.9	0
O. surinamensis resistant	0	1.6	0	0.8	1.6	0.8	0	0	2.4	0	4.9	0
S. granarius susceptible	0	0	0	0	0	0	0	0	0	0.8	0	0.8
S. granarius resistant	0	0	0	0	0	0	0	0	0	0	0	0.8
R. dominica susceptible	1.5	0	0	1.6	0	0	0.8	0.8	2.4	0	1.6	0.8
R. dominica resistant	2.5	0	0.8	0	0	0	0	0	0	0	3.8	0

Table 41 : Mean % population inhibition and ranges of each beetle species exposed to 4 doses of azadirachtin at different times after treatment on wheat stored at 25°C and 75% rh

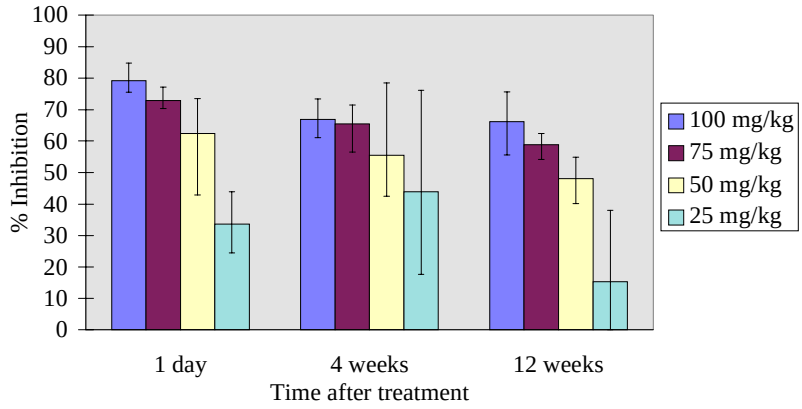
Figures in square brackets are the mean % of the F1 adults that were dead (n=5*)

Species/ Strain	One day after treatment					4 weeks after treatment					12 weeks after treatment				
	Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)				Mean control	Mean % Inhibition (ranges)			
	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg	nos (range)	100 mg/kg	75 mg/kg	50 mg/kg	25 mg/kg
O. surinamensis susceptible	80.8 [7.9] (67 - 93)	100	100	100	100	59.4 [10] (45 - 72)	100	100	100	100	23.4 [2.6] (9 - 45)	99.1 [100*] (95.7 - 100)	100	100	100
O. surinamensis resistant	61 [12.3] (41 - 92)	100	99.7 [100 *] (98.4 - 100)	100	99.7 [100*] (98.4 - 100)	92.6 [17.4] (75 - 107)	99.8 [100*] (98.9 - 100)	100	100	100	50 [6.6] (28 - 73)	100	100	100	100
S. granarius susceptible	294.2 [0.4] (190 - 480)	79.2 [6.7] (84.7 - 75.5)	72.9 [7.6] (70.4 - 77.2)	62.4 [3.2] (42.9 - 73.5)	33.7 [2.4] (24.5 - 43.9)	301 [0] (207 - 381)	66.8 [5.1] (61.1 - 73.4)	65.4 [6.3] (56.5 - 71.4)	55.5 [4.8] (42.5 - 78.4)	43.9 [2.8] (17.6 - 76.1)	266 [0.2] (105 - 380)	66.2 [2.8] (55.6 - 75.6)	58.8 [4.4] (54.1 - 62.4)	48.1 [3.3] (40.2 - 54.9)	15.3 [1.5] (0 - 38)
S. granarius resistant	269.4 [0] (195 - 341)	74.8 [3.4] (66.6 - 81.1)	67.7 [3] (61 - 75.5)	57.9 [3.6] (38.4 - 66.2)	44.6 [1.8] (16.8 - 80.3)	249.4 [0.2] (132 - 442)	64.4 [1.7] (53.9 - 88)	67 [3] (54.7 - 87.2)	51.9 [2.2] (58.2 - 42.7)	29.6 [1.1] (5.4 - 40.8)	261.8 [0.1] (222 - 304)	62.8 [1.1] (51.1 - 74)	53.6 [2.6] (43.9 - 66)	35.2 [1.5] (30.5 - 45)	22.5 [1.4] (12.1 - 38.1)
R. dominica susceptible	480.6 [0.5] (434 - 522)	99.1 [6.7] (98.3 - 99.6)	99.1 [33.3] (98.8 - 99.6)	96.8 [16.8] (95.8 - 97.9)	83.1 [13.7] (79.4 - 88.3)	432.4 [0.3] (322 - 562)	98.6 [20.1] (97.9 - 99.3)	98.6 [42.4] (98.1 - 99.1)	91.4 [22] (88.7 - 93.8)	74.1 [14] (68.1 - 78.5)	448 [0.3] (408 - 513)	88.4 [24.7] (85.3 - 92.6)	89.8 [22.3] (88.4 - 91.1)	70.6 [14.3] (51.3 - 79)	47.9 [8.9] (39 - 55.6)
R. dominica resistant	457.2 [0.3] (357 - 531)	99.3 [53.3] (98.7 - 99.8)	99.3 [49] (98.9 - 99.8)	97.2 [20.8] (96.1 - 97.8)	91.1 [4.4] (86.4 - 93.4)	453.8 [1] (361 - 503)	98.2 [39.4] (97.1 - 99.1)	98.8 [29.8] (98.2 - 99.3)	95.4 [14.1] (96 - 94.7)	82.6 [6.8] (77.7 - 85.5)	414 [0.6] (351 - 460)	83.5 [11] (80.4 - 86.5)	87.7 [14.1] (82.4 - 92.5)	60.5 [7.8] (58 - 65)	47.8 [3] (39.8 - 52.2)

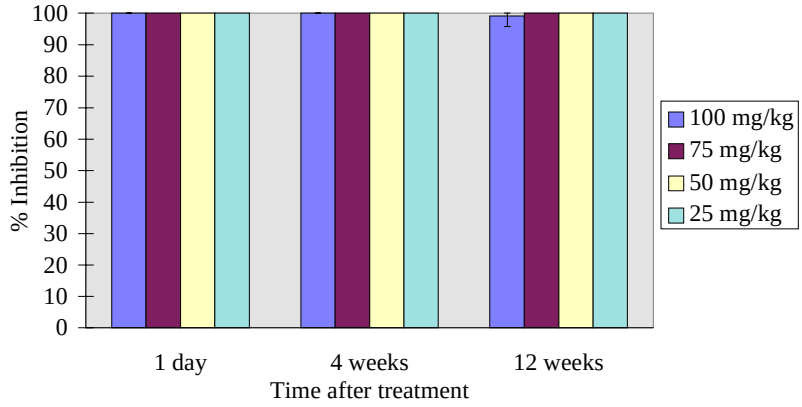
* - n = < 5 where 100% inhibition occurred in one or more replicates

Figure 40 : Mean % population inhibition (and ranges) of susceptible strains of insects exposed to 4 doses of Azadirachtin on wheat stored at 25°C and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

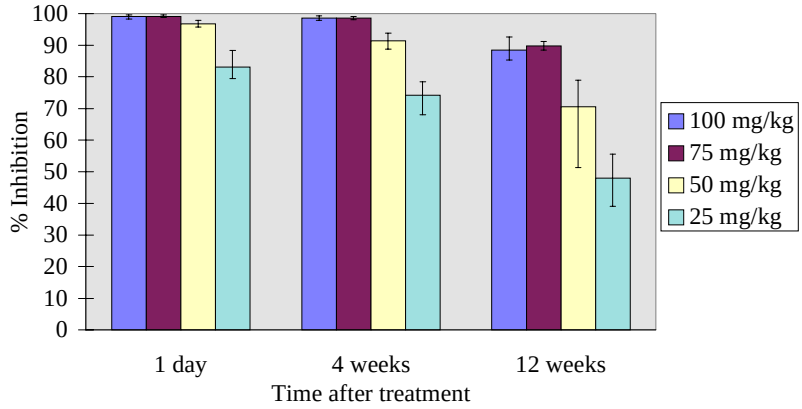
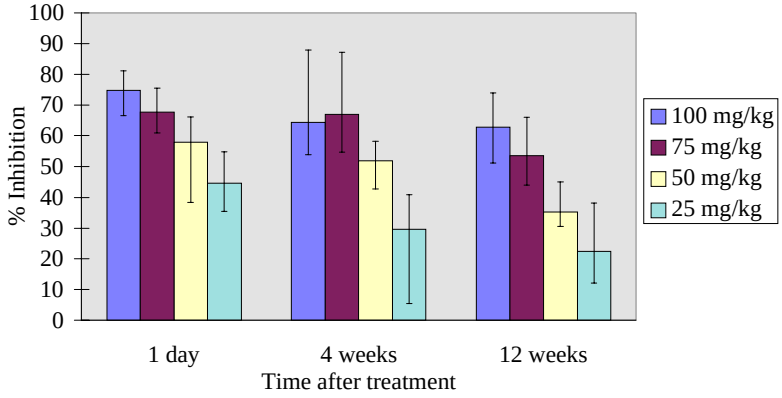
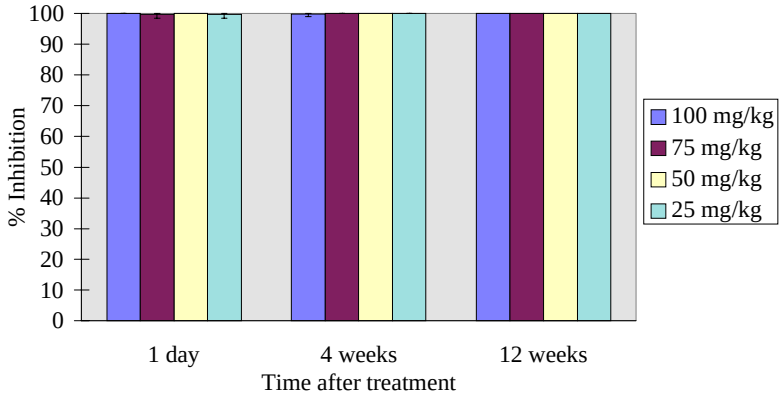


Figure 41 : Mean % population inhibition (and ranges) of OP resistant strains of insects exposed to 4 doses of Azadirachtin on wheat stored at 25ºC and 75% rh (n=5)

Sitophilus granarius



Oryzaephilus surinamensis



Rhyzopertha dominica

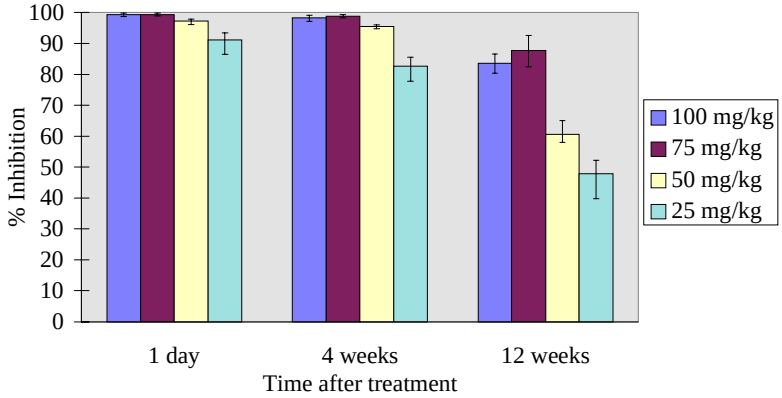


Table 34 - Doses required of each pesticide to achieve mean inhibitions of > 95% of each pest species

Pesticide	Temperature	Pest species	Strain	Effective doses achieving mean inhibitions of > 95%		
				One day	4 weeks	12 weeks
Protect-it	25oC	*A. siro*	susceptible	> 3 g/kg	3 g/kg	1 g/kg
			resistant	> 3 g/kg	3 g/kg	> 3 g/kg
		L. destructor	susceptible	> 3 g/kg	3 g/kg	1 g/kg
			resistant	> 3 g/kg	1 g/kg	1 g/kg
		T. putrescentiae	susceptible	> 3 g/kg	3 g/kg	1 g/kg
			resistant	> 3 g/kg	3 g/kg	3 g/kg
		S. granarius	susceptible	> 3 g/kg	> 3 g/kg	> 3 g/kg
			resistant	> 3 g/kg	> 3 g/kg	> 3 g/kg
		O. surinamensis	susceptible	1 g/kg	1 g/kg	0.5 g/kg
			resistant	1 g/kg	0.5 g/kg	0.5 g/kg
		R. dominica	susceptible	3 g/kg	3 g/kg	3 g/kg
			resistant	3 g/kg	3 g/kg	3 g/kg
	10oC	*A. siro*	susceptible	1 g/kg	0.5 g/kg	1 g/kg
			resistant	1 g/kg	0.5 g/kg	0.5 g/kg
		L. destructor	susceptible	1 g/kg	3 g/kg	1 g/kg
			resistant	1 g/kg	1 g/kg	0.5 g/kg
Bifenthrin	25oC	*A. siro*	susceptible	1 mg/kg	1 mg/kg	2 mg/kg
			resistant	2 mg/kg	2 mg/kg	> 2 mg/kg
		L. destructor	susceptible	1 mg/kg	1 mg/kg	2 mg/kg
			resistant	2 mg/kg	1 mg/kg	2 mg/kg
		T. putrescentiae	susceptible	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
			resistant	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
		S. granarius	susceptible	1 mg/kg	2 mg/kg	2 mg/kg
			resistant	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
		O. surinamensis	susceptible	< 0.25 mg/kg	< 0.25 mg/kg	< 0.25 mg/kg
			resistant	< 0.25 mg/kg	< 0.25 mg/kg	< 0.25 mg/kg
		R. dominica	susceptible	< 0.25 mg/kg	< 0.25 mg/kg	< 0.25 mg/kg
			resistant	< 0.25 mg/kg	< 0.25 mg/kg	< 0.25 mg/kg
	10oC	*A. siro*	susceptible	0.25 mg/kg	1 mg/kg	0.25 mg/kg
			resistant	1 mg/kg	1 mg/kg	1 mg/kg
		L. destructor	susceptible	1 mg/kg	0.5 mg/kg	0.5 mg/kg
			resistant	0.5 mg/kg	0.5 mg/kg	0.5 mg/kg

Table 34 continued - Doses required of each pesticide to achieve mean inhibitions of > 95% of each pest species

Pesticide	Temperature	Pest species	Strain	Effective doses achieving mean inhibitions of > 95%		
				One day	4 weeks	12 weeks
Flufenoxuron	25oC	*A. siro*	susceptible	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
			resistant	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
		L. destructor	susceptible	0.5 mg/kg	0.5 mg/kg	2 mg/kg
			resistant	0.5 mg/kg	0.5 mg/kg	2 mg/kg
		T. putrescentiae	susceptible	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
			resistant	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
		S. granarius	susceptible	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
			resistant	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
		O. surinamensis	susceptible	0.5 mg/kg	1 mg/kg	0.1 mg/kg
			resistant	0.5 mg/kg	0.5 mg/kg	0.1 mg/kg
		R. dominica	susceptible	> 2 mg/kg	1 mg/kg	> 2 mg/kg
			resistant	> 2 mg/kg	2 mg/kg	> 2 mg/kg
	10oC	*A. siro*	susceptible	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
			resistant	> 2 mg/kg	> 2 mg/kg	> 2 mg/kg
		L. destructor	susceptible	0.5 mg/kg	> 2 mg/kg	0.5 mg/kg
			resistant	0.5 mg/kg	> 2 mg/kg	0.1 & 1 mg/kg
Sodium polyborate	25oC	*A. siro*	susceptible	2 g/kg	> 2 g/kg	> 2 g/kg
			resistant	0.5 g/kg	2 g/kg	2 g/kg
		L. destructor	susceptible	0.5 g/kg	1 g/kg	2 g/kg
			resistant	0.5 g/kg	1 g/kg	2 g/kg
		T. putrescentiae	susceptible	0.5 g/kg	2 g/kg	> 2 g/kg
			resistant	0.5 g/kg	2 g/kg	2 g/kg
		S. granarius	susceptible	2 g/kg	2 g/kg	1 g/kg
			resistant	2 g/kg	1 g/kg	1 g/kg
		O. surinamensis	susceptible	1 g/kg	1 g/kg	1 g/kg
			resistant	1 g/kg	1 g/kg	1 g/kg
		R. dominica	susceptible	0.5 g/kg	0.5 g/kg	0.5 g/kg
			resistant	1 g/kg	1 g/kg	0.5 g/kg
	10oC	*A. siro*	susceptible	1 g/kg	2 g/kg	> 2 g/kg
			resistant	1 g/kg	1 g/kg	2 g/kg
		L. destructor	susceptible	< 0.25 g/kg	0.5 g/kg	0.5 g/kg
			resistant	< 0.25 g/kg	0.5 g/kg	1 g/kg

Table 34 continued - Doses required of each pesticide to achieve mean inhibitions of > 95% of each pest species

Pesticide	Temperature	Pest species	Strain	Effective doses achieving mean inhibitions of > 95%		
				One day	4 weeks	12 weeks
Benzyl benzoate	25oC	*A. siro*	susceptible	200 mg/kg	> 200 mg/kg	> 200 mg/kg
			resistant	200 mg/kg	> 200 mg/kg	> 200 mg/kg
		L. destructor	susceptible	< 50 mg/kg	200 mg/kg	200 mg/kg
			resistant	< 50 mg/kg	> 200 mg/kg	200 mg/kg
		T. putrescentiae	susceptible	200 mg/kg	> 200 mg/kg	> 200 mg/kg
			resistant	150 mg/kg	> 200 mg/kg	> 200 mg/kg
		S. granarius	susceptible	> 200 mg/kg	> 200 mg/kg	> 200 mg/kg
			resistant	> 200 mg/kg	> 200 mg/kg	> 200 mg/kg
		O. surinamensis	susceptible	200 mg/kg	> 200 mg/kg	> 200 mg/kg
			resistant	> 200 mg/kg	> 200 mg/kg	> 200 mg/kg
		R. dominica	susceptible	> 200 mg/kg	> 200 mg/kg	> 200 mg/kg
			resistant	> 200 mg/kg	> 200 mg/kg	> 200 mg/kg
	10oC	*A. siro*	susceptible	100 mg/kg	150 mg/kg	> 200 mg/kg
			resistant	100 mg/kg	150 mg/kg	> 200 mg/kg
		L. destructor	susceptible	< 50 mg/kg	100 mg/kg	> 200 mg/kg
			resistant	< 50 mg/kg	150 mg/kg	> 200 mg/kg
Azadirachtin	25oC	*A. siro*	susceptible	75 mg/kg	> 100 mg/kg	> 100 mg/kg
			resistant	75 mg/kg	> 100 mg/kg	> 100 mg/kg
		L. destructor	susceptible	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
			resistant	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
		T. putrescentiae	susceptible	100 mg/kg	> 100 mg/kg	> 100 mg/kg
			resistant	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
		S. granarius	susceptible	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
			resistant	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
		O. surinamensis	susceptible	< 25 mg/kg	< 25 mg/kg	< 25 mg/kg
			resistant	< 25 mg/kg	< 25 mg/kg	< 25 mg/kg
		R. dominica	susceptible	50 mg/kg	75 mg/kg	> 100 mg/kg
			resistant	50 mg/kg	50 mg/kg	> 100 mg/kg
	10oC	*A. siro*	susceptible	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
			resistant	> 100 mg/kg	> 100 mg/kg	> 100 mg/kg
		L. destructor	susceptible	75 mg/kg	> 100 mg/kg	> 100 mg/kg
			resistant	100 mg/kg	> 100 mg/kg	> 100 mg/kg

PART 3

THE EFFICACY OF FLUFENOXURON AND AZADIRACHTIN AGAINST MIXED MITE AND INSECT POPULATIONS IN SMALL BINS OF WHEAT

ABSTRACT

Batches of wheat were treated with flufenoxuron and azadirachtin, put into small bins, infested with mixed populations of mites and insects (*Acarus siro*, *Lepidoglyphus destructor*, *Sitophilus granarius* and *Oryzaephilus surinamensis*) and stored at 15^{o}C and 80% rh for 26 weeks. From the second week, efficacy was evaluated at 4 weekly intervals by the ability of the compounds to inhibit the development and survival of the pest species. No *A. siro* or *L. destuctor* were detected in the samples treated with flufenoxuron at or after 6 and 10 weeks respectively. In the azadirachtin treated samples no mites were detected from week 2 up to week 26 when *A. siro* were found in one of the bins. However, there were also no *L. destructor* detected in any of the untreated samples after week 14 which may have been due to competition from the faster breeding *A. siro*. Adult beetles were caught in the traps throughout the experiment with lower numbers of *O. surinamensis* trapped than *S. granarius*. The treatments reduced the numbers of *S. granarius* by approximately 75 %.

INTRODUCTION

Previous experiments screened 21 compounds for their potential as possible alternatives to OPs for the control of storage mites on grain (Part 1). From those experiments, six compounds were judged to be effective in controlling mites at doses likely to allow them to be used cost-effectively in the U.K. These six compounds were then further assessed for their efficacy, when admixed with wheat, against OP-susceptible and resistant strains of storage mites and insects, over extended storage periods, in temperatures covering the immediate post-harvest and long term storage conditions in the U.K (Part 2). From those experiments the chitin synthesis inhibitor, flufenoxuron, and the botanical, azadirachtin, were considered to be worthy of further investigation.

The aim of these experiments was to investigate the efficacy of flufenoxuron and azadirachtin on wheat stored in small bins in conditions considered to be favourable for the development of surface pest populations in aerated U.K. grain stores. Efficacy was evaluated by the ability to inhibit the development and survival of mixed populations of mites and insects over a 26 week storage period.

MATERIALS

Wheat : Pesticide-free English milling wheat of the 'Mercia' variety was used. The wheat was stored in plastic bags in a freezer for at least 21 days prior to use to ensure any mites coming in on the grain were killed. The moisture content (mc) was adjusted to 17% as determined using the oven method (ISO 712), by drying in a ventilated oven at 130^{o}C for 2 hours.

Insects : Laboratory susceptible strains of *Oryzaephilus surinamensis* and *Sitophilus granarius* were used. Both have been reared at the Central Science Laboratory (CSL) in constant conditions of 25^{o}C and 70% rh without exposure to pesticides. Known aged adults were tested, *O. surinamensis* when 0-2 weeks old and *S. granarius* when 2-4 weeks old.

Mites : Laboratory susceptible strains of *Acarus siro* and *Lepidoglyphus destructor* were tested. Both have been reared at the CSL in constant conditions of 15^{o}C and 90% rh without exposure to pesticides. Mixed stages of unknown age were used.

Pesticides : An emulsifiable concentrate of azadirachtin and a suspension concentrate of flufenoxuron were used; containing 1% and 30 g/l active ingredient respectively. Doses of 75 mg kg $^{-1}$ and 2 mg kg $^{-1}$ were applied of the respective compounds.

Test conditions : The wheat was stored in a controlled environment room set at 15^{o}C and 80% rh.

METHOD

Preparation and treatment of grain : The wheat was weighed out into six batches of 25 kg and six batches of 20 kg and put into plastic bags prior to treatment. Three of the 25 kg batches were treated with flufenoxuron with three of the 20 kg batches treated with azadirachtin. Different amounts of grain were treated with flufenoxuron and azadirachtin because an insufficient amount of azadirachtin was received from the supplier. The remaining batches were used as controls. The pesticide doses were prepared using distilled water as a diluent.

Each batch of grain was placed into a concrete mixer and the required amounts of pesticide solutions were applied to separate batches using a hand held 'De Vilbis' paint sprayer, whilst the grain was tumbling in the mixer. The control batches were similarly treated with distilled water alone.

The opening of the mixer was covered with a piece of plastic to ensure that there was no loss of the compound during the mixing process. The grain was then tumbled in the mixer for 15 minutes to ensure

thorough mixing. Each batch of grain was then put into galvanised steel bins, measuring 58 cm high and 46 cm diameter giving a grain depth of 20 and 16 cm for the different pesticides. The inner rim of the bins was previously coated with a layer of 'fluon' with a layer of petroleum jelly above this, to prevent insects escaping. The bins were then put into the CE room to condition overnight.

Twenty-four hours after treatment sufficient numbers of each species were added to each bin to give an infestation corresponding to approximately 100 mobile mixed stage mites and 10 adult beetles per kg. These were added by moving grain at the surface of the middle of each bin to the sides, creating a cone shaped hollow to a depth of 10cm. The beetles were put into the hollow and covered by the grain heaped at the sides. The mites were added to the centre of the surface using a microspoon and the bins were then covered with loose fitting metal lids.

Sampling of grain : After 2 weeks and then at 4-weekly intervals for 26 weeks grain samples were removed from each bin using a compartmental spear of 20 g capacity. Five equidistant samples were taken from the surface of each bin and then combined to give 100 g samples for each bin.

Each combined sample was then sieved over a 2mm mesh and the sievings were examined under a low power binocular microscope to count the number of live mites. If many live mites were observed (> approximately 80) the dust was transferred to a petri dish over a 'Solomon's disc' (a disc divided into 64 sectors of which 8 are blacked - Solomon 1962). The dust was spread evenly over the surface of the dish using a seeker, and the numbers of live mites on the blacked out sectors were counted. This number was then multiplied by 8 to provide an estimate of the total mite numbers. The accuracy of this method in estimating mite numbers has been validated by a series of experiments where known numbers of mites were added to a 'Solomon's disc' and then recounted on the disc (See Appendix).

If lower numbers of mites were initially observed (about one per black segment), the dust was transferred to a petri dish, formed into a thin line and teased away using a seeker whilst counting the numbers of live mites. After each assessment, the grain from each bin was put into a plastic bag and stored in a freezer to await m.c. analysis.

A single 'Pitfall Cone' (PC) trap was then inserted into the centre of the grain in each bin to monitor insect numbers. After one week the trap was removed and the numbers of insects inside each were counted. After counting, the insects were returned to the bins from which they were removed.

RESULTS AND DISCUSSION

Moisture content : In general the mc of the batches of grain in the control bins and those treated with flufenoxuron, were around the intended mc of 17% (77% e.r.h.) (Table 35). However, the mc of the batches treated with azadirachtin were approximately 1% lower, being closer to 16% (72.5% e.r.h.) (Table 35). The reason for this difference is unknown as all the bins were stored in the same CE room throughout the experiment. It may be that the initial mc of the grain was unintentionally lower than that required, and because the bins were fitted with metal lids during storage, the ability of the grain to absorb moisture would have been minimal. The mcs of the individual batches of grain were, however, seen to remain fairly constant throughout the experiment differing by no more than ± 0.4% (Table 35).

Mites : Numbers of *A. siro* in the untreated wheat increased throughout the experiment peaking at week 26, with median numbers of approximately 32,000 per kg (Figure 42). In the wheat treated with flufenoxuron, no *A. siro* were detected in the samples at or after 6 weeks storage, whereas no mites were detected in the wheat treated with azadirachtin from week 2 until week 26, when approximately 50 mites per kg were counted in one of the bins (Figure 42). These may have contaminated the grain from the control bins as there was no barrier to prevent cross contamination between bins. It also indicates that the compound may not be as effective at preventing mite infestations during long-term storage.

Numbers of *L. destructor* in the untreated wheat peaked at week 6, with a median population of approximately 600 per kg (Figure 43). However, after this time the numbers declined rapidly and no mites were detected in any of the untreated samples after week 14 (Figure 43). This decline in the numbers of *L. destructor* in the controls has been observed in other experiments with mixed infestations (Cook and Armitage, 2000). It has been suggested that the quicker breeding *A. siro* appears can out-compete *L. destructor* in some conditions, thereby preventing population build up (Cook and Armitage, 2000). In the treated samples no mites were detected at or after the 2 and 10 week assessments in wheat treated with azadirachtin and flufenoxuron respectively (Figure 43).

Insects : Adult insects were caught in the PC traps throughout the experiment, with in general, lower median numbers of insects in the treated wheat compared to the controls, although there was a good deal of overlap between the upper and lower limits. This lack of efficacy on adult mortality is not unexpected as both compounds act predominately on growth development of immatures and reproduction. Previous experiments also found low adult mortality when exposed to treated grain for 7 days (Part 2).

However, after week 18, there were significantly lower numbers of *S. granarius* in the treated and untreated wheat (Figure 44). In general there was a decrease in *S. granarius* numbers caught until week 18, with an increase in the control bins at weeks 22 and 26. This increase represents the emergence of adults of the next generation. There was also a slight increase in numbers in the flufenoxuron treatments. These are most

likely to be the progeny of adults that had not been affected by the flufenoxuron at the start of the experiment. It is known that other chitin synthesis inhibitors also exert a delayed effect on *Sitophilus* sp. (McGregor and Kramer, 1976). Some additional experiments found a significant reduction in the progeny produced from parents exposed to wheat treated with flufenoxuron for 21 days, compared to those exposed for 7 days. In the wheat treated with azadirachtin there was a general decrease in the numbers of *S. granarius* caught in the traps throughout the experiment (Figure 44).

Lower numbers of *O. surinamensis* than *S. granarius* were caught, and although, in general, there were lower median numbers in the treated compared to the untreated wheat, there was again overlap between the upper and lower limits (Figure 45). No progeny were observed with this species as it is unable to complete development in the experimental conditions. However, previous experiments (Part 2) found both compounds to be highly effective at preventing population development under favourable breeding conditions.

CONCLUSIONS

Both compounds were effective at inhibiting mite population development. However, the presence of *A. siro* in one of the azadirachtin bins at the end of the experiment may indicate that efficacy decreases during long term storage.

The compounds were ineffective against adult beetles, but prevented the development of a subsequent generation. However, the adults remained visible in the grain, and in U. K. storage conditions it may take a considerable amount of time for the insects to die naturally.

It may therefore be necessary, if these compounds are to be considered as alternatives to OPs, that they are used in conjunction with methods that will rapidly eradicate any adult stages as part of an integrated pest management program.

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Solomon, M E (1962). Notes on the extraction and quantitative estimation of the Acaridae (Acarina). Progress in soil zoology. Proceedings of a colloquium on research methods in soil zoology. Rothamstead. 1 : 305-307.

Table 35 : Moisture contents (%) of samples taken from each bin over the experimental period

Bin number / Treatment	Time after treatment (weeks)						
	2	6	10	14	18	22	26
Control Bin 1	17	16.9	16.9	16.9	17	16.8	17.1

Control Bin 2	17.1	16.6	16.6	16.7	16.8	16.6	16.8
Control Bin 3	16.7	16.7	16.8	16.8	16.9	16.7	16.9
Control Bin 4	16.9	17	16.9	17	17	16.8	17
Control Bin 5	16.6	16.7	16.7	16.8	16.7	16.7	16.9
Control Bin 6	17.1	17.2	17.1	17.2	17	17.1	17.3
Flufenoxuron Bin 1	17	16.8	16.9	17	17.1	16.7	16.9
Flufenoxuron Bin 2	16.9	17	17.2	17.2	17.2	17	17.2
Flufenoxuron Bin 3	16.6	16.8	16.9	16.8	16.9	16.7	16.8
Azadirachtin Bin 1	16.2	16.4	16.3	16.3	16.2	15.9	16.2
Azadirachtin Bin 2	15.9	16	15.9	15.9	15.8	15.7	15.9
Azadirachtin Bin 3	16.1	16.4	16.3	16.3	16.3	16.2	16.3

Figure 42 : Median numbers and ranges of *Acarus siro* in 100 g samples from control and treated wheat over experimental period

Acarus siro

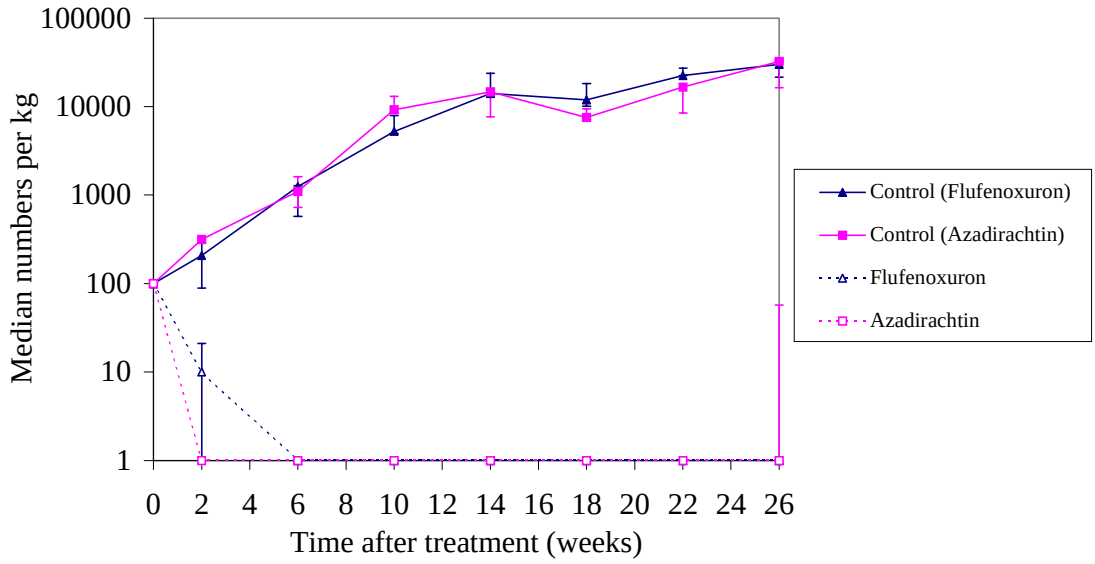


Figure 43 : Median numbers and ranges of *Lepidoglyphus destructor* in 100 g samples from control and treated wheat over experimental period

Lepidoglyphus destructor

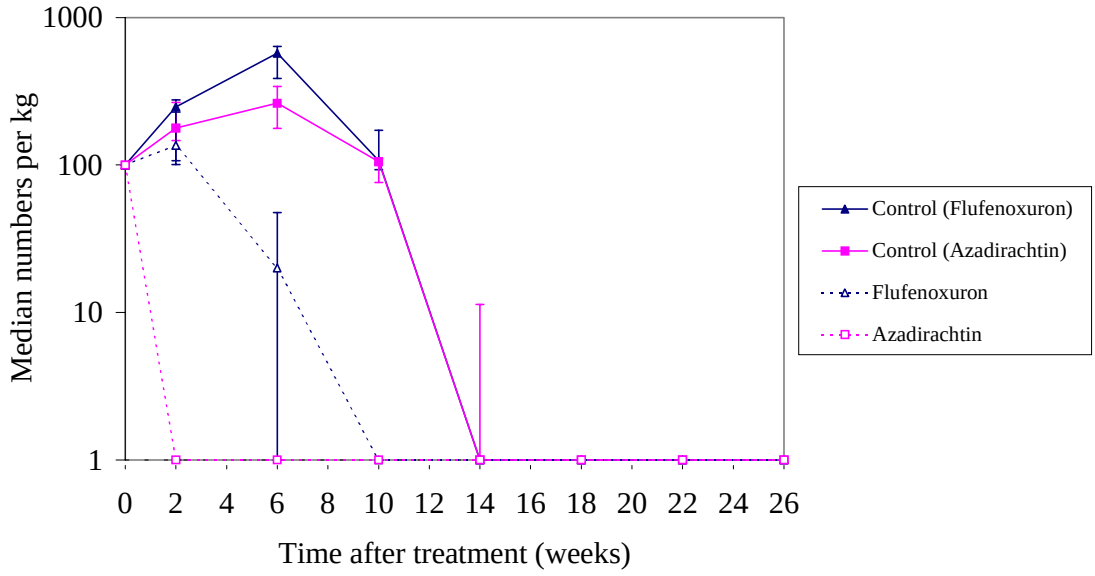


Figure 44 : Median numbers and ranges of *Sitophilus granarius* caught in PC traps placed in control and treated wheat over experimental period

Sitophilus granarius

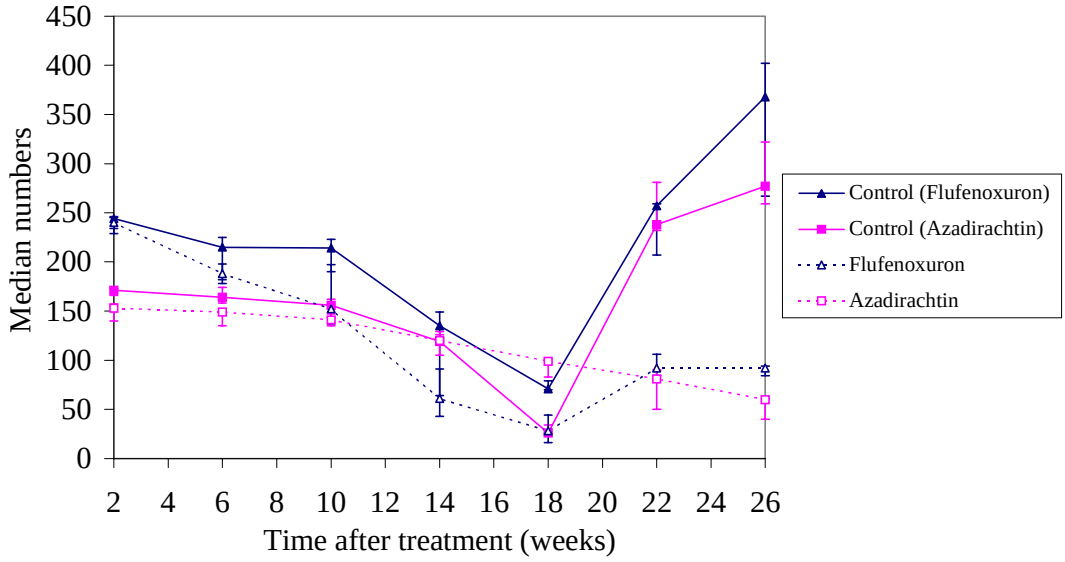
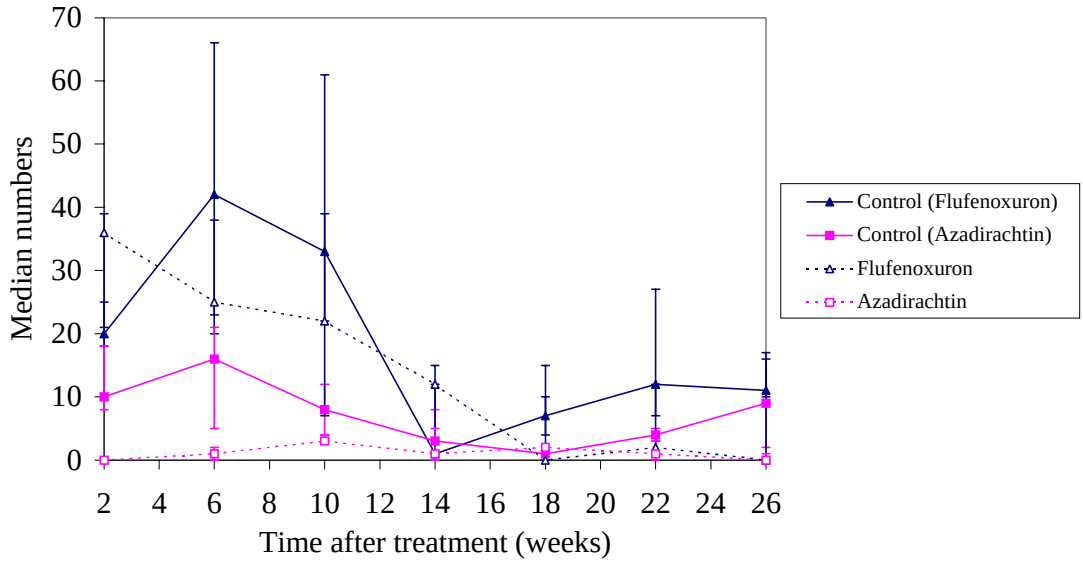


Figure 45 : Median numbers and ranges of *Oryzaephilus surinamensis* caught in PC traps placed in control and treated wheat over experimental period

Oryzaephilus surinamensis



PART 4

THE EFFICACY OF FLUFENOXURON, AZADIRACHTIN AND A DIATOMACEOUS EARTH,WHEN ADMIXED WITH OILSEED RAPE, AGAINST STORAGE MITE PESTS

ABSTRACT

The efficacy of flufenoxuron, azadirachtin and the diatomaceous earth 'Protect-it' were assessed, when admixed with oilseed rape, against *Acarus siro*, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae*. Population inhibition was evaluated in experimental conditions of 15^{o}C and 80% rh. 'Protect-it' appeared the most effective treatment against all the mite species with a dose of 3 g kg $^{-1}$ inhibiting the populations by > 96 %. Flufenoxuron was highly effective at all doses against *L. destructor*, with azadirachtin appearing the least effective against all the species at the doses assessed.

INTRODUCTION

Previous experiments in this report have investigated the efficacy of alternatives to organophosphorus (OP) pesticides when admixed with wheat (Parts 1, 2 and 3); but OPs have also been used heavily for the protection of stored oilseed. However, pirimiphos-methyl (Actellic) lost its approval for use on oilseed rape earlier this year, and although batches of 'Actellic' with the existing label may be used legally until March 2002 (Abel, 2000), after that date, phosphine will be the only pesticide available. However, phosphine requires application by specialist fumigation companies, and this sole reliance on one pesticide may lead to resistance problems in the future.

The aim of these experiments was therefore to investigate the efficacy of three compounds, that had been shown in previous experiments, to be effective against mites when applied to wheat. The diatomaceous earth 'Protect-it', flufenoxuron and azadirachtin, were admixed with oilseed rape and assessed against 3 species of storage mites. Efficacy was evaluated by the ability to inhibit the development of mite populations in conditions considered to be favourable for the development of surface pest populations in aerated U.K. stores.

MATERIALS

Oilseed rape : Pesticide-free, oilseed rape of the Capricorn variety was used, with a moisture content (mc) of about 9.5 % as determined by BS4289. The oilseed was stored in plastic bags in a freezer for at least 21 days prior to use to ensure any mites coming in on the rape were killed.

Mites : Laboratory susceptible strains of *Acarus siro*, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* were used. All have been reared at the CSL in constant conditions of 15°C and 90% rh without exposure to pesticides. Mixed stages of unknown age were used.

Pesticides : The details of the pesticides and doses applied are given below in table 36.

Table 36

Active ingredient	Product name	Formulation	Active ingredient concentration	Doses applied
Diatomaceous earth	Protect-it	Dust	100 %	0.5, 1, 3 and 5 g kg^{-1}
Flufenoxuron	Motto	Suspension concentrate	30 g/l	0.25, 0.5, 1 and 2 mg kg^{-1}
Azadirachtin	Fortune AZA	Emulsifiable concentrate	3 %	20, 40, 60 and 80 mg kg^{-1}

Test conditions : The oilseed rape was stored in a controlled environment room set at 15°C and 80% rh.

METHOD

Preparation and treatment of oilseed rape : The oilseed rape was weighed out into 500 g batches and put into 1.5 l 'Kilner' jars.

The liquid formulations were prepared by serial dilution of the highest dose using distilled water as a diluent. Each batch of oilseed rape was spread evenly over an enameled tray and sprayed with 5 ml of the required dose (starting with the lowest) using a hand held 'De Vilbis' paint sprayer and returned back to the jars. A batch of oilseed rape was similarly treated with 5 ml of distilled water only, to act as a control.

The diatomaceous earth was applied by weighing out the appropriate amount of dust and adding directly to the oilseed rape in jars. A batch remained untreated to act as a control.

After treatment the batches of treated and control rape were mixed on a tumbler for 15 minutes to ensure an even distribution and stored at room temperature overnight. The jars were then tumbled for a further 10 minutes.

Bioassay : Each batch of treated and control oilseed rape was divided into approximately 40 g lots, put into 120 ml bioassay jars and closed with a filter paper lid. Five replicate jars were prepared for each pesticide dose and mite species. The jars were left to equilibrate in the test conditions for 24 hours.

The mite cultures were prepared in the following way : The initial culture population density was assessed by taking a level microspoonful of culture and counting the numbers of live mixed stage mites under a binocular microscope using the Solomon's disc method described below. Food medium was then added to each culture flask, to adjust the proportion of food to mites, so that a level microspoon contained in the range of 400 - 600 mixed stage mobile mites. This was confirmed by recounting as before and recording those numbers (n=3). The added food served as an initial food supply for the mites to aid establishment on the oilseed rape and prevent population crashes.

A level microspoon of adjusted mite culture of each species was then placed into separate jars and the jars were re-closed with a filter paper lid. The jars were incubated in the test conditions for periods long enough to include the passing of at least one generation. Since the different mite species have different developmental rates from egg to adult, exposure periods varied for each species. Therefore, assessments of the F_1 generation took place at approximately the following number of days after exposure to the oilseed :

A. siro	25 days
L. destructor	40 days
T. putrescentiae	45 days

The contents of each jar were then sieved over a 710 µm mesh and the sievings were examined under a low power binocular microscope. If many live mites were observed (> approximately 80) the sievings were transferred to a petri dish over a 'Solomon's disc' (a disc divided into 64 sectors of which 8 are blacked - Solomon 1962). The sievings were spread evenly over the surface of the dish using a seeker, and the numbers of live mites on the blacked out sectors were counted. This number was then multiplied by 8 to provide an estimate of the total mite numbers. The accuracy of this method in estimating mite numbers has been validated by a series of experiments where known numbers of mites were added to a 'Solomon's disc' and then recounted on the disc.

If lower numbers of mites were initially observed (about one per black segment), the sievings were transferred to a petri dish, formed into a thin line and teased away using a seeker whilst counting the numbers of live mites. After each assessment, the oilseed rape from each jar was combined, put into a plastic bag and stored in a freezer to await mc analysis.

The mean percentage inhibition of each mite population was calculated for each treatment as a proportion of the numbers of mites in the controls.

RESULTS

Moisture content : The initial mc of the oilseed rape before treatment was measured as 9.47 %. Analysis of samples after exposure resulted in mcs ranging from 9.12 - 9.95 % (approximately 71.5 - 76.5 % e.r.h.), which were still within 0.5 % of the intended level of 9.5 %.

Bioassays : Against *A. siro*, a dose of at least 3 g kg $^{-1}$ Protect-it inhibited the population > 96 %, whereas the highest doses of flufenoxuron and azadirachtin achieved mean inhibitions of 93 and 66 % respectively (Table 37, Figure 46).

Flufenoxuron appeared the most effective treatment against *L. destructor*, with approximately > 95 % inhibition at doses of 0.5 mg kg $^{-1}$ and above (Table 37, Figure 46). Protect-it inhibited the population by > 97 % at doses of 3 g kg $^{-1}$ and above, whereas the top dose of azadirachtin produced a mean inhibition of 80 % (Table 37, Figure 46).

Against *T. putrescentiae*, Protect-it was also the most effective treatment, providing > 95% inhibition at doses of 1 g kg $^{-1}$ and above (Table 37, Figure 46). The top doses of azadirachtin and flufenoxuron inhibited the populations by 66 and 47 % respectively (Table 37, Figure 46).

DISCUSSION

Under the conditions of this experiment, 'Protect-it' appeared the most effective treatment against all the mite species with a dose of 3 g kg $^{-1}$ inhibiting the populations by > 96%. In similar experiments on wheat at 15^{o}C and 75% rh, > 99% inhibition of *A. siro* and *T. putrescentiae* was achieved at doses of 0.5 g kg $^{-1}$ and above, with at least 3 g kg $^{-1}$ required to inhibit *L. destructor* (Part 1). This suggests that higher doses are required on oilseed rape to achieve the same degree of protection as on wheat, which is also the case with OPs. It has been suggested that this may be due to the greater surface area provided by the smaller seeds, which would tend to reduce the effective dose, and also the rapid uptake of pesticides by the oil, which reduces availability to the mites (Good et al., 1977).

The dust did, however, appear to adhere to the oilseed to a greater extent than when applied to wheat, as when the mites were sieved off the oilseed, very little dust was removed. This may prove to be a

disadvantage however, as the dust was very noticeable on the oilseed changing it from black to more of a white colour. When applied to wheat, nearly 99 % of any dust is removed during normal milling processes for flour production (Desmarchelier et al., 1996) but this may be more difficult to achieve on oilseed. However, during crushing it is unlikely that the dust would contaminate the oil.

Consistent with previous experiments on wheat (Part 2), flufenoxuron was highly effective at all doses against *L. destructor*, with a reduction in efficacy against *A. siro* and *T. putrescentiae*. There was also a noticeable number of unhatched mite eggs in the treated replicates. Acylureas, of which flufenoxuron is one, affect the immature stages of mites undergoing moults and also prevent hatching of eggs. Against spider mites, it has also been found that treated females lay sterile eggs (Anderson et al, 1986). This may therefore explain the large number of unhatched eggs observed.

At the doses assessed, azadirachtin appeared the least effective compound against all of the species, suggesting that higher doses may be required for complete inhibition.

In a field situation, exposure is unlikely to be limited to certain periods of time, therefore efficacy may be greater in more practical situations. Experiments on wheat have found that, over a 6-month storage period, azadirachtin and flufenoxuron were highly effective at preventing mite population development in small bins of treated wheat (Part 3).

CONCLUSION

With the withdrawal of approval for the use of pirimiphos-methyl on oilseed rape, phosphine will remain the only pesticide available for use on the commodity. However, this sole reliance on phosphine may lead to resistance problems in the future. These experiments have shown that there are potential alternatives to OPs for the control of storage mites in oilseed rape that may prove effective when used as surface treatments, in conjunction with cooling and drying, as part of an integrated pest management program.

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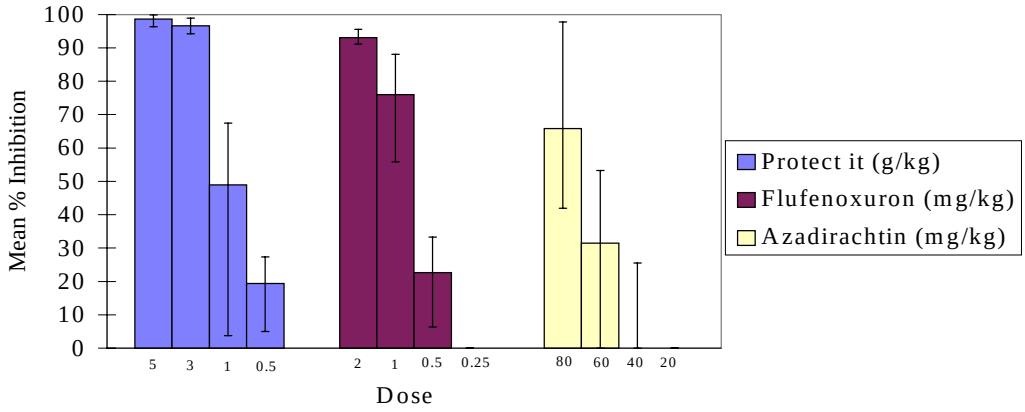
Table 37 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of Protect-it, flufenoxuron and azadirachtin on oilseed rape stored at 15^{o}C and 80% rh (n=5)

Treatment	Mite species	Mean control nos (range)	Mean % Inhibition (ranges)			
			5 g/kg	3 g/kg	1 g/kg	0.5 g/kg

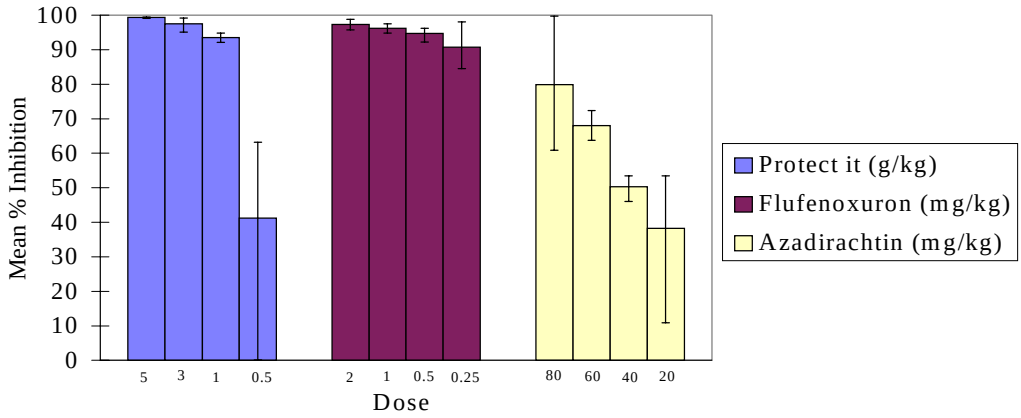
Protect-it	*A. siro*	1254.4 (648 - 2032)	98.6 (96.3 - 99.9)	96.6 (94.2 - 98.9)	48.9 (3.7 - 67.5)	19.4 (5 - 27.3)
	L. destructor	1433.6 (1264 - 1608)	99.3 (99.1 - 9.5)	97.5 (95.1 - 99.2)	93.5 (92.1 - 94.8)	41.2 (0.1 - 63.2)
	T. putrescentiae	1243.2 (1072 - 1400)	99.9 (99.9 - 100)	99.8 (99.7 - 100)	95.2 (94 - 96.9)	44.4 (25.4 - 58.2)
Flufenoxuron			2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
	A. siro	923.2 (640 - 1144)	93.1 (91.2 - 95.6)	76 (55.8 - 88.1)	22.7 (6.4 - 33.3)	0
	L. destructor	1392 (1176 - 1696)	97.3 (95.7 - 98.8)	96.2 (94.8 - 97.5)	94.7 (92.2 - 96.2)	90.7 (84.5 - 98.1)
	T. putrescentiae	1140.8 (800 - 1480)	46.6 (21.5 - 67.7)	24.5 (7.4 - 40.4)	4.8 (0 - 50.9)	0 (0 - 3.9)
Azadirachtin			80 mg/kg	60 mg/kg	40 mg/kg	20 mg/kg
	A. siro	923.2 (640 - 1144)	65.8 (41.9 - 97.8)	31.5 (0 - 53.2)	0 (0 - 25.5)	0
	L. destructor	1392 (1176 - 1696)	79.9 (60.9 - 99.7)	68 (63.8 - 72.4)	50.3 (46 - 53.4)	38.2 (10.9 - 53.4)
	T. putrescentiae	1140.8 (800 - 1480)	65.5 (52.7 - 72.4)	59.3 (48.6 - 72.6)	0.1 (0 - 24.3)	0 (0 - 3.2)

Figure 46 : Mean % population inhibition and ranges of each mite species exposed to 4 doses of ‘Protect-it, flufenoxuron and azadirachtin on oilseed rape stored at 15^{o}C and 80% rh (n-=5)

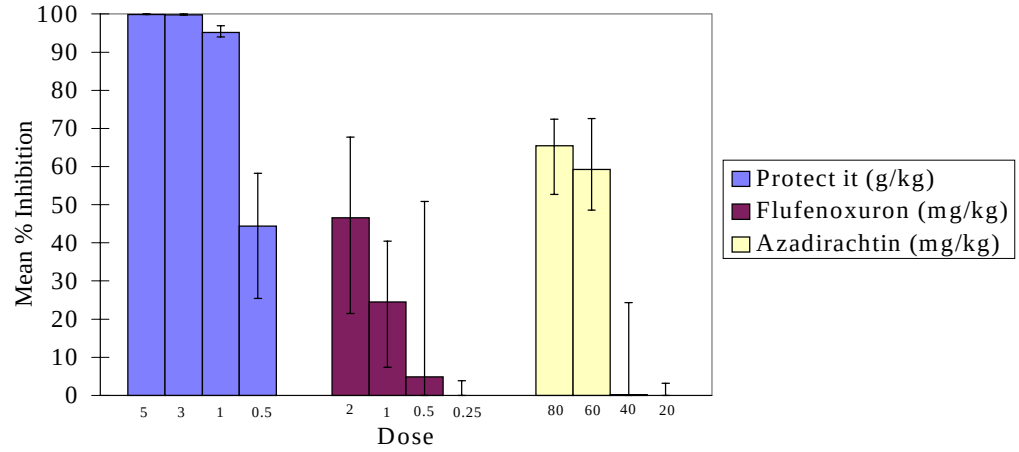
Acarus siro



Lepidoglyphus destructor



Tyrophagus putrescentiae



PART 5

FARM SCALE EXPERIMENT -

SURFACE TREATMENT OF AN EXISTING MITE INFESTATION WITH A DIATOMACEOUS EARTH

ABSTRACT

Unpublished MAFF-funded work has studied surface treatments with the DE, 'Dryacide' on a farm and commercial scale. The surface of a 250 t grain store was divided into quadrants, two of which were treated with 2g/kg DE. Laboratory bioassays of samples taken from the surface confirmed the efficacy against both insects and mites. Mite and insect populations were also compared in two 20 t bins of wheat treated at 1g/kg, two treated at 3g/kg and two untreated bins. These results suggested that the lower dose could be effective in practical circumstances. Therefore, for this project, the surface of a mite infested 20 t bin was divided into quadrants. Two were treated with 1g/kg of the DE; 'Protect-it' and two were left untreated. The mite population at the surface of the treated quadrants fell to near zero within a fortnight although the effect at a depth of 0.25m was much less marked.

INTRODUCTION

Of all the alternatives to organophosphorus (OP) pesticides studied in this project, one of the most promising proved to be the diatomaceous earth (DE), 'Protect-it' which was also the generic representative nearest to being marketed. DEs are registered as grain protectants or for treating grain and storage structures in Australia, Canada, China, Croatia, Denmark, Germany, and USA and in the UK are marketed for treatment against poultry mite and domestic pests.

In the UK, considerable attention has been paid to laboratory, farm and commercial scale experiments on the use of DEs as a prophylactic top-dressing to cooled grain as a replacement strategy to raking in OP dusts. These have been carried out as part of MAFF-funded research but as their publication is unlikely to be immediate, it is appropriate to summarise some of the findings here.

Published laboratory work on a 50 g scale, indicated that the Australian DE, 'Dryacide' was effective at temperatures as low as 10°C but that high doses of 3-5 g/kg were required to control all species of mites at moisture contents (mcs) close to 17% which is likely to be the mc of surface grain in the UK during the winter. (Cook et al., 1999). The work that comprised the first part of this HGCA funded study indicated that there were only minor differences in the efficacy toward mites of different DEs (Armitage et al., 1999). Work on a 20 kg scale, indicated that at 15°C, 3g/kg required 5 weeks for total mite suppression and 13 weeks to control adults of the most DE tolerant insect, *Sitophilus granarius* (L.) (Cook and Armitage, 2000).

In the first farm-scale trial, carried out in 1997-8, reported in Cook et al. (1999) comparison was made of surface populations of mites and insects in three 20 t bins top-dressed at 3g/kg with 'Dryacide' but, although psocid populations were 90% lower in the treated bins, the low mc of the wheat used in these experiments did not allow the development of high mite populations in the untreated bins, so no definite conclusions could be made.

In 1998-9 a MAFF-funded commercial-scale experiment was designed to establish whether surface mite and insect populations could be prevented by a top-dressing of a silicaceous dust under fluctuating UK winter conditions.

Mite and insect numbers were compared in four quadrants of a cooled 3 m deep floor store, approx. 9.9 x 8.75 m holding 250 tonnes of feed wheat at about 14% mc. Two were top-dressed with 'Protect-it' a silicaceous dust at 2 g/kg, raked in to 0.3 m. Monitoring of insect numbers was carried out using 12 surface PC traps, and 12 probe traps at 1 m. Mite numbers were estimated from nine 200 g spear samples at each of 1 m and 2 m depths and temperatures were measured by two rows of thermocouples in each quadrant at the surface, 1 m and 3 m depths, attached to hourly-recording data loggers. The grain was cooled using the existing underfloor aeration system. Initial populations of *S granarius*, *Acarus siro* L. and *Lepidoglyphus destructor* (Schrank) at 0.8 / kg, 0.3 / kg and 0.4 / kg were achieved by introduction at 3 depths and 10 columns in each quadrant.

Grain temperatures started at 13-16°C at the start in November , were already below 10°C one month later and 4-6°C in January. Mean numbers of *Sitophilus* in the treated quadrants were one-fifth to one-tenth those in the untreated, except for the initial sampling when they were about 40 x higher !

Surface moistures only peaked at about 16% in February, too low and too late to permit significant mite infestations, so samples of the surface treated grain were used for laboratory bioassays.

Five 1.5 kg samples were scooped from the surface of each treated and untreated quadrant and used to set up 50 g insect and mite bioassays, with the remainder used to determine dose variability and grain percentage dockage. Each sample provided one of five replicates for each species. Bioassays were carried out at temperatures of 10, 15 and 25°C and moisture contents of 16-17% for 4 species but only if they were able to complete life-cycle development. Bioassays results were used to calculate mean percentage population inhibition. For the mite tests, treatment achieved 100% inhibition for both *A. siro* and *L. destructor* at all temperatures with the exception of the latter at 25°C, where a few mites survived treatment giving 99.999% inhibition. For the insects, 100% inhibition was achieved for *Oryzaephilus surinamensis* but developing stages of *S. granarius* escaped treatment resulting in inhibition of 73.2%. However, this reduction in

population compared to the controls showed that the treatment clearly effected the number of eggs laid. Further studies showed that the mean percentage dockage for all quadrants was high at 6.63% and very variable, ranging from 3.16-17.57%. Mean 'Protect-it' doses for the treated quadrants was 2.01 g/kg with no significant difference between the two quadrants. However doses across the surface were vary variable and were estimated to range between 0.55 and 3.46 g/kg. Although treatment was uneven there was no indication that this affected efficacy.

In 1999-00, mite and insect numbers were compared monthly in six cooled bins of feed wheat. Two were top dressed with 3g/ kg 'Protect-it', two were treated at 1g/kg and two remained untreated. Saw-toothed grain beetle (*O. surinamensis*) and grain weevil (*S. granarius*) were introduced at 1.2 / kg and flour mites (*A. siro*) and cosmopolitan food mites *(L. destructor*) at 3 / kg on to the grain initially at 20-28°C and 15-16% mc. Insects were trapped using 9 PC traps at the surface and 5 probe traps at 1m in each bin and mite numbers and moistures were estimated from five 200 g spear samples from the surface and depths of 1 and 2 m. Temperatures were recorded by data logger and a central column of thermocouples at 3 depths in each bin.

Mite numbers at the surface peaked after 12 weeks, coinciding with surface mcs of 18-19% when numbers of *A.siro* in the untreated bins were 2,600 and 19,000 / kg compared to less than 1 / kg in all the treated bins. Numbers of insects in the surface traps fell in treated and control bins throughout the experiment but after 17 weeks there were still 10 weevils and 12 sawtooth beetles in the untreated bins with only individuals of each species being found in the treated bins.

Laboratory assessments were made by scooping five 1.5 kg samples from the surface of each paired treated bin at 1 g/kg and 3 g/kg and the paired controls to set up 50 g insect and mite bioassays. Each sample provided one of five replicates for each species. Bioassays were carried out at temperatures of 10, 15 and 25°C and equilibrium relative humidity (erh) of 75% for 4 species but only if they were able to complete life-cycle development. Bioassay results were used to calculate mean percentage population inhibition. For the mite tests, 3 g/kg achieved 100% inhibition for both *A.siro* and *L. destructor*. At 1 g/kg there was 100% inhibition for *L. destructor* at all temperatures but only at 10°C for *A. siro* where a few mites survived treatment giving 99.99% inhibition at 15 and 25°C. For the insects, 100% inhibition was achieved for *O. surinamensis* but developing stages of *S. granarius* escaped treatment at 25°C resulting in inhibition of 61% at 1 g/kg and 87% at 3 g/kg, with the 15°C tests as yet uncounted. However, this reduction in population compared to the controls showed that the treatment clearly affected the number of eggs laid. This data is corroborated by results from the previous year which examined the effect of a commercial - scale treatment at 2 g/kg.

Although the laboratory assessments indicate the limited effectiveness of the fractional dose of DE, the farm-scale results indicate that the low dose could be commercially effective. Accordingly, the experiment described here used the fractional dose of 1g/kg and was the first to determine the effect of a DE treatment on existing mite infestations, rather than as a prophylaxis.

METHODS

The experiment was carried out in a 3 m x 3 m x 3 m bin containing 20 t of feed wheat at about 16% mc which was a 'control' bin from the 1999-00 MAFF-funded experiment on fractional DE dose reported above. A sizeable mite infestation was present at the surface of the grain.

The surface was divided into quadrants and 200 g spear samples were taken from five points in each, at the surface and at a depth of 0.25 m, making ten samples from each quadrant, forty samples in all.

Mite numbers were determined by sieving the samples over a 2 mm mesh and examining the resulting dust under a low-power binocular microscope. Where numbers were high, a disc divided into areas was used (Solomon, 1962). The moisture content of each sample was then determined by ISO 712, drying in a ventilated oven at 120°C for 2 h.

Two of the quadrants were then treated at 1g/kg with the DE, 'Protect-it' by sprinkling 0.45 kg of the dust as evenly as possible by eye onto the grain surface and raking in with a rake with 0.3 m tines. Four further fortnightly samplings were then carried out, as already described , for 2 months.

On each sampling occasion, spot readings of temperatures at 0.25 m at the centre of each quadrant were recorded using a hand meter, while the surface temperature at the centre of the bin was recorded using a thermocouple attached to a data logger.

RESULTS

The moisture content at the grain surface fell from around 17%, to below 16% during the 2 months of the experiment (Figure 47). At 0.25 m, the mean mc of the four quadrants started at 16.1-16.5% and by the end of the experiment was 16.4-16.5% (Figure 48). The mean surface temperature of the bulk rose from 7.0°C initially to 11.3°C after a fortnight, to 13.4°C after a month and were 14.4°C by the end of the test.

Temperatures at 0. 25 m were initially at 7.3°C, after a fortnight they were 11.0-11.2°C, after one month they were 12.6°C and by the end of the experiment they were between 14.9 and 15.1°C.

The mite infestation mainly comprised of *A. siro* although there were much smaller numbers of *L. destructor*, *Cheyletus eruditus* (Schrank), Gamasidae and Tydeidae. At the start of the experiment, numbers of *A.siro* varied between 1100 and 3400 / kg in the quadrants. Two weeks after treatment, they fell to below 10 / kg in the treated areas and did not recover during the subsequent 2 months. Numbers in the untreated areas fluctuated somewhat but the trend was of gentle decline (Figure 49). At 0.25 m numbers of mites were between 2700 and 5700 / kg in the quadrants. Thereafter numbers were lower in the untreated quadrants on 7 / 8 occasions but by the end of the experiment they were still quite high (770 and 1500 / kg) although lower than in the untreated areas (5900-8000 / kg) (Figure 50). Populations of *L.destructor* followed a similar trend but were approximately a factor of ten lower.

DISCUSSION

While the equilibrium relative humidity at the surface of the grain fell from about 75 to 72%, mainly due to the surface layers desorbing moisture in the late Spring, at 0.25 m, the erh rose from about 72 to 75 %, mainly due to the grain warming up. As a result, the numbers of mites at the surface of the treated areas gradually declined during the experiment whilst the numbers beneath generally increased.

The surface treatment of 1g / kg was successful in reducing a considerable surface infestation, mainly of *A. siro*, to negligible proportions within a fortnight. Although our laboratory results tend to show a higher dose is necessary for complete control, it should be borne in mind that the surface treatment was calculated on the assumption that the top 0.3 m would be treated with this dose. In practice, the raking in probably did not achieve such an even distribution so the dose at the surface would have been much higher. This is borne out by the only slight effect on populations noted at 0.25 m. It may be that the particles of DE, which are larger and 'stickier' than those of an OP dust, do not penetrate so deeply into a grain bulk.

It is not clear whether control was achieved by killing the mites at the surface or whether they were merely repelled by the DE. Conspicuously large numbers of dead mites were not present in the dust samples but it is usual for mites treated with DE to become coated with the dust and hence become indistinguishable.

In our previous tests, *L.destructor* was found to be less susceptible than *A.siro* to DEs but in this experiment, there were relatively few of this former species. However it should be noted that the germ-feeding *A.siro* is the species that forms much higher infestations, predominates on damper grain and is more destructive than the adventitious *L.destructor* (Parkinson, 1990*)*.

This treatment was effective in Spring, when surface mc is declining as the ambient r.h. falls with the warmer weather (Armitage and Cook,1999). It may be advisable to repeat the test on a surface infestation in mid-winter when surface mcs are at their peak or against substantial infestations of the predatory mite, *C. eruditus* which is more resistant than the astigmatid pest species to desiccation (Solomon, 1961) and conventional pesticides (Zdarkova, 1997).

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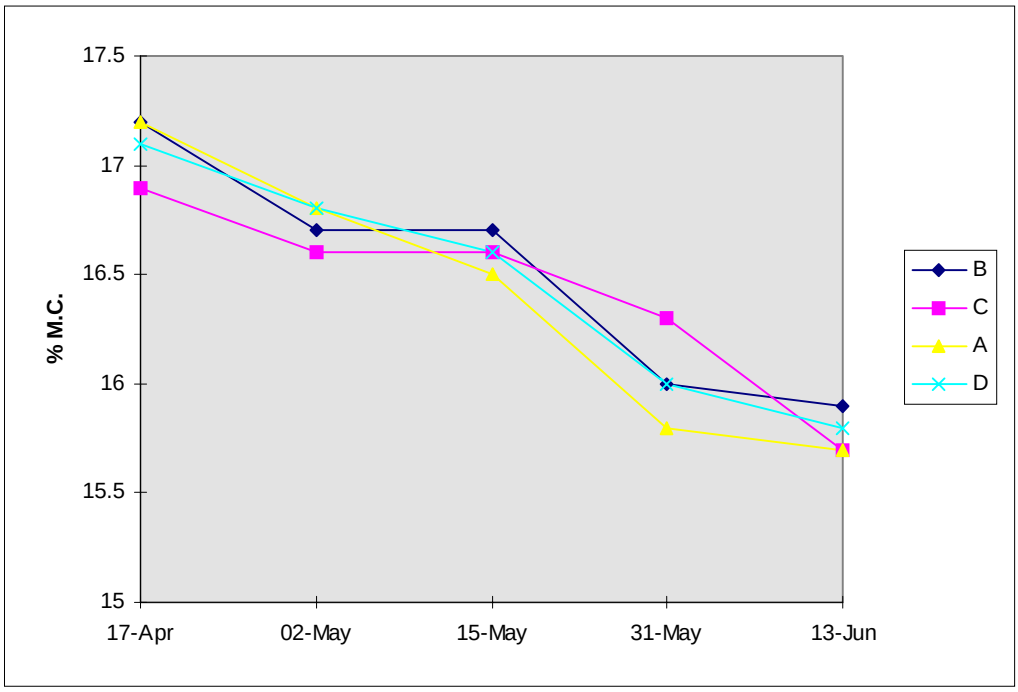

Figure 47 - Moisture contents at the surface of DE treated (A, D) and untreated (B, C) quadrants of a 20 t bin of wheat.

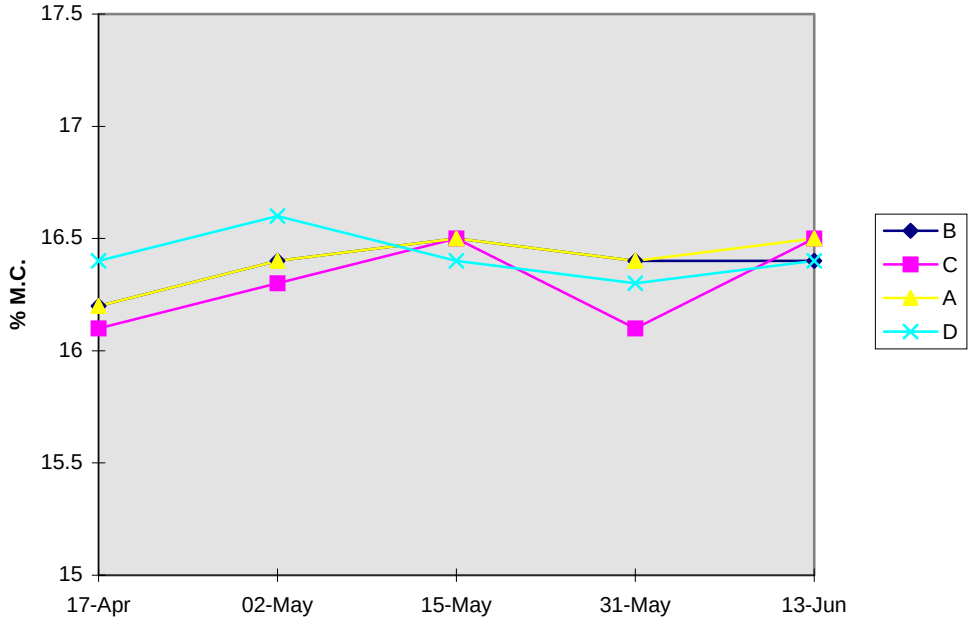

Figure 48.- Moisture contents at a depth of 0.25m in DE treated (A, D) and untreated (B, C) quadrants of a 20 t bin of wheat.

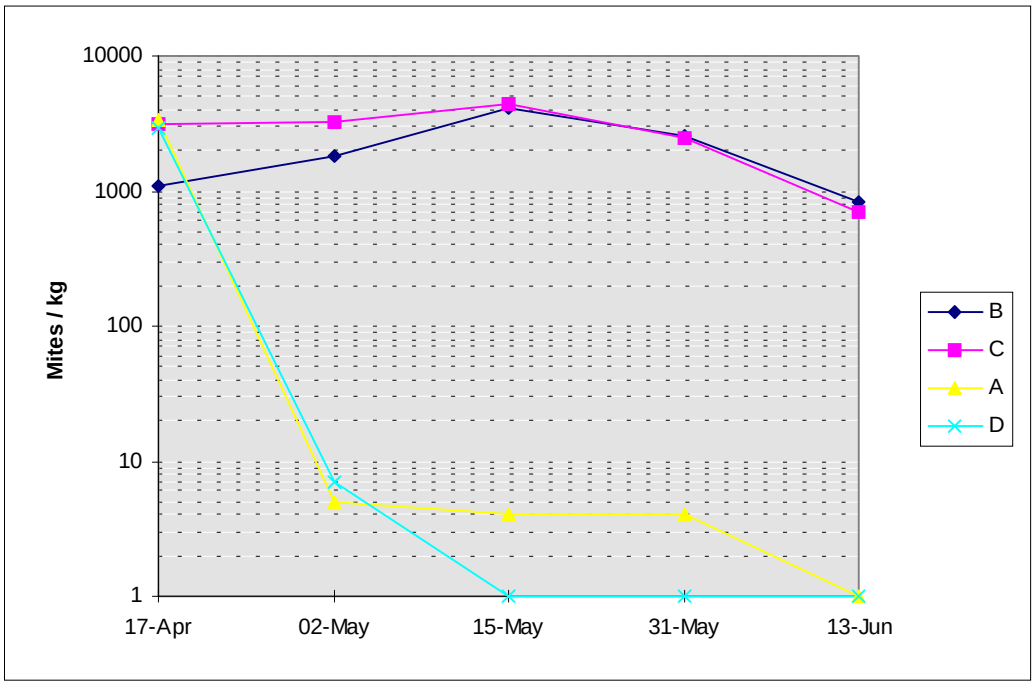

Figure 49- Numbers of *Acarus* at the surface of DE treated (A, D) and untreated (B, C) quadrants of a 20 t bin of wheat.

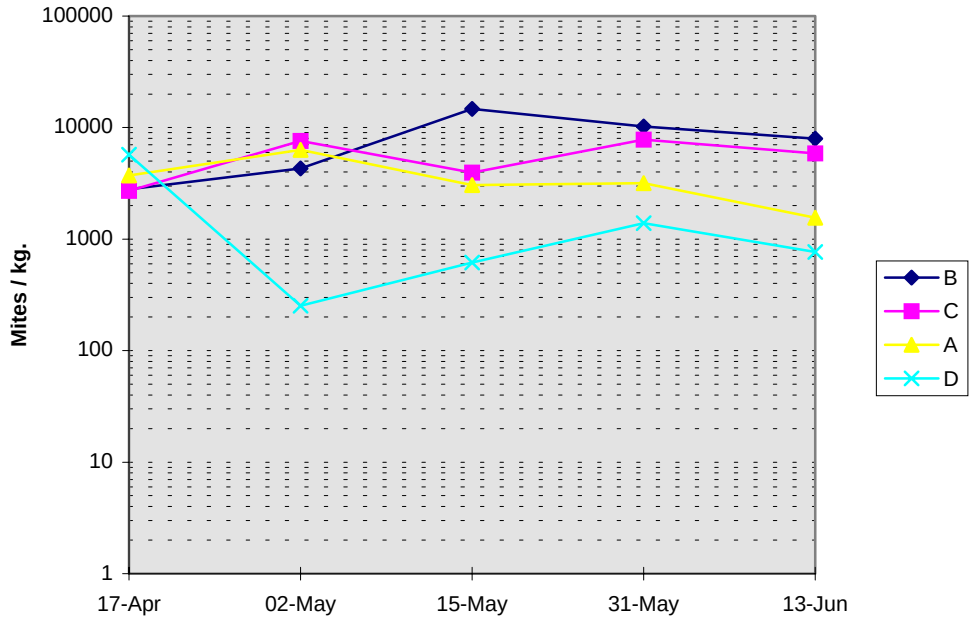

Figure 50.- Numbers of *Acarus* at a depth of 0.25m in DE treated (A, D) and untreated (B, C) quadrants of a 20 t bin of wheat.

OVERALL CONCLUSIONS

This project has demonstrated that there are potential alternatives to OPs for the control of storage mite pests. They have shown not only to be effective against mites, but also against insect pests during prolonged storage, under typical U.K. conditions.

Of all the compounds assessed the chitin synthesis inhibitor, flufenoxuron, the plant extract, azadirachtin and the diatomaceous earth, 'Protect-it' appeared the most promising. These compounds do not produce a rapid kill effect but act more slowly, with the former two compounds affecting growth development and fecundity. Effects on population development are now considered more desirable than quick knock-downs. All have low mammalian toxicity with commercial formulations available, however, none are registered for use on stored commodities in the U.K.

In the U.S. products containing 3 % azadirachtin are registered for greenhouse, nursery, turf and forestry uses on vegetable, fruit and tree crops. They also have approval for organic crop use, and there are current negotiations to expand to post harvest and domestic uses (J Immaraju - personal communication).

Flufenoxuron is not registered for use in the U.S. but in Europe is registered for use on citrus, cotton, grapes, maize, non-cropland, ornamentals, tree fruits (including apples) and vegetables (Farm Chemicals Handbook 2000). In the U.K., a product containing a mixture of flufenoxuron and a pyrethroid has HSE approval as a professional insecticide for public hygiene pest control in domestic and public premises (Pesticides 2000).

It is difficult to calculate and directly compare the costs of treating grain with these new compounds, compared to existing OP treatments, as more information on the dose rates and protection periods in field situations are required. However, it is likely that the product costs will be higher than the current OP prices. However, the cost of any treatment needs to be considered in relation to the cost of grain rejection.

The nearest to the U.K. market are probably the diatomaceous earths, products of which are registered for storage use in several countries including USA, Canada, Australia and Germany. However, when compared to OPs, the rates of 'Dryacide' effective against mite pests in the U.K., are about 1,000 times higher than those required for OPs and also cost more. In the U.K. Cook and Armitage (1999) recommend dressing the top 0.3 m with 'Dryacide' at a rate of 3 g/kg in conjunction with aeration. Based on material costs of 2.3 USD (U. S. dollars) per kg, it would cost 13.8 USD to top dress a 20 t bin or 207 USD to top dress a 1000 t store, approximately four times the cost of an OP top dressing but much cheaper than admixing the entire bulk (Cook and Armitage, 1999).

The availability of any new compounds as grain protectants will be largely dependant on the registration process, the small U.K. market and the cost of registration. However, with the inevitable demise of OPs there is a real demand for effective replacements. The future of any potential alternatives to OPs is likely to lie in their ability to be incorporated into an integrated pest management programme. A targeted approach may be required where knowledge of the environment and pest biology are important factors in deciding the appropriate control measures, with a move away from sole reliance on one method.

RECOMMENDATIONS FOR FUTURE WORK

Further investigation of some of these alternative compounds is required in larger field-scale experiments to assess efficacy against existing infestations and when used in conjunction with cooling and drying, and also to provide more information on dose rates and protection periods.

A previous HGCA funded project investigated the efficacy of shroud materials on the grain surface to limit moisture uptake and prevent mite infestation. Plastic sheeting over the surface decreased the rate of moisture absorption and reduced mite numbers compared to uncovered controls. A spin off from this work and an extension to the current project, may be to investigate the efficacy of alternative pesticide impregnated materials to act as a barrier to incoming infestations and as a surface treatment to control existing infestations.

This work has produced considerable interest from commercial Companies interested in extending the practical uses of their products, and this may increase if OPs are gradually withdrawn from the market.

ACKNOWLEDGEMENTS

The author would like to thank all the commercial Companies who provided test materials used in this project with particular thanks to Jamie Robinson (BASF plc), John Immaraju (Fortune Biotech) and Colin Muirhead (CMI Ltd). This project was funded by the Home Grown Cereals Authority.

APPENDIX 1

RE-EVALUATION OF SOLOMONS DISC TECHNIQUE FOR COUNTING LARGE NUMBERS OF LIVE MITES.

A. Buckland

INTRODUCTION

Mite bioassays assessing the efficacy of pesticides on stored foods, require the counting of live mobile stages to determine the effect of treatment (Collins, 1999). After exposure, live mites are recovered from treated grain by sieving and numbers in the sievings are counted in a petri-dish under a low-powered binocular microscope. These mite numbers can range from tens to thousands, often making counting difficult and time consuming. When numbers are high, random areas of a divided disk can be used to count a standard fraction of the whole sample, based on the method of Solomon (1962). Numbers in the fraction are multiplied to give an estimate of numbers in the whole sample. At the Central Science Laboratory, a disk pattern giving a fractional count of 1/8 (Appencix Figure 1) has been used for a number of published studies (Burrell and Havers, 1970; Burrell and Havers, 1976; Armitage, 1980; Hurlock *et al.*, 1980; Armitage *et al*., 1982; Armitage, 1986; Armitage *et al*., 1994; Armitage *et al*., 1996; Cook & Armitage, 1996; Cook & Armitage, 1999; Collins *et al*., 1999).

Solomon's method used a pattern of disk giving a fraction of 1/30 (Appendix Figure 2). His validation of this disk showed that for a sample of 12,000 mites, the arithmetic mean of 10 counts was 12,036 and the standard deviation was 1,074, i.e. accurate to 8.9% of the mean. The 1/8 pattern now used should be at least as accurate, since a higher proportion of the area is counted. The aim of this study is to evaluate the accuracy of the 1/8 pattern disk for 2 species of mite, *Acarus siro* and *Lepidoglyphus destructor* and to determine guidelines for when to switch between counting the whole sample to the fraction.

MATERIALS

Mites

Pesticide susceptible strains of *A. siro* (9266/1) and *L. destructor* (G6) were used. Both strains have been maintained at CSL at conditions of 15° C and 80% rh, without exposure to pesticides and are fed on a food medium of 3 parts yeast to one part wheatgerm.

METHOD

Preparation of mites

Mites were extracted from the food medium according to CSL operating procedure EFF/057 (J. Dunn, 1999). A sample of mite culture was placed within a 6 cm diameter glass ring with a glass sheet underneath. Another glass sheet was placed on top of the ring to prevent any mites escaping. All this rested on a moistened filter paper placed on a small inverted petri dish, within a larger crystallising dish containing ice and water. The ice was used to cool the mites prior to and during their transfer which slowed them down and made them easier to manage, particularly in the case of fast moving *L. destructor*. The water acted as a moat to prevent mite escape and also maintained a high humidity. Mites were separated from the food by picking up the glass ring keeping the sheets in place, turning it over and tapping what was the bottom sheet, leaving just the mites on the underside. This sheet was placed on another inverted petri dish with an ice and water moat, and placed under a low powered microscope. When not in immediate use a crystallising dish with moistened tissue paper was placed over the over the whole structure so that the mites did not become too dry.

Assessment of mite numbers

Using a single hair brush, individuals of mixed mobile mite stages were picked up and placed evenly on the surface of the Solomon's disk. The disk rested in a petri dish with a moat of ice and water to keep the mites inactive. When the required number had been transferred, the disk was put under a microscope in warmer water and the mites allowed to naturally distribute across the disk for several minutes. The mites on the shaded areas of the disk were then counted. This number was multiplied by eight and compared to the known number of mites introduced. A range of mite numbers was investigated, designed to test the accuracy of the disk at high and low mite densities. The range of mite numbers tested were 75, 150 and 500 individuals. Five replicates were run for each sample size and for each species.

RESULTS

The method overestimated *A. siro* numbers by 15%, 29% & 37% for densities of 75, 150 & 500 individuals respectively (**Table 1**). The greater the mite numbers assessed, the more this method over-estimated. However, after transforming the proportion data (asin of "% of original") and comparing using one way ANOVA, there was no statistically significant difference between the over-estimation for each assessment number ($p=0.6$).

Table 1. Estimates of *Acarus siro* numbers using Solomon's disk at 3 population densities.

Mite nos.	75		150		500	
Replicate	mite estimate	% of actual	mite estimate	% of actual	mite estimate	% of actual
1	112	149%	248	165%	632	126%
2	88	117%	216	144%	736	147%
3	56	75%	200	133%	680	136%
4	80	107%	168	112%	448	90%
5	96	128%	136	91%	568	114%
Mean	86	115%	193	129%	682	137%

This method also over-estimated numbers of *Lepidoglyphus destructor*, by 13% at the lowest mite density and by 30% at the highest (**Table 2**). Again, after transformation and analysis using ANOVA, there was no significant difference between the over-estimation for each assessment number (p=0.3).

Table 2. Estimates of *Lepidoglyphus destructor* numbers using Solomon's disk at 3 population densities.

Mite nos.	75		150		500	
Replicate	mite estimate	% of actual	mite estimate	% of actual	mite estimate	% of actual
1	72	96%	176	117%	568	114%
2	72	96%	160	107%	672	134%
3	96	128%	216	144%	704	141%
4	112	149%	112	75%	728	146%
5	72	96%	168	112%	584	117%
Mean	85	113%	166	111%	651	130%

Two-way ANOVA comparing density and species, again showed no significant difference for the over-estimation for all assessments (p=0.4).

Moreover, this over-estimation makes no or little difference to the estimate of population inhibition -

$$100 - \left[\frac{\text{mean number of live mites per treatment} \times 100}{\text{mean number of live control mites}}\right] = \% \text{ mortality.}$$

The data can be corrected by dividing the Solomon's estimation by % of actual (from **tables 1 and 2**) multiplied by 100.

eg. for *A. siro*, an estimate of 75 mites: 75/ 115 * 100 = 65

Population inhibition was calculated for the uncorrected and corrected data (**Table 3**).

Table 3. The effect of estimation error using Solomon's disk on calculations of population inhibition (c= control, t1/t2 = treatments).

Species	As counted	After adjusting for error
A. siro	C = 500 t1 = 150 t2 = 75 137% 129% 115% t1 = 100-(150/500*100) = 70% t2 = 100-(75/500*100) = 85%	C = 365 t1 = 116 t2 = 65 137% 129% 115% t1 = 100-(116/365*100) = 68% t2 = 100-(65/365*100) = 82%
L. dest.	C = 500 t1 = 150 t2 = 75 130% 111% 113% t1 = 100-(150/500*100) = 70% t2 = 100-(75/500*100) = 85%	C = 442 t1 = 135 t2 = 66 130% 111% 113% t1 = 100-(135/442*100) = 69% t2 = 100-(66/442*100) = 85%

Here differences for the % population inhibition were between 0-3%.

When the worst-case correction for each species is applied to a count of 1000, again there is little difference for the % inhibition (**Table 4**).

Table 4. The effect of estimation error using Solomon's disk on calculations of population inhibition (c= control, t1/t2 = treatments).

Species	As counted	After adjusting for error
A. siro	C = 1000 t1 = 150 t2 = 75	C = 730 t1 = 116 t2 = 65

		137% 129% 115% t1 = 100-(129/730*100) = 82% t2 = 100-(65/730*100) = 91%
	t1 = 100-(150/1000*100) = 85% t2 = 100-(75/1000*100) = 93%	
L. dest.	C = 1000 t1 = 150 t2 = 75 t1 = 100-(150/1000*100) = 85% t2 = 100-(75/1000*100) = 93%	C = 770 t1 = 135 t2 = 66 130% 110% 113% t1 = 100-(135/770*100) = 82% t2 = 100-(66/770*100) = 91%

CONCLUSIONS

The original Solomon's validation work was done using 12,000 mites at a time, estimated volumetrically. The margin of error for this work was 9%. When compared to this latest validation, the original disk pattern may have appeared to be more accurate because plate seeding was estimated rather than actual, perhaps enhancing the results. Since accuracy at a population of 75 mites is as good as for 500, a simple rule for switching from counting the whole sample to an 8th fraction for high numbers, may be when numbers are >80, ie. 1 mite per blacked out segment.

ACKNOWLEDGMENTS

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Figure 1. Current pattern of counting disk, 1/8 fraction.

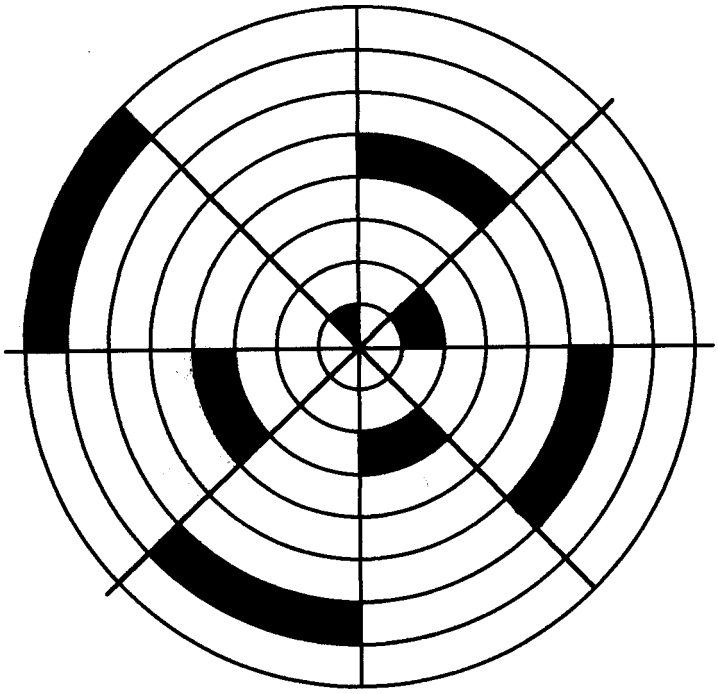

Figure 2. Original pattern of counting disk, 1/30 fraction (Solomon 1962).