

EVALUATION OF ETRIMFOS AS AN ACARICIDE ON STORED OILSEED RAPE

Lorna M. Stables, Elisabeth A.M. Good, S.J. Chamberlain

Ministry of Agriculture, Fisheries and Food, Agricultural Science Service, Slough  
Laboratory, London Road, Slough, Berkshire

Summary The admixture of etrimfos emulsion to two ten-tonne lots of stored oilseed rape controlled a widespread infestation of Acarus siro and Glycyphagus destructor in 3-4 weeks, and afforded protection against infestation for up to 24 weeks. The mean initial level of etrimfos on the seed was shown to be 11.8 ppm.

Little breakdown of etrimfos on the seed occurred during the trial, but preliminary results indicate that 95-100% of the compound was lost during a simulated commercial extraction and refinement of the oil.

#### INTRODUCTION

Production of oilseed rape, Brassica napus L., continues to expand in England and Wales. There has been an increase from 5,130 ha grown in 1971 to 65,000 ha in 1978, and the increase is expected to continue. The seed is grown for its oil which is used mainly for the manufacture of edible oils and fats. Some oil is also used in industry, and the residue remaining after extraction is converted into animal food.

Increased production has led to extended storage periods for the seed, and consequently a greater risk of mite attack, which may occur if the moisture content exceeds 8%. Mite infestations in stored oilseed rape have been reported from Canada and France (Mills, 1976; Fleurat Lessard and Anglade, 1973) but they seem to be particularly important in the UK, where even if the seed is initially dried to a safe moisture content, it tends to reabsorb atmospheric moisture during storage (Anon, 1977; Good, Stables and Wilkin, 1977). The principal mites involved in the UK are Acarus siro and Glycyphagus destructor.

In a heavy infestation, the entire contents of many seeds are consumed and rejection by buyers is likely. The effect of mite infestations on the quality of the oil produced is less well known. Mills, Sinha and Wallace (1978) found no direct relationship between the fat acidity value of the seed, and increased mite populations. They concluded that mites do not directly degrade the fat component, but may add moisture to the environment to promote growth of fungi.

Control of the mites can be achieved by re-drying the seed, but this is not always practicable. Chemical control in the UK was until recently restricted to gamma-HCH admixture, but widespread resistance to this compound in UK strains of A. siro and G. destructor necessitated a search for alternative compounds (Wilkin, 1975). Good et al. (1977) reported that in laboratory tests, pirimiphos-methyl was effective against both gamma-HCH susceptible and resistant strains of these species, and that it successfully controlled a heavy surface infestation on farm-stored seed. This compound is now cleared by the UK Pesticides Safety Precautions Scheme for safe use as a pesticide for admixture with oilseeds.

Another compound found to be promising as an acaricide by Stables (in press) and by Good et al (1977) was etrimfos (O-6-ethoxy-2-ethylpyrimidin-4-yl-O,O-dimethyl phosphorothionate). Like pirimiphos-methyl, it controlled both gamma-HCH susceptible and resistant strains of A. siro and G. destructor, and was effective against laboratory strains of Tyrophagus longior and T. putrescentiae. T. longior has occasionally been recorded on English farm-stored oilseed rape while T. putrescentiae was recorded as the dominant species in French stores (Fleurat Lessard and Anglade, 1973). The potential of etrimfos as an acaricide applied to stored oilseed rape was assessed in a field trial. The breakdown of etrimfos on the seed and during the refinement of the extracted oil was also investigated. The fungal flora of treated and control seed was compared.

## MATERIALS AND METHODS

### Delivery and treatment of the seed

Forty tonnes of mite-infested oilseed rape, variety Primor, were obtained in January, 1978, from a farm in South Humberside and transported by lorry in two loads to the Laboratory at Slough. The seed was divided into four 10-tonne lots and stored in a nest of 4 metal bins. One lot of seed from each load was sprayed with etrimfos emulsion as it was discharged into the bin, at an intended dose of 10 ppm (Bins 2 and 3). The two other 10-tonne lots were left untreated as controls (Bins 1 and 4). Despite the fact that the seed had twice been subjected to conveying, and to a road journey of about 200 miles, sampling revealed that the mean numbers of mites in the two loads of seed as they were discharged into the bins were 2,600/kg and 600/kg. Most were A. siro, with very few G. destructor. However, the infestation had been very much higher on the farm.

### Physical and biological assessments

At intervals after treatment (Table 1) samples of approximately 200 g were taken using a grain spear, from two sites per bin, and at depths of  $\frac{1}{4}$  m and 1 m at each site. The temperature at each position was recorded by means of a thermistor probe, and the moisture content of each sample was determined using an electrical resistance meter. The samples were weighed and then sieved using a 710  $\mu$ m aperture sieve. After coning and quartering the sievings to a suitable fraction, the number of live mites/kg were counted and identified to genus. A representative number of adults were also mounted on slides and identified to species.

On every occasion additional samples were taken from each position in the treated bins. From these samples, two 25 g replicates of seed were taken for residue analysis.

At the end of the trial, one sample per bin was taken for mycological examination by the dilution plating technique of Niles (1972).

Although the trial terminated at 24 weeks, the seed was not disposed of immediately, and some further observations on the mite population were made.

### Residue analysis

Etrimfos was removed from the whole seed by cold extraction with n-hexane. The extracts were analysed by gas-liquid chromatography on a 1 m glass column, packed with 5% OV-17 + 0.02% Epikote 1001 on Diatomite CLQ mounted in a Perkin-Elmer F33 gas chromatograph, using a phosphorus-specific thermionic detector. In addition, some preliminary trials using the extraction and refinement process reported by Good et al. (1977) were carried out. Residues in the extracted and

refined oil were analysed using a column similar to that described above, but mounted in a Perkin-Elmer F17 gas chromatograph, fitted with a flame photometric detector. Residues in the spent meal were also assessed.

## RESULTS

### Changes in the numbers of mites

The mean numbers of A. siro found at  $\frac{1}{4}$  m and 1 m positions in the bins are shown in Table 1.

Table 1

Effect of etrimfos admixture on the A. siro population in stored oilseed rape (mites/kg)

	Bin 1 (control)		Bin 2 (treated)		Bin 3 (treated)		Bin 4 (control)	
	$\frac{1}{4}$ m	1 m	$\frac{1}{4}$ m	1 m	$\frac{1}{4}$ m	1 m	$\frac{1}{4}$ m	1 m
DAY 1	18539	8354	3190	3344	1078	2382	5855	2467
WEEK 1	9779	10627	646	979	474	229	2308	1910
" 2	12595	2743	762	152	0	381	11447	1904
" 3	15582	3275	154	154	82	0	1195	343
" 4	12724	13638	48	10	0	10	5562	1257
" 6	19746	6938	114	0	38	38	3635	590
" 8	14503	2258	19	19	19	0	2744	969
" 12	17862	13068	0	0	0	0	3589	675
" 16	137752	33810	0	0	19	0	24381	1409
" 20	56238	64000	0	0	0	0	13486	1219
" 24	88381	31695	0	19	0	0	46933	17986

All results are the mean of two determinations

One day after treatment, there were more mites in the bins than expected from the counts made during loading. However, the population in the treated bins fell to a negligible level of under 200/kg about 3 weeks after the application of etrimfos, and remained at this level throughout the trial. G. destructor was not found in samples from the treated bins during the trial. The numbers of mites in the control bins increased and reached a maximum of over 137,000/kg at  $\frac{1}{4}$  m in bin 1 at 16 weeks and over 46,000/kg at  $\frac{1}{4}$  m in bin 4 at 24 weeks. A. siro was far more numerous than G. destructor, which never constituted more than 2% of the population.

Eight weeks after the end of the trial, a heavy infestation of Tarsonemus granarius had developed in both treated bins, only very few being found in one control bin. The numbers of A. siro had declined to near zero on the untreated seed, and a heavy infestation of Cheyletus eruditus had developed.

### Fungal flora

Altogether 28 species of fungi were isolated, mostly in low to barely detectable numbers, apart from a high count of Aspergillus versicolor (maximum level in treated seed  $5.9 \times 10^7$  colonies per g), and Penicillium verrucosum var. cyclopium (maximum level in treated seed  $3.3 \times 10^5$  colonies per g). There

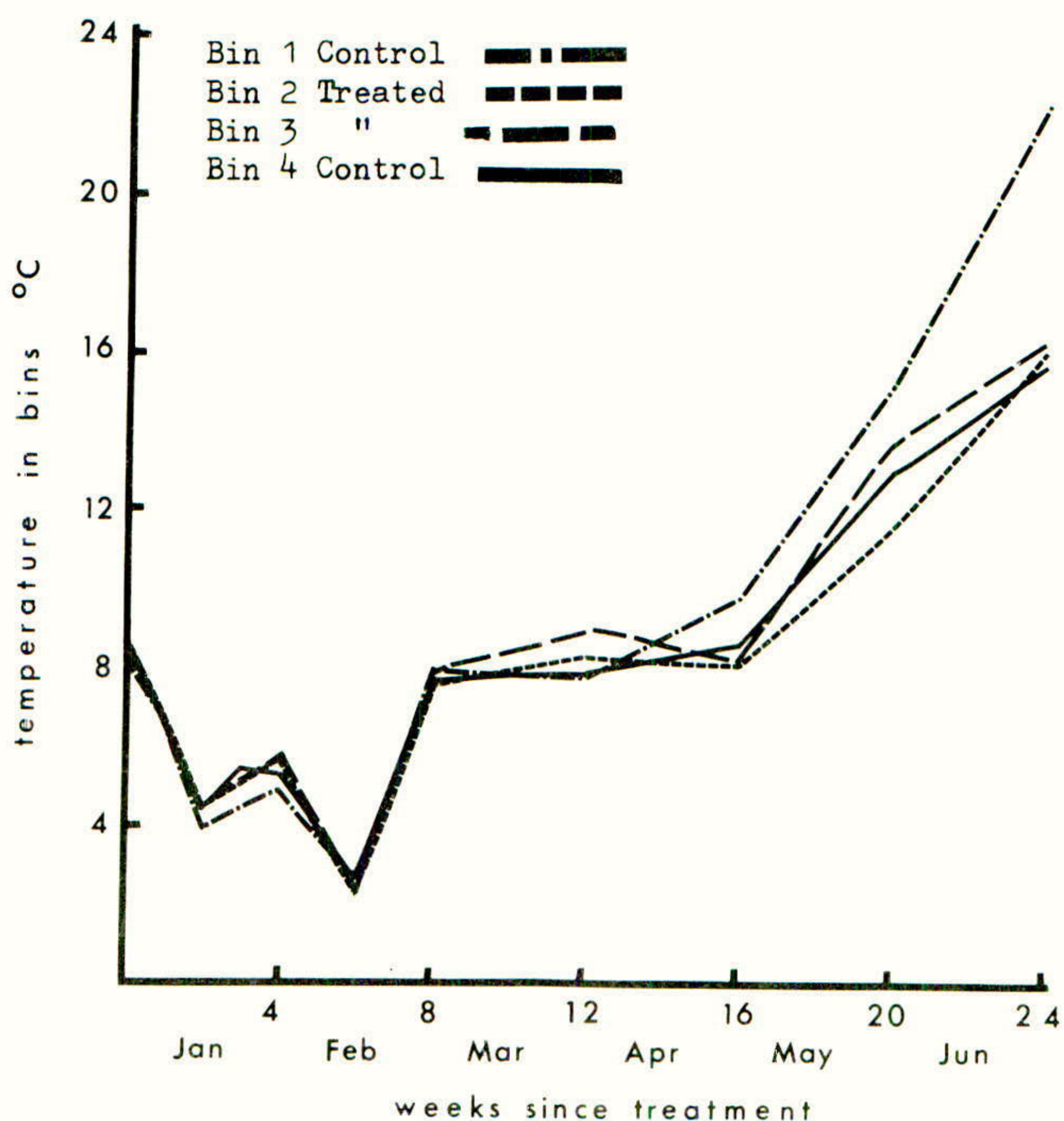
appeared to be no significant differences between either the species found or their numbers in the treated and untreated seed.

### Physical conditions

The changes in temperature at individual sampling points generally reflected changes in the ambient temperature. During the weeks in which the mite numbers were decreasing on the treated seed, the temperatures in these bins ranged from  $3.0^{\circ}\text{C}$  -  $9.3^{\circ}\text{C}$ , the mean being  $6.1^{\circ}\text{C}$  (Fig. 1).

Figure 1

Mean temperatures in the control and treated bins



There was no significant difference between temperatures in control or treated bins until week 16 when the mean temperature of bin 1 (control) began to rise more rapidly than the other bins. The temperature continued to rise to  $21^{\circ}\text{C}$  -  $23^{\circ}\text{C}$  at 24 weeks, a value about  $6^{\circ}\text{C}$  higher than those in the other bins ( $P = < .001$ ). This was possibly caused by the very heavy mite population, which rose to a much higher level in this bin than in the other control bin (Table 1). Although the heating of cereals as a result of insect activity is well known (Howe, 1962) there appears to be a lack of published evidence of heating in any commodity caused by mites.

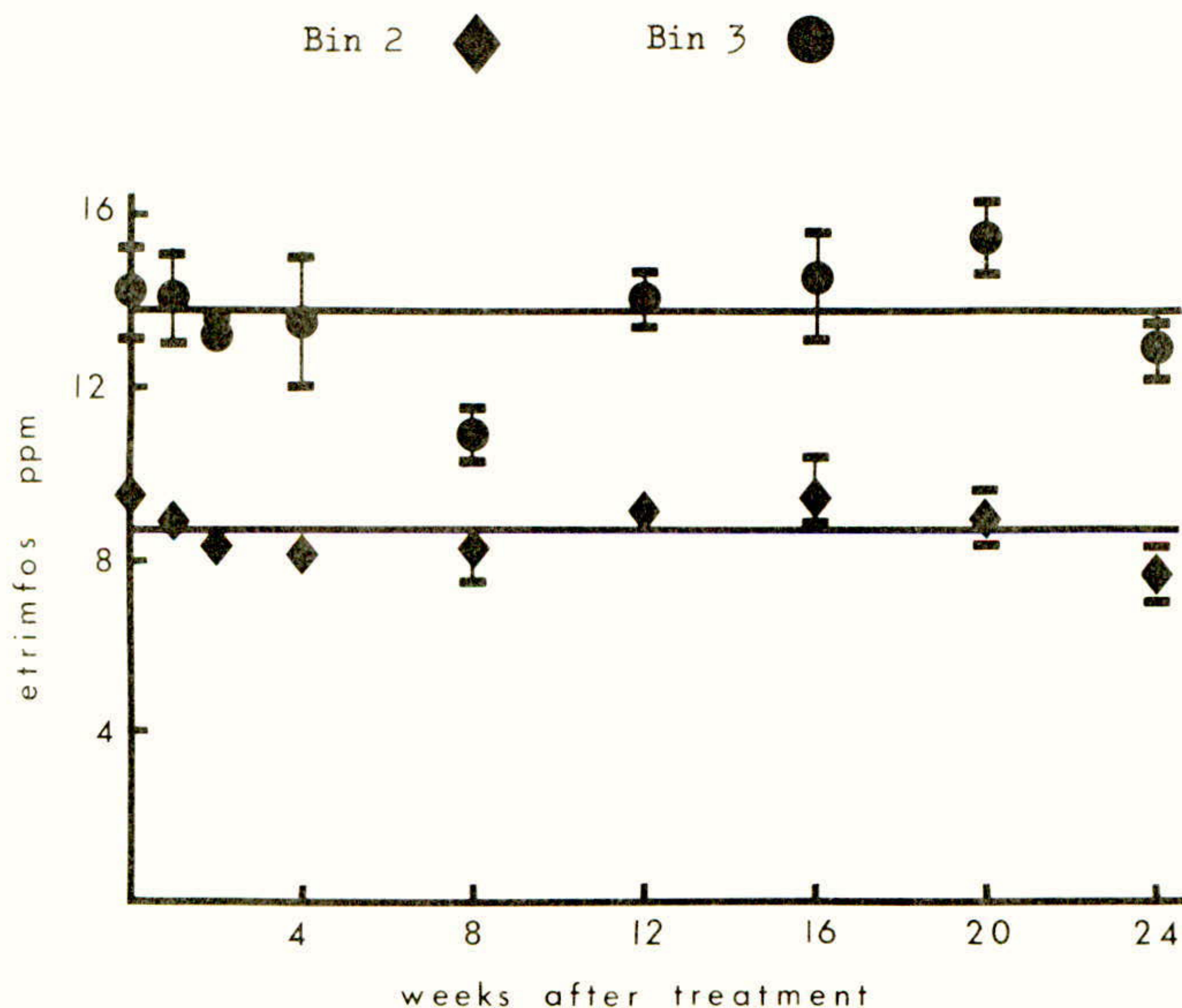
The moisture content of the seed at delivery was high, ranging from 9.3% - 11.2%, which is a favourable level for mite development. The seed in the first load, corresponding to bin 1 (control) and 2 (treated) was slightly damper than in the second load. Throughout the trial the moisture content did not change appreciably at any of the sampling points.

#### Etrimfos residues in the seed and oil

The mean initial level of etrimfos in the seed was 9.5 ppm (9.1-10.0) in bin 2, and 14.2 ppm (11.3-14.6) in bin 3. Similar variation was found, both between sampling points and at individual points, throughout the trial (Fig. 2).

Figure 2

Etrimfos residues on whole seed (mean of 4 determinations  $\pm$  S.E.)



After 24 weeks, no significant breakdown of etrimfos had occurred. When received, the free fatty acid level (FFA) was 1.2%, a level acceptable to commercial crushers. Although no FFA analysis was done at 24 weeks, preliminary analyses of samples of seed retained until 15 months after treatment showed that the FFA level was high, having approximately doubled.

Preliminary results indicated that 95-100% of the etrimfos was lost during the refinement of the oil and that the greatest losses occurred by degradation during bleaching and steam distillation. No residues were detected in the spent meal.

## DISCUSSION

This study demonstrated the effectiveness of etrimfos on an infestation of A. siro and G. destructor spread throughout a bulk of oilseed rape. A previous trial by Good et al. (1977) involved admixture of 8 ppm pirimiphos-methyl dust to only the surface of a bulk. They found that a heavy surface infestation of A. siro was controlled in two to three weeks, at a temperature of about 8°C. The control achieved by etrimfos at similar temperatures was comparable. It is probable that in warmer conditions, both compounds would have acted faster, since low temperatures are known to retard the action of acaricides (Wilkin and Haward, 1975). In addition to controlling the established infestation, etrimfos also prevented re-infestation of the seed for up to 24 weeks, despite high numbers of mites in the adjacent control bins. The trial involving pirimiphos-methyl only lasted for about 4 weeks and so it was impossible to assess the full potential of this compound as a protectant.

The persistence of etrimfos in the seed was similar to that of pirimiphos-methyl, another organophosphorus compound (Good et al., 1977). The stability of these compounds is enhanced at low pH levels, and it is possible that the free fatty acids in the seed contributed to the persistence of etrimfos. The degradation of both pesticides during the refinement of the oil appears to be comparable, with most of the losses occurring during bleaching and steam distillation, and only very low levels being detected in the refined oil.

It is interesting that there was no significant difference between the fungi in the treated or the untreated seed, despite the fact that a very heavy infestation of mites existed in the control bins, and both species present are known to be mycophagous (Hughes, 1976). Another interesting result was the development of the Tarsonemus infestation 8 months after treatment of the seed. These mites also thrive on fungi (Hughes, 1976). Since the fungal flora of the treated and untreated seed was not significantly different, it is not clear why the Tarsonemids did not breed to such an extent in the controls. One possibility is that the Cheyletus population, which developed in these bins, prevented the build-up of T. granarius. It is possible that the fungi degraded some of the oil, and contributed to its FFA level. Lipolytic action was not demonstrated in a strain of A. versicolor, but fungi closely related to P. verrucosum var. cyclopium were shown to be capable of degrading refined rape oil (J.H. Clarke, personal communication).

The potential of etrimfos as a stored-products acaricide has been clearly demonstrated. It has the advantage of a relatively low mammalian toxicity, but has not yet been cleared by the UK Pesticides Safety Precautions Scheme for admixture to oilseeds and cereals.

### Acknowledgements

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