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Field trials using ethyl formate as grain surface and empty silo treatments

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ABSTRACT

Ethyl formate has been recently studied as an alternative to phosphine and methyl bromide for the fumigation of stored products and as a structural disinfectant. The compound has been found to have a quick action and breaks down to safer chemicals. Under the current study, three experiments were performed to test efficacy of ethyl formate in controlling psocids on grain and also as a structural treatment. The trials were conducted at a farmer's site with a natural infestation of psocids in grain and in empty silo. Firstly, ethyl formate was applied to the surface of infested wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), with the aim of testing its efficacy in situation where insect infestation was localized. Secondly, it was applied to infested empty silos. Thirdly, it was applied to infested walls, floors and rails. In each case, ethyl formate was applied as a solution in water, without additives. In each experiment after treatment 100% disinfestation was achieved. But as expected, because of the large number of psocids in the structures and in the grain, the disinfested grain became reinfested. The workspace concentration even during spraying, was detected to be below Threshold Limit Value of 100 ppm and v/v. The highest air concentration in grain or in silos was less than 4% of the lower flammability limit of ethyl formate. Samples for residue testing in wheat and barley were taken 24 h after application. Residues in treated grain were below the detectable limit of GC-FID. It can be concluded that ethyl formate can be safely used for disinfestation, and is an alternative to dichlorvos, phosphine and methyl bromide in many situations.

Key words: Ethyl formate, Fumigation, Grain, Psocids, Structural disinfestation

There are many fumigants, such as carbon disulphide, chloropicrin, dichlorvos, ethylene oxide and methyl bromide have been eliminated for use as commercial fumigants due to unfavourable properties, especially with regards to chemical residues, work safety and environmental issues (Banks and Desmarchelier,1984; Banks, 1990; UNEP, 2006). Dichlorvos has been, and continues to be, widely used as a space or as a structural spray to disinfest structures. An alternative would provide a strategy to minimize resistance. Second, there are potential problems with dichlorvos in the areas of residues on grain and occupational health (Banks and Desmarchelier, 1984; Bond, 1984; Banks 1990).The increasing use of phosphine in preference to residual insecticides and resistance has been accompanied by an increase in the proportion of infestations due to psocids (Rees, 1998). Rees has also demonstrated the movement of *Liposcelis decolor* Pearman between grain and structures, including grain under SIROFLO® fumigation in open-cell structures, and has evaluated trapping procedures for estimating changes in populations in grain and in structures. The number of psocids in structures near grain cells, such as rails and catwalks, varies with diurnal conditions, and their exposure to phosphine is minimal. It is therefore necessary to control insects in structure if fumigation in open-top cells is to remain viable. The alternative is control

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failure, due to inadequate exposure to phosphine, and an increased probability of development of resistance, associated with inadequate dosing of large populations. There is now an urgent requirement for the development of a fumigant which can kill all stages of insects quickly, particularly for structural surface and headspace treatment, and is economic in comparison with existing methods.Ethyl formate is an old fumigant and originally has been developed to use for cereal grain fumigation and successfully used for package fumigation of dried fruits and was evaluated for grain protection in the 1980s (Muthu et al., 1984).Ethyl formate has been re-evaluated as a potential replacement for at least some uses of dichlorvos based on considerations of low mammalian toxicity, rapid loss of residues, solubility in water and natural presence in foodstuffs and in the environment (Desmarchelier, 1998). Commercial-scale trials with ethyl formate (90 mg L^{-1}) on wheat *Triticum* sp., completely killed mixed aged cultures of *Rhyzopertha dominica* (Fabricius) as well as adults and larvae of *Tribolium castaneum* (Herbst) (Desmarchelier et al., 1998) In contrast, previous laboratory and commercial-scale trials on wheat, barley, oats, fi ld peas and canola have shown that the internal larval stages of *Sitophilus oryzae* (L.) are difficult to control with EF (Desmarchelier et al., 1998; Mahon et al., 2003). Ethyl formate is one of the potential options to alternative of MeBr on agricultural commodities because of its fast kill, which is almost the same as MeBr, and safety for fumigators and customers (Ren and Mahon 2006). The aim in work reported in this publication was to assess ethyl formate as an alternative to dichlorvos.

MATERIALS AND METHODS

Criteria for evaluation

The main criteria of evaluation of the potential of ethyl formate were worker safety, residues and efficac against insects. The criteria for workspace safety were the extent to which workspace concentrations approached or exceeded the Threshold Limit Value (TLV) of 100 parts per million, v/v and the extent to which in-bin and other concentrations approached the lower flamma ility limit of 2.7% (approximately 89 g m^{-3}).The criteria for success with regards to residues were values after treatment with ethyl formate that were indistinguishable from those in the controls and residues less than the experimental Maximum Residue Limit for these trails of 1 mg kg^{-1}. The criteria for efficacy against insects were reduction in population numbers. This was based on a comparison between populations in treated and untreated situations, where such a comparison was possible, and by a comparison of population before and after each of the three types of treatment (admixture to the grain surface, space sprays and structural sprays).

Cell configuration and selection of experimental cells at Port Giles.

The cell configuration in Block 1 is shown in Fig. 1. On inspection of the site in Blocks 1 and 2, it was decided to evaluate structural treatments in a group of adjacent cells, cells 149,157 and 162, with an end wall adjacent to cells 161 and 162. The cells chosen for evaluation were those where the infestation appeared heaviest, as judged by a semi-quantitative count of psocids on the rails. The only empty cells in the complex were cells 159 and 235 (in Block 2); these

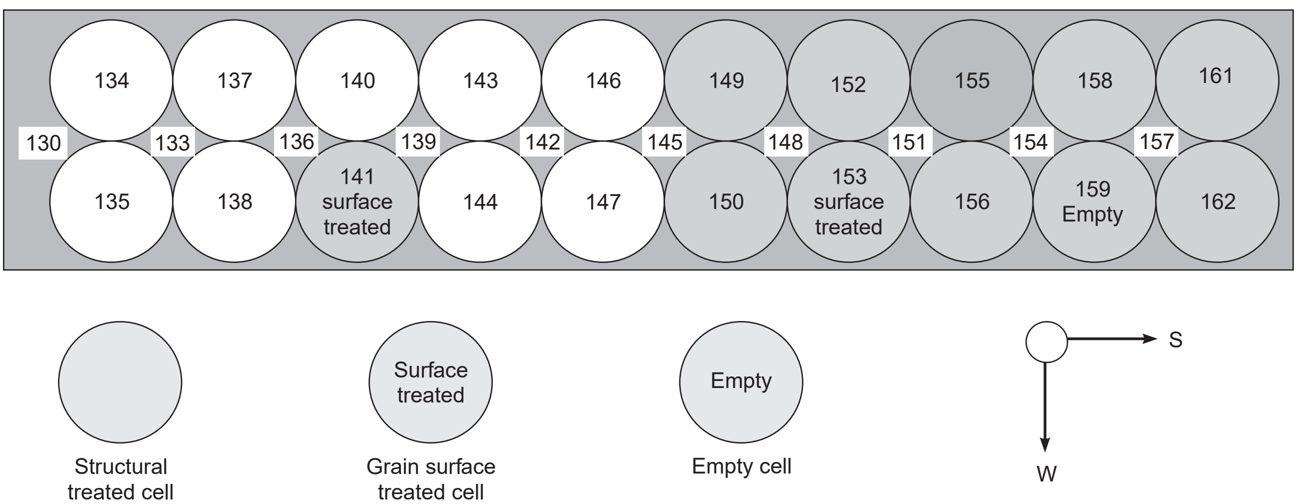

Fig. 1. Cell configuration in the trails at port Giles

were therefore selected for trials on ethyl formate as a space spray. The cell chosen for surface treatment, cell 141, was selected on the basis of being the full cell nearest the infested complex. All cells containing grain had been fumigated with phosphine, or had been under fumigation for 3 weeks, with the exception of cell 161, which were a half-empty cell containing grain previously fumigated at another site.

Measurement of chemical concentrations

Measurements of concentrations on ethyl formate in air: Ethyl formate concentrations in air were measured with Drager tubes for ethyl acetate (LF 0211).The limits of detection are approximately 40 ppm. Measurements of residues of ethyl formate on wheat and barley: Samples for residue determination were taken with bottom-emptying probes, to a depth of 1.5 m, based on standard industry sampling procedure. Grain was divided, and packed in jars which were sealed with lids. Grain was sampled from each of the 10 cells as shown in Fig.1, provided that the cell was full so that it was safe to take samples, and 3 samples from each bin which was surface-sprayed with ethyl formate. Samples were taken 24 h after end of experiments, kept cool, flown to Canberra and extraction solvent added within 24 h of taking of sample. The method of analysis was as per Desmarchelier et al. (1999), and involved extraction with aqueous propanol, followed by determination of ethyl formate in the headspace over the solvent by Gas Chromatography, after extraction periods of both 24 and 48h. The level of detection was set by the natural level (and/or interference), which is approximate 0.1 mg kg^{-1} on aged samples of wheat and barley, but up to 3 mg kg^{-1} on freshly-harvested barley and wheat. It is believed that natural levels of ethyl formate in grains are high at time of harvesting, and then decline, though it is also possible to regard natural levels as due to interfering chemicals.

Application of ethyl formate

Application to the surface of grain: Ethyl formate was applied to the surface of wheat in each of two 2,000 tonne bins from a 5 L spray, containing an 8% solution, v/v, in water. The amount applied was 10 L per 25 m^2 of wheat, to give a nominal application of 29 g m^{-2}. The experimental permitted 110 g m^{-3}, calculated over a depth of 0.5 m. On this method of calculation, the applied rate was 58 g m^{-3}.

Application to empty cells: Ethyl formate was applied to the base of empty cells by funneling 10 L of aqueous solution through a plastic pipe of 5 mm diameter. The concentration was 4%, v/v, in cell 159 and 8%, v/v, in cell 235. The aim was to apply ethyl formate at the bottom part of the cell. The amount applied was considerably less than the experimental permit of 110 g m^{-3}, calculated across the whole cell, but was close to that amount, if the volume was defin d as the surface area (66 m^2) times a depth of 100 mm.

Ethyl formate was applied, for reasons of safety, only from the area between the rails (the catwalk) such that it was applied only to approximately one-third of the bottom of the cell area. As there was no grain in the empty cells, samples could not be taken for residue testing. Besides, entry to cells was restricted as it was a confined space.

Application to metal rails and concrete floors In preliminary trials, ethyl formate was sprayed on psocids on rails in a 2% solution in water. Insects were also sprayed with water. The method of assessment was to mark out an area of less than 0.5 m containing 50 psocids, to spray the rails 2–3 m on either side of the marked area, and to count psocids in the marked area at intervals of time after application. On this assessment, psocid number was greatly reduced after application with ethyl formate, and to a more marked degree than after application with water. In the main experiment, metal rails and concrete floors were sprayed with a 2% solution, v/v, of ethyl formate in water. The area sprayed was the area able to reach from the catwalk around 10 cells, each of diameter 8.5 m. It included the outer rim of each cell, all rails, both horizontal and vertical, and various columns, light fittings, etc. The spray was applied to below run-off, at a rate of approximately 2.5 L/100 m^2 (0.45 g m^{-2}). The spray was repeated 5 times, with assessment after each time, with the aim of assessing the effect of repeated space sprays, as is widely used with dichlorvos. The amount of structure sprayed was only a small proportion of the total structure.

Biological assessments

General procedures: The methods of assessment were based on procedures developed to measure psocid population on grain and in structures (Rees, *personal communication*). The methods were:

- Counting of psocid numbers in marked areas. The areas examined were 10 mm of hand rails and an area of 200 mm × 100 mm on three floor of the catwalk.
- Leaving cardboard traps (150 mm × 100 mm) for defined periods, shaking out the psocids into boxes and counting. Traps were placed on the surface of grain, on the bottom of empty cells and on the catwalk.
- Sampling grain with a bottom-opening probe, sieving and counting insects.

Psocids were counted, in each assessment method, twice daily, at times of high numbers, between 6 AM and 7 AM and between 8 PM and 9 PM.

Assessment of application to the surface of grain in two cells: One method of assessment was to measure natural level of psocid infestation before and after spraying, using both cardboard traps on the surface of the wheat and bulk grain probes. In addition, grain samples were taken with a spraying. Grain was sieved and number of live and dead psocids counted and recorded.Insects in cardboard surface traps were counted in bins before application of ethyl formate, and also in neighbouring bins. Traps were removed from all bins immediately before spraying, and replaced 60 min after spraying. Trap numbers were assessed for a further period, in both treated and neighbouring bins.

Assessment of efficacy of space sprays in empty cells: Psocid numbers in cardboard traps at the bottom of cells were counted before and after application of ethyl formate. There were no control untreated empty cells, because none were available in the complex.

Assessment of efficacy of structural sprays on metal rails and concrete floors Psocid numbers were assessed before and after spraying by three methods. One method was to count psocid numbers at specified points on rails. Insects on 30 sampling positions on rails were counted twice daily. A second method was to count psocids in cardboard traps on the floor between silos. Ten traps were counted twice daily. A third method was to count number of live psocids on the floo .

Assessment by in-situ bioassays: Infested rails were sprayed with ethyl formate, the structure was left 3–4 min until it was nearly dry. Psocids were transferred from the middle of a sprayed rail to vials, and counted after approximately 1 and 24 h. For control, psocids were transferred to vials before spraying, and numbers counted.

RESULTS AND DISCUSSION

Measurement of chemical concentrations

Workspace concentrations: No concentration in the workspace was detected, at a detection limit of approximately 40 ppm (less than the TLV). This applied even to concentrations taken near the face of an operator during spraying of structures and of grain surfaces.

In-bin concentrations: The highest recorded value was 1,000 ppm, v/v. This value, 0.1% v/v, is less than 4% of the lower flammability limit of 2.7%, v/v.

Residues: Residues and/or interfering substances before and after application are shown in Fig. 2. There

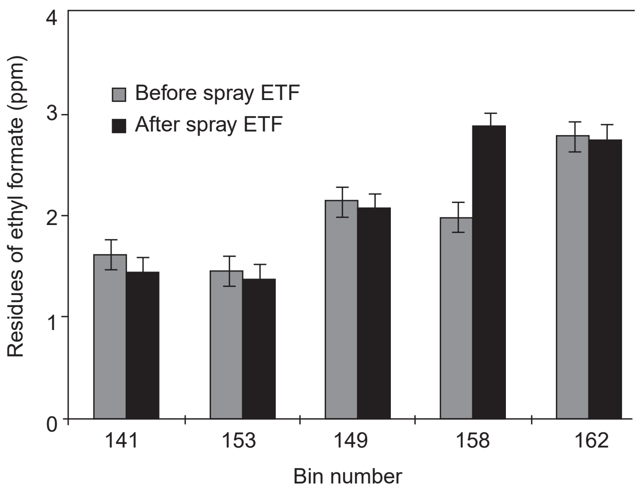

Fig. 2. Residues of ethyl formate and/or interfering substances before and after application.

was a natural level of ethyl formate and/or interfering substance in each bin before and after application as shown in Fig. 2. There was a natural level of ethyl formate and/or interfering substance in each bin before application. This level, after application and a 24 h withholding period, was not significantly different from the level before application. The residue from treatment, that is, difference between values of ethyl formate and/or interfering substance before and after application, was less than 1 mg kg^{-1} in each cell.

Biological efficacy

Efficacy of application to the surface of grain: Data for insect numbers before and after surface spraying of wheat are shown in Fig. 3. Insects were found in cardboard surface traps and in grain probes at all times before fumigation. No live insects were found in either cardboard traps or in grain probe samples after fumigation, although 2 dead psocids were found in a grain probe sample after treatment. In neighboring (untreated) cells, numbers in cardboard traps remained relatively constant before and after the time at which the experimental bins were treated. No grain probe samples were taken in the neighboring (control) bins.

Efficacy of application as a space spray in empty cells: Insect numbers in cardboard traps in empty cells before and after fumigation are shown in Figs. 4, 5 and 6. The number after fumigation declined to zero, but re-infestation subsequently occurred.

Efficacy as structural spray on rails and floors The efficacy of ethyl formate as a disinfestant was tested in two ways. One method involved transferring insects after spraying of rails to containers, and counting the number dead and alive. The second method consisted of counting insect numbers before and approximately 12 h after a series of sprays, conducted twice daily.

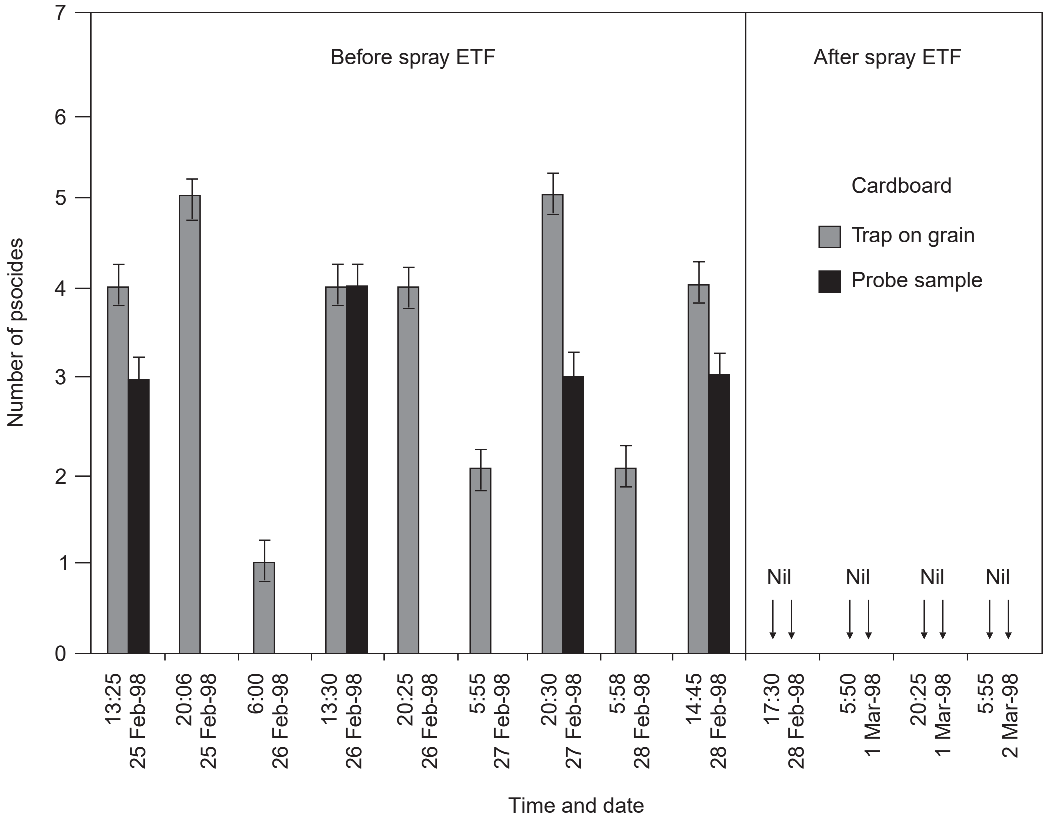

Fig. 3. Insect numbers in grain probe traps and cardboard surface traps before and after surface spraying of cell 141

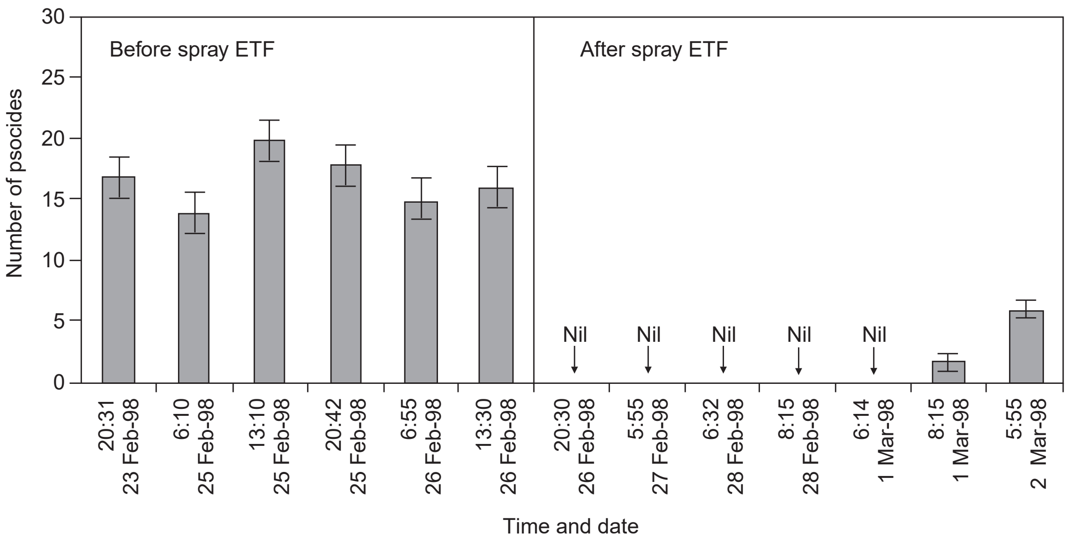

Fig. 4. Insect numbers in cardboard traps at the bottom of empty cell 159 before and after application of 10L

Interpretation of the first method of assessment is relatively straightforward, whereas interpretation of the second method of assessment is complex, and a full interpretation is not possible.

Assessment from in-situ *bioassays:* For the treated samples, all psocids died, from a total of 6 replicates each containing approximately 200 insects. Control mortality was low, but was not precisely assessed.

A*ssessment from changes in population on rails and in cardboard traps:* The number of psocids found on rails and in cardboard traps before and during a series of sprays, conducted twice daily, is shown in Fig. 6. Before spraying, the number found near dusk and dawn were greater than those found during daylight hours. This result is in agreement with a more extensive study (Rees, 1998).The number of insects

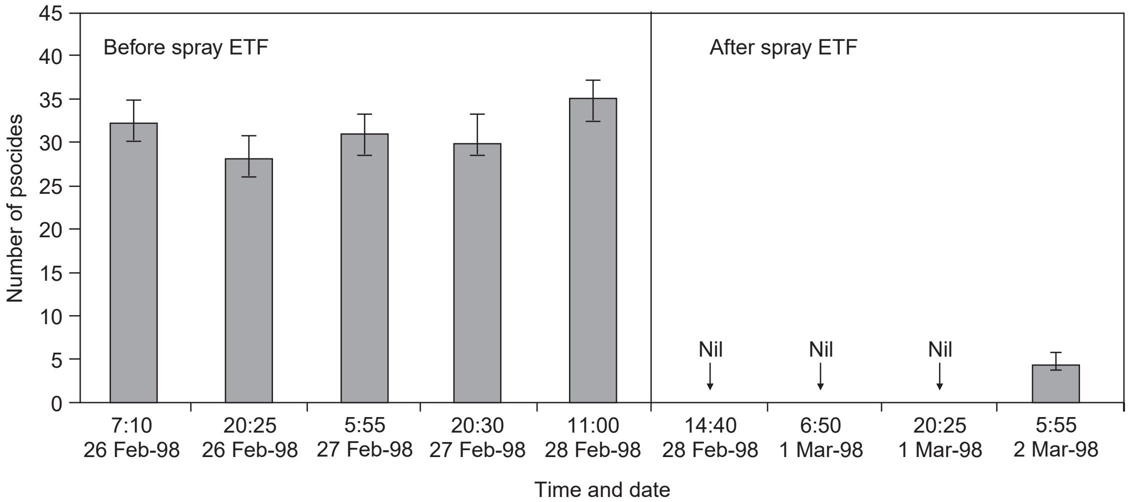

Fig. 5. Insect numbers in cardboard traps at the bottom of empty cell 235 before and after application of 10L of a 4% solution of ethyl formate in water

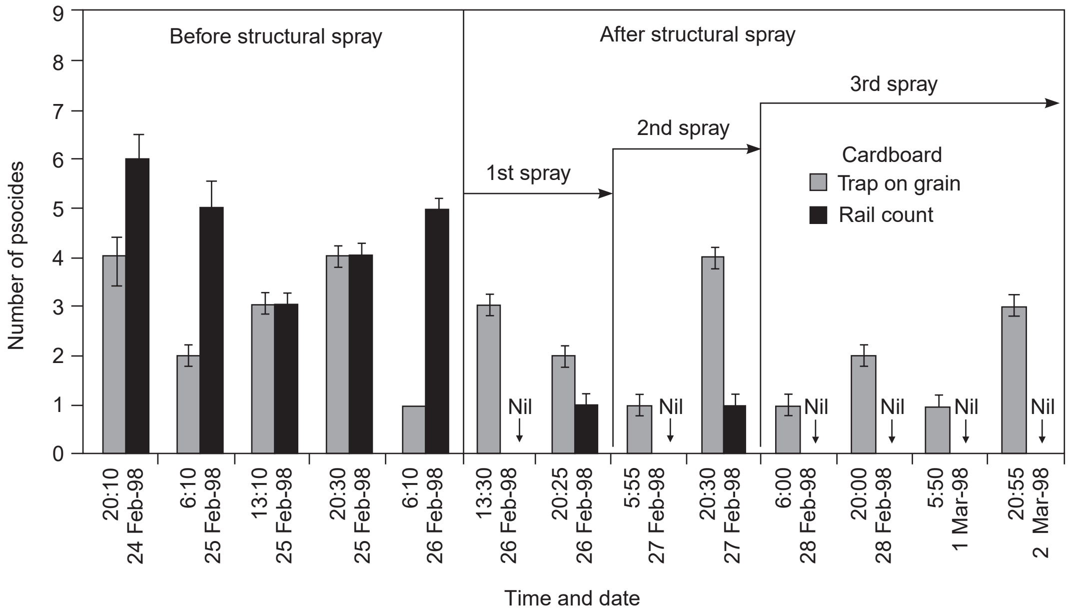

Fig. 6. Numbers of psocids in cell 149 from rail counts and in grain surface cardboard traps before and after

in surface cardboard traps did not greatly change subsequent to spraying of the structure. The number of insects in surface cardboard traps depends on a number of factors, related to population density and environmental factors. However, one would not expect a structural disinfectant to have a significant effect on numbers in traps on grain surfaces (unless a significan proportion of insects in a grain mass had migrated to the structure at time of spraying). During a series of twice-daily spraying, the number of insects on rails declined (Fig. 6). In addition, the number of insects found in cardboard traps between bins also declined (Figs. 7 and 8). Where initial psocid numbers had been relatively low, e.g. cells 149, psocid numbers on rails and on floor traps fell to zero. Where numbers had started at much higher level, psocid numbers declined, but still remained high.

CONCLUSION

Thus, ethyl formate can be used as disinfectant, because it can be applied safely as a spray in water, because workspace concentrations did not exceed the TLV, it disinfests and residues after application were indistinguishable from those before application. Ethyl formate met the selected assessment criteria in 3 situations, namely application to the surface of

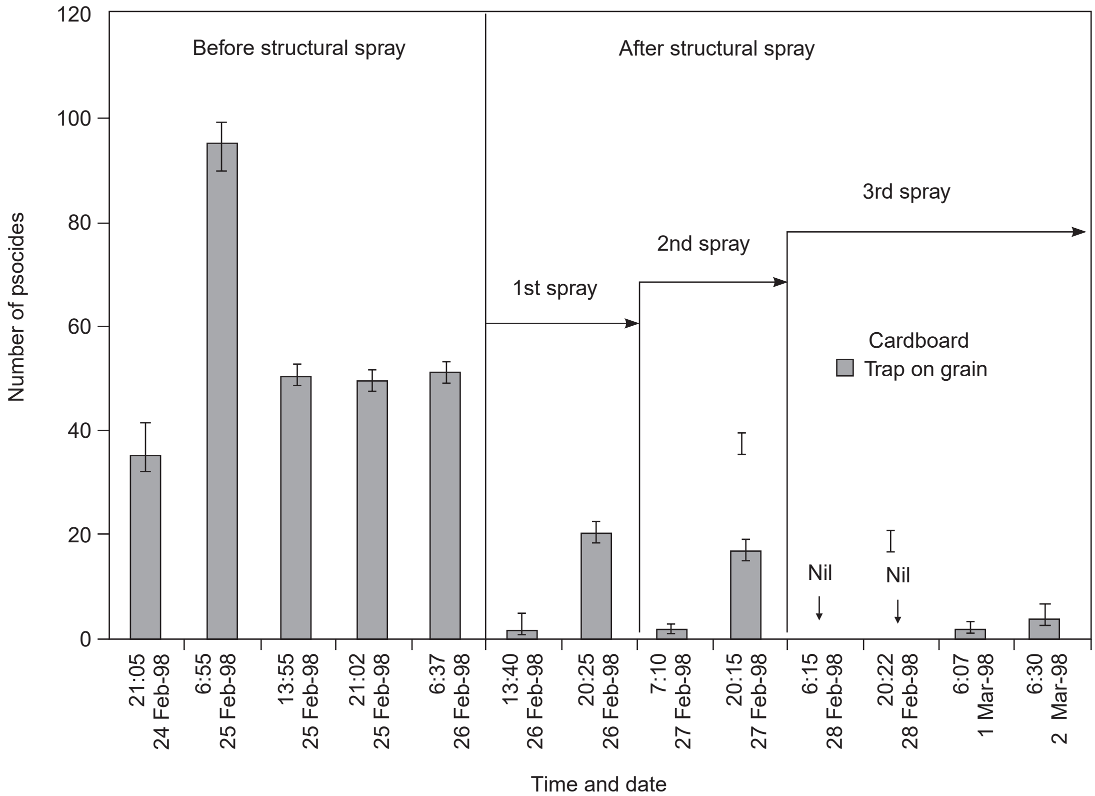

Fig. 7. Number of psocids in floor cardboard traps near star cell 157

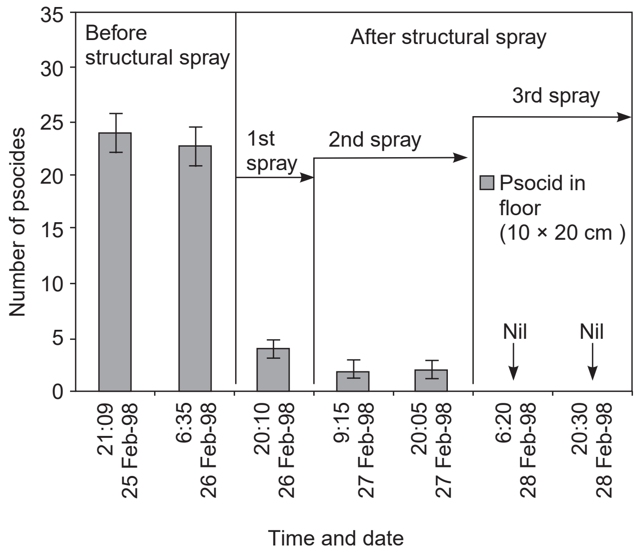

Fig. 8. Number of psocids in floor (10 × 20 cm) between cell 161 and cell 162

wheat, application to an empty cell and application to structures. The data on decline of insect numbers with regular spraying (assessment from changes in population on rails and in carboard traps) provide some evidence that routine space sprays could be useful under certain circumstances. Certainly, dichlorvos is used as a routine space spray. There is an argument that routine space spraying is rarely successful (whatever the chemical used), and this general argument should be considered before routine space spraying is adopted.At the 1997 meeting of the working party on grain protection, it was suggested that ethyl formate could fulfil many of the functions that are, or were, performed by dichlorvos. This general contention has been supported by the data.

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