THE BEHAVIOUR OF INSECTS IN THE PRESENCE OF INSECTICIDES: THE EFFECT OF FENITROTHION AND MALATHION ON RESISTANT AND SUSCEPTIBLE STRAINS OF TRIBOLIUM CASTANEUM HERBST

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ABSTRACT: Observations on susceptible and resistant strains of *Tribolium castaneum* (Herbst) have shown that certain strains exhibit different behaviour patterns with respect to activity levels and refuge seeking.

These behavioural differences may be reinforced by the presence of a contact insecticide and an avoidance of fenitrothion by a resistant strain of *T. castaneum* has been demonstrated.

Modification of behavioural patterns because of selection pressure by contact insecticides is discussed and a behavioural resistance mechanism is postulated.

INTRODUCTION: Field observations have shown that pockets of live insects which are protected in refuges in the fabric of a building can survive a thorough treatment of the building with a residual contact insecticide and lead to subsequent infestation of newly stored produce[1][2][3]. In one such occurrence regular fabric spraying with malathion wettable powder of two maltings in the U.K. failed to eradicate infestations of *Tribolium castaneum* (Herbst) [4]. Even though large numbers of insects were killed by each treatment, survivors caused heavy infestations of newly-stored malt. Substitution of fenitrothion for malathion did not markedly improve the situation although bioassays on the treated surfaces showed that the deposits achieved would normally be regarded as effective. Laboratory tests confirmed the efficacy of the dosage rates applied even though one of the strains of *T. castaneum* exhibited a high level of non-specific malathion resistance.

An investigation is proceeding to identify factors contributing to the survival of insects under such circumstances and to determine their relative importance. Laboratory simulation of residual populations of *Oryzaephilus surinamensis* (L.) and *Sitophilus granarius* (L.) have shown that the effect of fenitrothion can be greatly reduced in the presence of a refuge containing food[5]. It was decided therefore to determine the significance of food refuges in the control of *T. castaneum* while investigating the effects of malathion and fenitrothion on the maltings resistant strain (TLM) and a laboratory susceptible strain (FSS).

METHOD: Experimental arenas and food refuges were constructed as described by Pinniger[5] as shown in Figure 1. Access to each end of the food refuges was provided by 2 mm aperture gauze, and each refuge contained 8 g of coarsely kibbled wheat.

Fenitrothion and malathion wettable powder were diluted to 1.25% active ingredient with distilled water and applied to filter papers using a turntable sprayer. The papers were treated with 465 mg/m^2 fenitrothion, 400 mg/m^2 malathion or 800 mg/m^2 malathion.

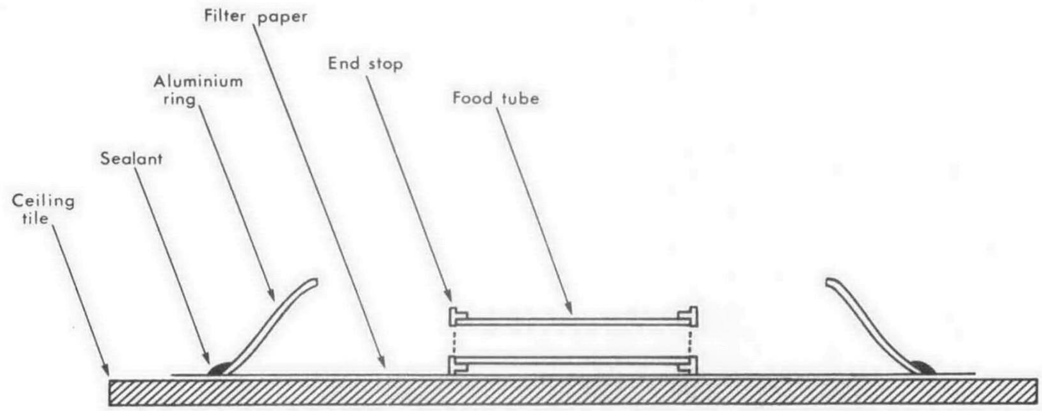

FIGURE 1. Section through experimental arena (after Pinniger 1974)

The insects used were from cultures of susceptible FSS and resistant TLM *T. castaneum* maintained at 25°C and 70% r.h. Thirty 2-4 week old adults were placed in each of the arenas which were kept at 25°C and 70 r.h. in darkness. Over a period of 28 days insects visible in the arenas were counted at a fixed time each day and dead insects removed, observations being made with low wattage lighting to minimise disturbance.

For each strain of *T. castaneum*, four arenas with refuges and four arenas without refuges were set up for the controls and each of the two insecticides. For each replicate provided with a refuge the insects were placed in a 12 cm dia. crystallizing dish with a clean filter paper in the bottom and left for 48 hours with the food refuge. After this time the refuge was carefully transferred to the arena together with those insects which had not wandered into the refuge. Insects were placed directly in the arena for those replicates without refuges.

RESULTS AND DISCUSSION: Mortality - Control mortality was less than 2% over the 28 day period and can be regarded as negligible. All the susceptible insects were killed by day 2 and all the resistant insects by day 9 on 800 mg/m^2 malathion treated arenas without a food refuge. Where a refuge was provided mortality at 28 days was 96% for the susceptible strain and 22% for the resistant strain (Figure 2). Fenitrothion treated arenas without refuges killed all the susceptible insects by day 2 and all the resistant insects by day 7. Where a refuge was provided mortality at 28 days was 98%

for the susceptible strain and 42% for the resistant strain (Figure 3).

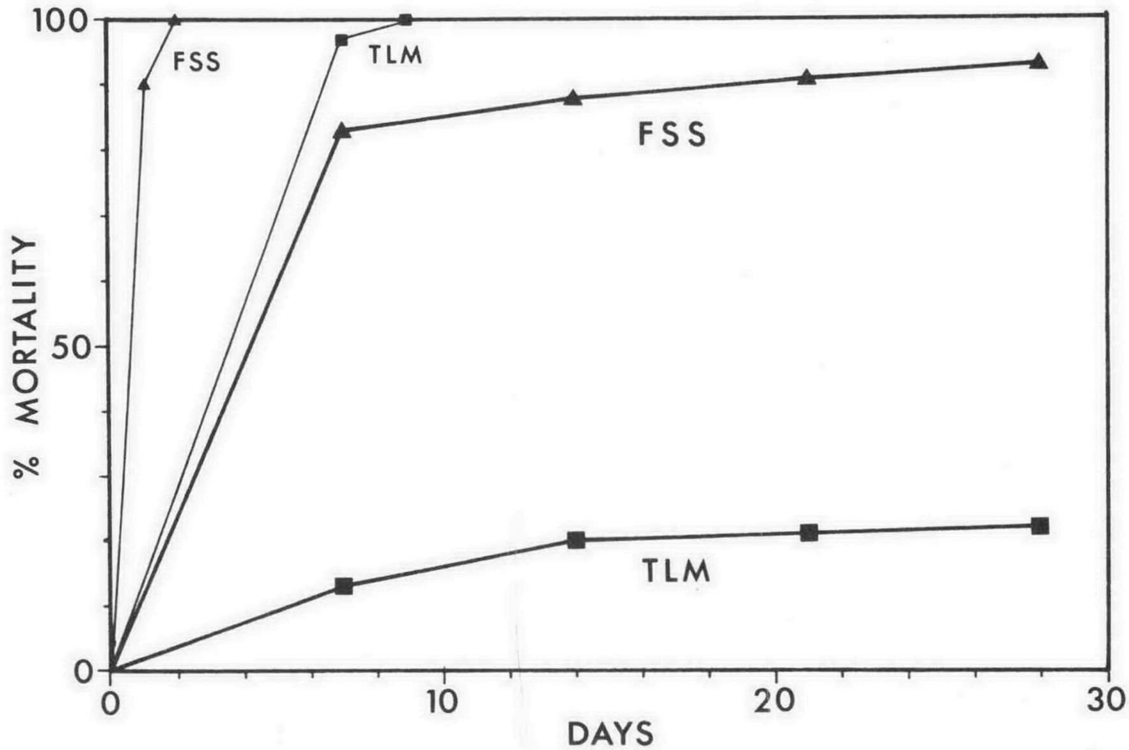

FIGURE 2. Mortality of *T. castaneum* on malathion 800 mg/m^2

▲ FSS)) with refuge ■ TLM)   ▲ FSS)) without refuge ■ TLM)

<u>Insects in arenas with refuges</u> - Figure 4 shows the percentage of insects visible in the control arenas over the 28 day period, the mean for susceptible insects being 81% and for resistant insects 57.5%. The percentage of surviving resistant insects visible in the treated arenas is also shown in Figure 4. There was a steady decrease in the percentage of insects in the treated arenas and by day 28 this had reached 3% on malathion 400 mg/m^2, 1% on malathion 800 mg/m^2 and 0.5% on fenitrothion 465 mg/m^2.

A comparison between the mortality of *T. castaneum* on treated arenas without refuges and treated arenas with refuges shows the effect of the food refuge in reducing the kill of insects (Figure 2). The difference in mortality between the susceptible and resistant strains was also demonstrated (Figures 2 and 3). Although fenitrothion was more effective than malathion, neither insecticide gave 100% control of susceptible or resistant strains. The results indicate that the presence of refuges in the fabric of a building would make either strain difficult to control with a single treatment of malathion or fenitrothion.

When the percentage of insects present in the susceptible

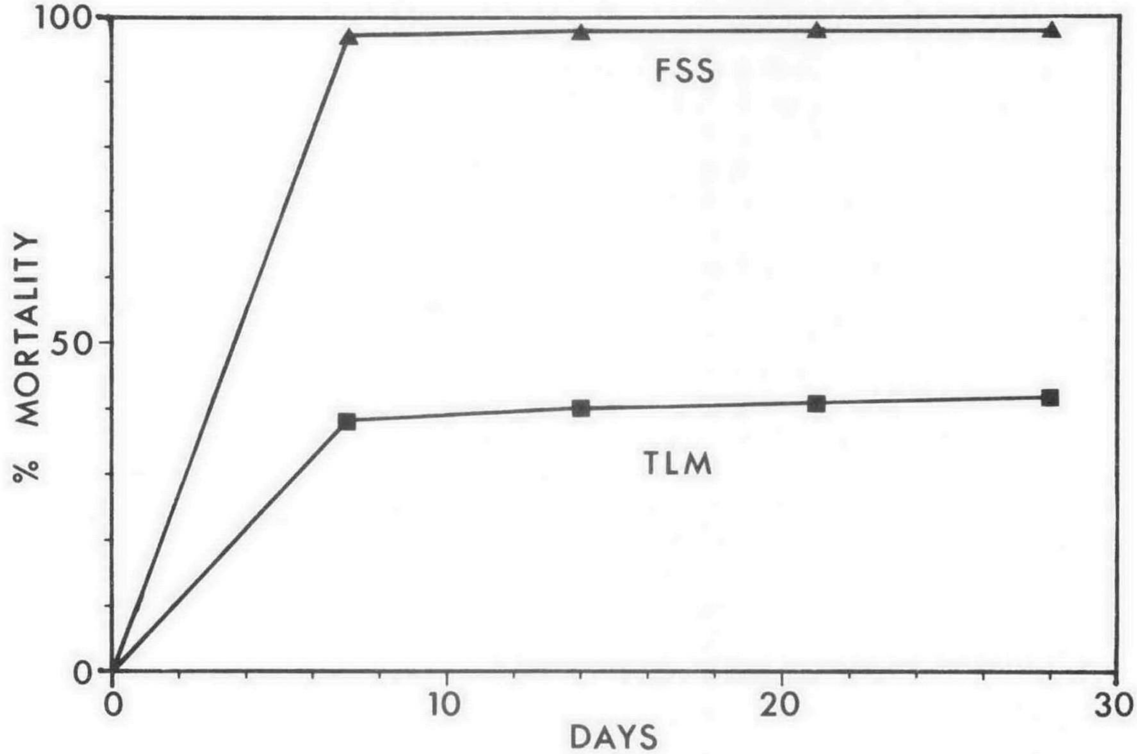

FIGURE 3. Mortality of *T. castaneum* on fenitrothion 465 mg/m^2

strain control arenas is compared to the percentage in the resistant strain control arenas (Figure 3) it can be seen that there is a tendency for more resistant insects to remain inside the food refuge, the number of insects in the arena being 20% lower than the susceptible insects. It was noticed that very few insects of the resistant strain were seen in the arenas of the treated replicates and that by day 28 more than 95% of the surviving insects were inside the food refuge at the times of count (Figure 4). In order to investigate the effect of the insecticide on the wandering activity of the resistant strain a further series of tests was set up with 32 replicates, 8 with fenitrothion 465 mg/m^2, 8 with malathion 800 mg/m^2 and 16 untreated. After 3 weeks observations, 4 refuges with insects were transferred from malathion treated arenas to untreated arenas and 4 refuges with insects from untreated arenas were transferred to the malathion treated arenas and observed for a further four weeks. Similarly 4 refuges with insects were transferred from arenas treated with fenitrothion to untreated arenas and 4 refuges with insects were transferred from untreated arenas to those treated with fenitrothion. Eight refuges remained undisturbed on control untreated arenas for 7 weeks and 4 refuges on each insecticide were undisturbed for 7 weeks. The results are shown in Figures 5 and 6. The percentage of insects in the undisturbed untreated arenas ranged between 44% and 71% with a mean of 61.5% over the 7 week period. The percentage of insects in the

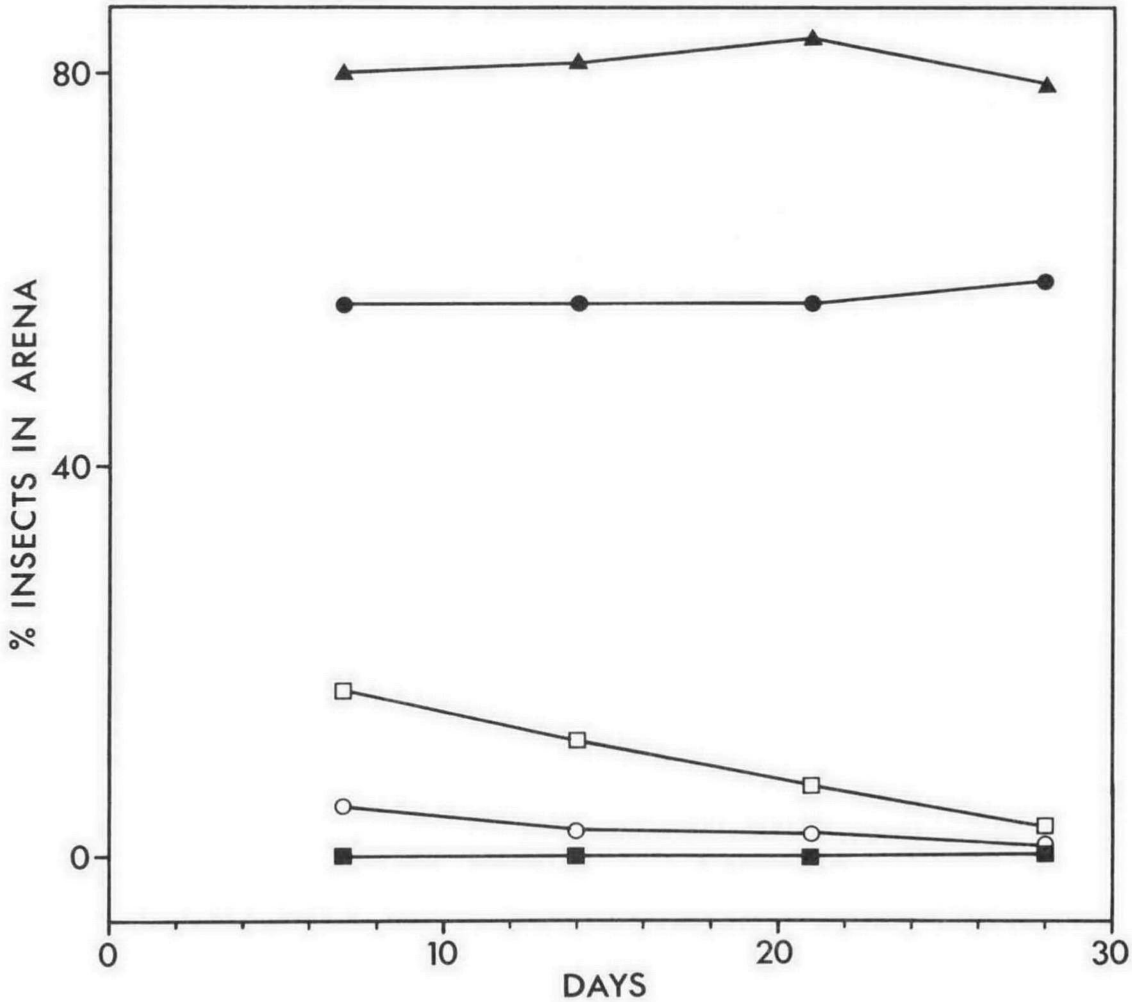

FIGURE 4. Percentage of insects in arenas with refuges

□ TLM Malathion 400 mg/m²   ▲ FSS Control
○ TLM Malathion 800 mg/m²   ● TLM Control
■ TLM Fenitrothion 465 mg/m²

undisturbed treated arenas did not exceed 8% on malathion and 2% on fenitrothion. The transfer of refuges and insects from untreated arenas to those treated with malathion reduced the percentage of wandering insects to 23% in the first week and to less than 1% by the fourth week after transfer. Conversely the transfer of insects and refuges from malathion treated arenas to untreated arenas increased the percentage of wandering insects to 40% in the first week and 68% by the fourth week after transfer.

Fenitrothion showed a similar but more pronounced effect, the absence of the insecticide increasing wandering activity to control levels in the first week after transfer and the presence of

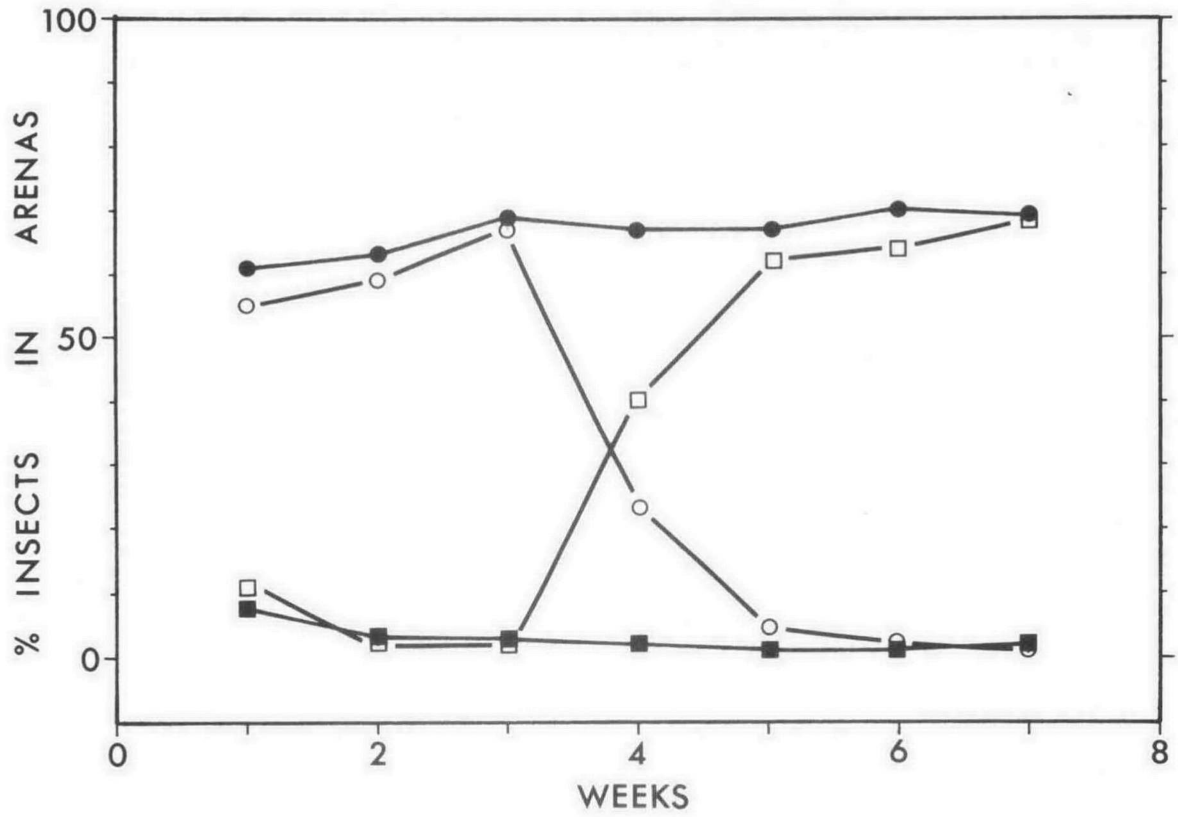

FIGURE 5. Percentage of resistant insects in arenas with refuges

● Untreated

■ Malathion 800 mg/m^2

○ Untreated transferred to malathion

□ Malathion transferred to untreated

the insecticide reducing the wandering to less than 5% in the same period (Figure 6).

The experiments demonstrated that the resistant strain of *T. castaneum* showed a greater tendency to remain inside a food refuge in an untreated environment when compared to the susceptible strain. When exposed to an insecticidal environment this aspect of the behaviour of the resistant strain was considerably reinforced and wandering activity further reduced. This may indicate some of the reasons for the practical control failure experienced with this strain of *T. castaneum* in that the presence of an insecticide modifies and limits the wandering activity and reduces the contact between insect and the toxic surface. The low mortalities achieved by the relatively high doses of insecticide used in this experiment underline this. In these tests the insecticide may have acted as a repellant but even in an untreated environment there was a difference in refuge seeking between the resistant and susceptible

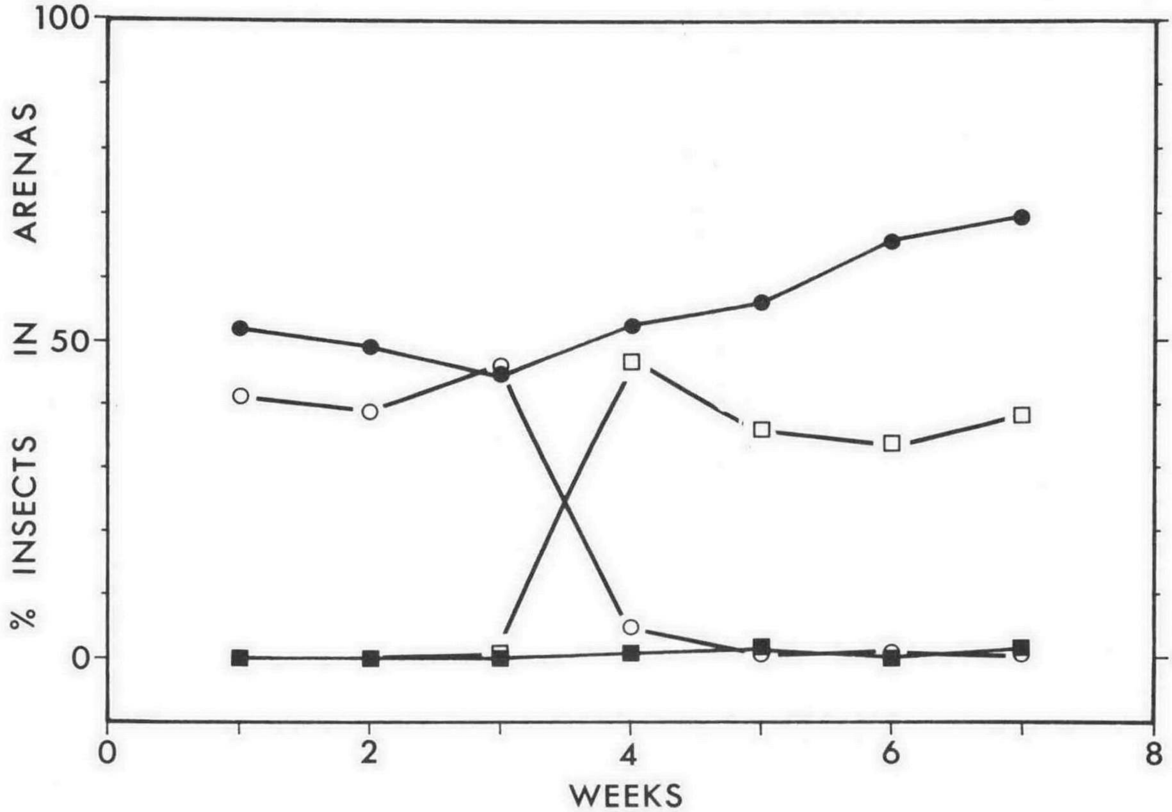

FIGURE 6. Percentage of resistant insects in arenas with refuges

● Untreated

■ Fenitrothion 465 mg/m^2

○ Untreated transferred to fenitrothion

□ Fenitrothion transferred to untreated

strains. During the daily observations a further difference in behaviour was observed between the resistant and susceptible strains. The susceptible strain tended to exhibit a high rate of movement and even a small disturbance initiated a period of intensive activity. In contrast, the rate of movement of the resistant strain appeared to be slower, the insects spent long periods of time apparently motionless and were affected much less by disturbance.

Selection by insecticides which has resulted in biochemical mechanisms of insect resistance are well established[6][7]. The behavioural differences demonstrated by this experiment may also be the result of selection by insecticides. Individuals in a population show a range of activity levels and the most active members will be more likely to come into contact with the insecticide and be eliminated. Those individuals with a tendency to remain in refuges until the environment is less toxic are more likely to survive and if this characteristic is carried genetically it will be passed to subsequent generations. Laboratory selection of refuge-seeking survivors of both resistant and susceptible strains is being carried out to investigate whether this characteristic can be carried genetically.

The differences in behaviour between the resistant and susceptible strains may be unconnected with biochemical resistance if there is a simple strain difference which coincidentally confers an additional survival advantage to the resistant strain. However, it is possible that a behavioural mechanism evolved sequentially with the biochemical resistance mechanism, with both mechanisms acting to reinforce resistance and to produce insects which are able to survive in insecticidal environments.

ACKNOWLEDGEMENTS: I should like to thank J. A. Hope who carried out many of the experimental observations and C. E. Dyte for criticism of the manuscript.

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