

*EFFECTS OF SOME RESIDUAL INSECTICIDES AND THEIR MIXTURES
ON STORED GRAIN PESTS*

BY

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*TO THE MEMORY OF MY PARENTS
AND TO MY DAUGHTER HEBA-ALLA*

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ABSTRACT

Malathion (4% w/w), etrimfos (2% w/w), and primiphos methyl (2% w/w) dust formulations were applied to whole wheat which was then stored at 25° C and 15% moisture content and at 30° C and 10% moisture content. The decline in insecticidal activity was monitored for 20 weeks by bioassaying samples of treated wheat against *Rhyzopertha dominica* and *Sitophilus granarius*, and the loss of active ingredient was followed over the same period using gas liquid chromatographic analysis of wheat extracts. The results are discussed in relation to inactivation of residues of the insecticides on the wheat.

Formulations of malathion (4% w/w), pirimiphos methyl (2% w/w), etrimfos (2% w/w), gamma-HCH (2% w/w), and deltamethrin (2% w/w) were bioassayed alone and as binary mixtures against a number of coleopterous pests of stored grain, including a strain of *Tribolium castaneum* specifically resistant to malathion and a strain of *Sitophilus granarius* resistant to gamma-HCH, using two bioassay methods. The hypothesis of additive action between pairs of insecticides was tested using a computer program based on the model and algorithm described by Plackett and Hewlett (1963) for two toxicants acting by simple similar action with complete correlation of tolerances. In most cases the data was consistent with the additive model.

Examples of the use of a linear programming technique to determine optimal relative concentrations of insecticides applied as mixtures to control a number of pest species with different susceptibilities to the components are exhibited, using the above bioassay data. The method depends on additivity of action between the components of the mixture.

Sitophilus granarius and *Rhyzopertha dominica* were exposed for 7 days to wheat treated with LC5, LC10, and LC20 concentrations of dust formulations of malathion, pirimiphos methyl, and their 1:1 w/w mixtures and then removed. The number of progeny produced by *R.dominica* survivors were significantly lower than that of unexposed populations for all treatments, whereas there was no difference between treated and untreated populations of *S.granarius*. The results are discussed in relation to the use of residual treatments of insecticides to protect wheat from attack by stored product insects.

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CHAPTER 1

General Introduction

1.1 Problems in the use of insecticides to control pests of stored grain

Wheat, rice, and other cereal seeds which are stored for periods of months or years prior to consumption are subject to infestation by a variety of insect species. Such infestations lead to a reduction in the nutritive value of grain used for human or animal food and to a reduction in germination of seed grain. Consequently, the market value of cereals is very sensitive to the presence of small numbers of insects detected in samples, and if inspection of a sample reveals the presence of live, or in some cases dead, insects a batch of grain may be rejected for purchase or for export, or it may be downgraded to a use suitable for a lower quality product.

Protection of the commodity against insect attack can be achieved in a variety of ways, but currently the cheapest and most effective method is to use residual synthetic insecticides admixed with the grain prior to deposition in the storage bin or silo. There are a number of problems associated with such prophylactic treatments: organic insecticides decay

or are otherwise lost from the grain during the storage period, so that the protection they offer from pest attack diminishes as the storage period is extended; current products approved for use in stored cereals are toxic to man as well as to insects, so that some assurance is needed that the residues of any particular compound will have declined to safe levels under local storage conditions at the end of the proposed storage period; insect populations may respond to intensive local selection pressure over a large number of generations by acquiring some degree of resistance to the insecticides used to control them; and individual insecticides may not give adequate protection at economic or safe application rates against infestation by all species which are locally present. In developing countries there may be additional logistical problems of insecticide supply and quality assurance, trained manpower, and environmental conditions which favour rapid breakdown of insecticides and development of infesting populations.

The work reported in this thesis deals with the response of insect pests of stored grain to insecticides under environmental conditions which are typical of those found in countries of the Southern Mediterranean region, of which Egypt is one example. The principal stored grain pest species found in Egypt are shown in Table 1.1.

Table 1.1

Cereal production in Egypt¹ and the importance of major pests of stored cereals²

Production (metric tons x 10 ³)		So	Sz	Sg	Rd	Tc	Tco	Os	Om
wheat	1874	A	-	C	A	A	C	B	-
barley	150	A	-	C	A	A	C	B	-
millet	650	A	-	-	A	A	C	B	-
rice, paddy	2312	C,A	-	C,C	B,A	A,A	C,C	C,C	-
maize	3982	A	-	C	A	A	C	B	-

Notes: A major importance
C minor importance

B moderate importance
- occurs rarely or absent

So= *Sitophilus oryzae*

Sz= *Sitophilus zeamais*

Sg= *Sitophilus granarius*

Rd= *Rhyzopertha dominica*

Tc= *Tribolium castaneum*

Tco= *Tribolium confusum*

Os= *Oryzaephilus surinamensis*

Om= *Oryzaephilus mercator*

¹ Data from FAO production yearbook 1986

² Champ and Dyte (1976)

1.2 Residues of insecticides in stored grain

1.2.1 Effects of temperature and moisture content on the rate of loss of insecticide residues from treated wheat

The loss of insecticide residues from grain at a particular temperature and grain moisture content has been shown, in some instances, to follow a first order rate law. For example, Desmarchelier (1978a) demonstrated a reasonable agreement between data on the loss of fenitrothion from wheat, oats, sorghum, and rice and a first order rate model at grain moisture contents and temperatures (in the case of wheat) in the range 9.6 - 12.6% and 20 - 35° C. The dependence of rate constants on temperature was in close agreement with that predicted on the basis of the Arrhenius equation, and true rate constants, which were independent of grain type and grain moisture content, could be obtained by dividing rate constants determined at different grain moisture contents by the corresponding activity of water in the grain. These results were interpreted as showing that the rate determining step in fenitrothion decay was a transport process, involving desorption of the compound from the grain and replacement by a water molecule. The hypothesis is not consistent with data for all insecticides, since malathion decays at greatly different rates on heat-sterilised and untreated sorghum (Kadoun and LaHue, 1972) implying some enzymic participation in the rate determining step.

The first order decay model can be applied to much published data on loss of residues from grain (see Section 3.2.6), particularly when grain moisture content is relatively high. It was explicitly used by Noble *et al.* (1982) and Noble and Hamilton (1985) to describe the breakdown of mixed or single isomers of the pyrethroids permethrin, phenothrin, fenvalerate, deltamethrin, cypermethrin and cyfluthrin on stored wheat, and by Samson *et al.* (1988) to describe breakdown of fenitrothion on stored maize at a number of different moisture contents.

Such predictability in the behaviour of insecticides on grain in the relatively constant environmental conditions of storage bins and silos should in principle make it possible to calculate the residue remaining at any time after application, given a knowledge of the initial concentration in the grain and the storage conditions, and in some instances this has been achieved with adequate accuracy (Desmarchelier and Bengston, 1979).

Degradation of malathion applied as a dilute emulsion to stored wheat has been investigated as a function of temperature and moisture content by a number of workers (Strong and Sbur, 1960; Minnett *et al.*, 1968; Kadoum and LaHue, 1969; Kadoum *et al.*, 1978, Quinlan *et al.*, 1979, Kadoum and LaHue, 1979; ; Mensah and Watters, 1979; Watters and Mensah, 1979; Abdel-Kader *et al.*, 1980). Malathion is relatively unstable on wheat, especially as the moisture content increases to the range 14 - 16% and at

temperatures above 20°. Pirimiphos methyl degradation on stored wheat has not been as intensively studied as that of malathion. Kadoun *et al.* (1978) reported pirimiphos methyl residues in wheat and milled fractions of wheat following treatment with emulsions at 7.3 and 14.6 mg/kg and storage at 12.5% wheat moisture content and 26° C, and Mensah and Watters (1979) compared residues as a function of time in wheat of two different moisture contents (12% and 16%) (see also Mensah *et al.*, 1979). The stability of this insecticide when applied to wheat as an emulsion is reported to be greater than that of malathion, particularly at higher moisture contents. Etrimfos is a comparatively recently introduced insecticide for stored grain protection, and the only published data relating to its decomposition on wheat is that of Thomas *et al.* (1987). These workers compared the effectiveness of chlorpyrifos-methyl, etrimfos, fenitrothion and pirimiphos methyl emulsions when applied to wheat under bin storage conditions in the U.K. and at mean temperatures which varied through the experiment but were maximally 18° C. Residues of pirimiphos methyl and etrimfos were found to remain substantially constant and close to the initial application rate under these conditions throughout the 36 weeks storage period.

1.2.2 Effect of formulation on loss of residues from treated wheat

Insecticides are generally applied to wheat as aqueous emulsions or dusts. A survey carried out by MAFF in the UK in 1982 indicated that many commercial grain stores routinely treated incoming grain with insecticide, pirimiphos methyl being the compound most frequently used, and dust formulations being preferred to emulsions (Wilkin, 1985). Preferences for formulations and insecticides were thought to reflect their local availability, frequency of application, existing machinery, and appreciation of the resistance status of pest species.

There have been surprisingly few studies on the decline of insecticide residues resulting from treatment with dust formulations of insecticides used for stored grain protection, given their widespread use. There are likewise few studies in which the relative efficacy of insecticidal dust and emulsion formulations have been directly assessed. There is some evidence from early work on pyrethrum that the insecticidal action of dust formulations lasts longer than that of emulsions (Floyd and Smith, 1953; Lindgren *et al.*, 1954) and Rowlands (1967) quoted unpublished results showing considerably greater persistence of malathion dust formulations (on white wheat flour) than water-based emulsions on wheat at both low and high moisture contents. However, work involving comparisons between other formulation types (emulsions, solutions in organic solvents, wettable

powders, microcapsules) have generally showed no great difference between either residue decay or insecticidal effect over time (Strong *et al.*, 1961; LaHue and Kadoum, 1979). Dust formulations differ from those in which the insecticide is applied in a liquid carrier in both the rate and extent to which the chemical is absorbed by the grain (Rowlands, 1971).

1.2.3 Penetration and distribution of residues in grain

The location of residues of insecticides in grain is important for three reasons: insects which are susceptible to the contact action of insecticides are most effectively controlled if diffusion of the compound into the grain interior is slow, while conversely there may be some control of cryptic larval stages of some species by compounds which accumulate at appropriate locations inside the grain; there is much evidence to indicate that a variable proportion of the insecticide applied to wheat becomes progressively unavailable to insects, whether moving over the seed coat or feeding or developing as larvae in the endosperm, presumably due to diffusion into inaccessible sites in the grain (see Sect. 3.2.5); and thirdly the toxic hazard of residues of insecticides to consumers of milled flour will depend on the distribution of those residues among the various milling fractions.

Studies of the distribution of insecticide residues in wheat were initiated by Rowlands (1966a, 1966b) and Rowlands and Horler (1967). Penetration of lipophilic insecticides varied with point of application to the wheat, the back and attachment region being the areas of slowest and most rapid diffusion respectively. Labelled malathion was applied to these regions as a hexane solution, and the pericarp, germ and endosperm separated by manual dissection. Moisture content was found to have very little effect on initial penetration when applied to the back of the wheat seed, but the amount reaching the germ increased markedly with decreasing moisture content when malathion was applied at the attachment region. Subsequent redistribution of the insecticide tended to minimise these differences, leading to a distribution pattern (endosperm > pericarp > germ) which was similar for both application sites. The distribution pattern for other organophosphorus insecticides was qualitatively similar, while DDT, lindane and cyclodiene compounds tended to accumulate in the germ fraction (Rowlands, 1967, 1970).

Many workers have looked at the distribution of insecticides in grain indirectly by measuring residues in different fractions of milled wheat. These fractions are generally distinguished as bran (corresponding to pericarp, seed coat and aleurone layer), middlings (corresponding to fine bran particles - "shorts" in American terminology, germ, and coarse flour), and flour (corresponding to endosperm). This work has been comprehensively reviewed up to 1974 by Rowlands (1967, 1971, and 1975). In

the case of malathion, results from this type of experiment have uniformly indicated the greatest residue to be located in the bran fraction, with comparatively low residues in the flour. The apparent contradiction between these findings and those of Rowlands following individual topical application of grains may result from a re-distribution of residues after the first few days of storage, or rapid metabolism of those residues which accumulate initially in the endosperm. Work subsequent to 1974 confirms this distribution pattern under a greater variety of storage conditions and for other organophosphorus insecticides. For example, Mensah *et al.* (1979) found between 6 and 25 times as much pirimiphos methyl in the bran as in the flour fraction of wheat stored either at 12% or 16% moisture content for 1 to 6 months, and similar results were obtained by Kadoum *et al.*, (1978).

1.3 Loss of insecticidal activity from insecticide-treated grain

The biological effectiveness of an insecticidal treatment is not simply proportional to the residue of insecticide present. Insecticides in grain are inactivated during storage such that an aged residue is less effective than an equivalent residue freshly applied (Desmarchelier, 1978a ; 1978b ; Hargreaves *et al.*, 1982). Also, residues on grain of high moisture content are generally less effective than when moisture content is low (Samson *et al.*, 1987, 1988). The magnitude of the inactivation

phenomenon, which is thought to be due to diffusion of the insecticide into regions of the grain inaccessible to the insect, depends on the species being bioassayed and its life stage. Samson *et al.* (1988) correlated the biological activity of fenitrothion on stored maize towards adult *T. castaneum* with the chemical concentrations of the compound which could be extracted by using a 60 s rinse of the grains with methanol, implying that the difference between toxic and total residue corresponded to material which had penetrated into and through the seed coat and was therefore unavailable to this surface browsing species.

Desmarchelier has presented a model for prediction of the biological activity of residues based on the assumption of a first order decay of chemical residues, correlation of chemical residue levels with biological activity, and a linear log(equivalent dose)/probit(response) relationship for the species in question (Desmarchelier, 1978b). His method was applied to results using dichlorvos, pirimiphos methyl, and chlorpyrifos methyl on wheat, and bioassays with *T. confusum* and *R. dominica*.

1.4 Effects of insecticides on survivors of treatments

Grain storage periods are generally longer than the generation time of most storage pest species. Because of the degradation of chemical residues over time, the progeny of any insects surviving treatment at the beginning of a storage period will continue to breed in an environment containing less insecticide, and although the level of infestation ultimately reached will probably be lower than that in untreated wheat the presence of even a few live insects may necessitate a costly fumigation or lead to rejection of a batch (Thomas *et al.* 1987). Consequently, the effect of an insecticide on the breeding success of survivors of treatments is a relevant factor influencing choice of compound and application rate. Recommended rates for insecticides are intended to provide complete control of adults and emerging progeny for a period of several months. However, in practice grain treatment is inevitably uneven, storage conditions may favour more rapid breakdown of insecticide than anticipated, and there may be continual infestation pressure from populations outside or in the fabric of the buildings.

There is good evidence from many studies that females survivors of applications of certain insecticides may produce fewer progeny than untreated insects (Loschiavo, 1955; Harein, 1960; Chadwick, 1962; Zettler and Lecato, 1974; and Taher and Cutkomp, 1983). However, only in a few cases has the cause of this

effect been investigated. Survivors of an insecticide treatment are selected on the basis of their tolerance to the treatment. Because biological attributes such as ability to mate and fecundity of females may be irreversibly altered by such treatments, the only method of determining the correlation between such attributes and insecticide tolerance in the unselected population is to measure their value in samples of individual insects prior to determining their insecticide tolerance. Such experiments have not yet been reported. It is therefore unclear whether the reduced fecundity observed in some cases following insecticide treatments is due to the continuing action of the insecticide on reproductive processes or to an inverse correlation between fecundity and insecticide tolerance in the initial population. Similar qualifications apply to other "sub-lethal" effects of insecticides.

Experimental investigation of the effects of insecticides on reproduction have generally taken two forms: empirical studies to determine whether or not residual concentrations of insecticides in wheat which are insufficient to kill adult populations can nevertheless restrict reproduction and the emergence of an F_1 generation under controlled or field conditions, and mechanistic studies designed to investigate the reasons for such effects when discovered.

Empirical studies generally involve an initial infestation of treated medium, the adults being allowed to mate and oviposit for a limited time and then removed from the medium. A proportion (up to 100%) of the adults may die during this exposure period, but may nevertheless lay eggs before succumbing to the insecticide. The medium is then incubated until adult emergence of any progeny. Such experiments may be continued through to the F_2 generation. For example, Harein (1960) obtained a 94% reduction in progeny production by exposing a mixed population of *S. oryzae* to an LC7 of fenchlorphos for 14 days in wheat, and determining adult emergence from eggs laid during the exposure period. Similarly, Bengston *et al.* (1983b) showed that methacrifos applied at 15 mg/kg and sampled 1 week after spraying onto sorghum gave 39% mortality of *Rhyzopertha dominica* after an exposure period of 26 days but 100% reduction in F_1 progeny, and Thomas *et al.* (1987) found that etrimfos, chlorpyrifos methyl, fenitrothion and pirimiphos methyl all greatly inhibited (though did not completely prevent) progeny production by *S. granarius* and *S. oryzae* at doses which gave > 95% mortality of these species over a 7 day period.

Such studies are valuable in establishing field application rates, but cannot distinguish mechanistically between the various effects which could give rise to such suppression of progeny production, including effects on mating, egg formation, oviposition, egg hatch, and subsequent larval development in a medium containing continuously decreasing levels of residue. The egg and larval stages of insects may be more or less

protected from the effects of insecticides applied to the surface of grain, as well as differing from adults in their susceptibility. Different species vary widely in these respects, depending on their pattern of development. For example, *R. dominica* is vulnerable to toxic residues on the surface of wheat grains during the first three larval instars prior to penetration of the grains, and again when adults emerge, while *Sitophilus* spp. are protected from such residues throughout their larval development and contact them only during and after emergence. Champ *et al.* (1969) have reported in detail on the stage susceptibility of larvae of these two species to malathion, diazinon, and fenitrothion applied to grain after adults had completed oviposition.

Mechanistic studies have attempted to isolate individual events during the reproductive process, and to investigate the effects of insecticides on them. Tenhet (1947) studied the effects of pyrethrum oil spray treatments at the LC15-LC37 level on mixed populations of the cigarette beetle *Lasioderma serricorne* (F.) and showed that survivors laid significantly fewer eggs than the untreated population, and Loschiavo (1955) showed reduced adult progeny production by mated females of *T. confusum* which had survived a 2 h exposure to DDT and were then maintained on fresh media. Parkin (1961) examined the action of pyrethrum dusts on the control of weevil infestations in grain. He found that the number of progeny of *S. granarius* was reduced if 86% of the adult population was knocked down within the first 24 h of exposure, and suggested that either

the weevils were so activated by the pyrethrins that they could not remain still long enough to complete the oviposition process, or the neuromuscular processes become so uncoordinated that the complex sequence of actions required to bore a hole in the grain, deposit an egg in it, and seal the opening could not be completed. Chadwick (1962) provided some evidence for the former hypothesis by showing that inhibition of progeny production was considerably reduced when insects exposed to pyrethrum-treated wheat were allowed to oviposit on untreated wheat. There is evidence from more recent studies that females lay fewer eggs following sub-lethal doses of a number of pyrethroid and organophosphorus insecticides (see Chapter 6).

1.5 The Use of Insecticide Mixtures to Control Insect Pests

Mixtures of insecticides may have certain advantages over the use of individual compounds when used to control insect pests. When insecticides are applied to protect a crop or commodity from attack by a number of different pest species which vary in their response to a particular compound, the use of mixtures can be an effective way of extending the spectrum of activity of the application. It is not uncommon for herbicide mixtures designed to control a broad range of weed species to contain four or more components; application of the same principle has rarely resulted in insecticide formulations containing more than two active

ingredients, possibly because insecticides are generally less selective in their action.

Mixtures of insecticides may also be used to control different life stages of an individual pest species, as for example the use of mixtures of diflubenzuron and methomyl on cotton to control, respectively, the egg and early larval instar stages of the cotton leafworm, *Spodoptera littoralis*. However, perhaps the greatest interest in the use of mixtures to control insect pests (at least in the published literature) originates in the concept of synergistic action, in which two insecticides applied together give a more effective control of a single species than would be expected on the basis of their activity when applied alone. There is a vast literature on this subject, but much of it is empirical with little effort being devoted to establishing causes for observed effects. Claims of synergistic action are always made relative to some model for the anticipated joint action of insecticides (Sect. 1.6).

Mixtures of insecticides to control pests of stored commodities are of interest both because of the wide diversity of pest species which infest these materials and the range of tolerance which exists within these species to the relatively few compounds approved for application. The situation is dynamic, since the tolerance of individual species to one or more compounds may alter with time as resistant strains develop.

In the context of the control of complexes of pests in stored grain in Australia, Desmarchelier (1977) pointed out that if a single insecticide is applied to protect grain from attack by a number of insects the application rate is determined by the need to control the most tolerant species. Thus the amounts of pyrethroids that have to be applied to grain to control *Tribolium castaneum* (Herbst) are in excess of the amount required to control *Rhyzopertha dominica* (Ardley and Desmarchelier, 1974; Carter *et al.*, 1975), and conversely the amounts of phosphorothioate insecticides such as malathion and fenitrothion which have to be applied to control *R. dominica* are in excess of amounts to control *T. castaneum*, *Tribolium confusum* Jacquelin du Val, *Sitophilus oryzae* (L.) or *Sitophilus granarius* (L.) (Champ *et al.* 1969; Bengston *et al.* 1975). Similarly, *Sitophilus* spp. have proved tolerant of many juvenile hormone analogues which are highly effective against *Tribolium* spp. and species of Lepidoptera, and the application rates required to control the former species make the use of these otherwise useful and safe compounds uneconomic in stored grain.

These considerations led the Department of Primary Industries in Australia to initiate a program of research into insecticide mixtures for stored grain protection, such mixtures comprising a broad spectrum organophosphorus insecticide and a pyrethroid or carbamate compound added specifically to provide control of *R. dominica*, including field strains with some resistance to malathion and other phosphorodithioate compounds.

The work required studies to identify pairs of compounds which had the toxicological and other characteristics necessary to reduce application rates for protection of stored wheat against the range of pests (which included strains resistant to some insecticides) present in Australia, to give a persistence of biological effect over a storage period of 6-9 months, to produce residues at the end of this time which conformed to existing or proposed international limits, and to achieve these requirements under the range of storage conditions which existed in Australian silos.

Ardley and Desmarchelier (1978) established that mixtures of synergised bioresmethrin (2 mg/kg) + fenitrothion (4 mg/kg) applied to stored wheat adequately controlled a resistant strain of *R. dominica* in the presence of field strains of *S. oryzae* and *T. castaneum* which were resistant to a number of organophosphorus insecticides. The level of control of adult *T. castaneum* and *R. dominica* was inadequate following 8 months storage, but progeny production was almost completely suppressed.

Subsequent reports of field tests with this mixture and also combinations of chlorpyrifos methyl + bioresmethrin, pirimiphos methyl + bioresmethrin, fenitrothion + (1R)-phenothrin (Bengston *et al.*, 1980a 1980b, Desmarchelier *et al.*, 1981), and pirimiphos methyl + carbaryl (Davies and Desmarchelier, 1981; Desmarchelier *et al.*, 1987) established suitable application rates which met the above requirements, the

combinations chosen always reflecting the need to control field strains of *R. dominica* in the presence of other pests (including malathion resistant strains). Of interest in the context of the present thesis was the apparently empirical approach to determining suitable ratios and application rates for the mixture components. On the one hand it can be argued that if a more structured approach had been used there would have been less chance of making chance discoveries such as the apparent synergistic suppression of progeny production by mixtures of synergised bioresmethrin and fenitrothion noted by Ardley and Desmarchelier (1978), but on the other hand it might have been possible to test fewer application rates and component ratios, with considerable reductions in time and cost.

Field trials have also established the ability of applications of fenitrothion (12 mg/kg) + fenvalerate (1 mg/kg) + piperonyl butoxide (8 mg/kg) and pirimiphos methyl (4 mg/kg) + permethrin (1 mg/kg) + piperonyl butoxide (8 mg/kg) to control common field strains of *S. oryzae* and *R. dominica*, as well as completely suppressing progeny production in *T. castaneum*, *T. confusum* and *E. cautella* (Bengston et al. 1983a). Subsequent studies have sought to establish comparable grain protectant mixtures and application rates to protect stored sorghum from a similar pest complex as that which attacks stored wheat in Australia. Of the above combinations, fenitrothion (12 mg/kg) + bioresmethrin (1 mg/kg) and pirimiphos methyl (4 mg/kg) + carbaryl (8mg/kg) controlled typical malathion-resistant strains

of *S. oryzae*, *R. dominica*, *T. castaneum* and *E. cautella*, while chlorpyrifos methyl (10 mg/kg) + pyrethrins (1.5 mg/kg) + piperonyl butoxide (12 mg/kg) and fenitrothion (12 mg/kg) + (1R)-phenothrin (1 mg/kg) were only partially effective (Bengston *et al.*, 1983b). Of interest in this latter work was the illustration that the approval of a new insecticide for stored grain protection can radically alter the range of possible mixtures which could be assessed to protect against a broad complex of pest species; methacrifos (15 mg/kg) was found to provide protection against all species except *E. cautella*.

A method for control of a broad complex of stored product pests including malathion-resistant strains which infested railway trucks and lorries in America was reported by Harein and Schesser (1975). This involved a mixture of dichlorvos and tetrachlorvinphos applied as an emulsion to surfaces, target species being *T. confusum*, *S. granarius*, *R. dominica*, and larvae of *Trogoderma inclusum* Le Conte. Various application rates of a 1 : 2 w/w mixture were tested, but no reason was given for the selection of this ratio of components. In this case, a constraint on application rate in addition to that of cost was that the vapour concentration of dichlorvos in the sprayed area should not exceed 1 mg/l, to protect the operator.

1.6 Joint action of insecticides

Models of the joint action of toxicants attempt to describe in quantitative form the quantal biological effects produced by toxicants applied together in terms of the quantal biological effects produced when they are applied singly. Two notions underlie all models for quantal responses, that of a target site at which the toxicant disrupts normal physiological events in the organism, and that of a threshold dose at the target site at which the capacity of the organism to compensate for this disruption is exceeded, leading to a response.

The first systematic classification of biological action resulting from the joint use of pairs of toxicants was that of Bliss (1939) who distinguished three types: independent joint action, similar joint action, and synergistic action. Independent joint action covered cases in which toxicants act at different target sites, producing failures of physiological systems which are unconnected with each other. An organism produces a response if the dose of either toxicant at its respective target site exceeds the capacity of that physiological system to withstand it. This concept of joint toxic action leads directly to an expression for the proportion of a population killed by a combination of two poisons A and B.

$$P_c = P_a + P_b(1 - P_a)(1 - r)$$

where P_c is the proportion killed by a combination of A and B, P_a the proportion killed by the same dose of A applied alone, P_b the proportion killed by the same dose of B applied alone, and r is a coefficient describing the degree of correlation between the susceptibility of the population to poisons A and B. When $r = 0$, no correlation exists, and in this form the equation is commonly used as a formula to correct for natural mortality (Abbott, 1925), B representing the toxicant and A the effect of natural mortality during an experiment. It may be noted that under the assumptions of independent action as defined by Bliss the probit(response)/log (dose) regression for a mixture will not necessarily be linear.

Similar joint action was used by Bliss to describe the effect of toxicants which act at the same physiological site so that in a mixture one can be substituted at a constant ratio for any proportion of the other without altering the toxicity of the combination. It was assumed that such a situation would always result in probit(mortality)/log(dose) regressions lines which were parallel since the variances of the tolerance distributions to the two toxicants would be identical. In these circumstances, an expression relating the dose D_c of a given mixture of A and B giving some specified response can be calculated from the dose D_a of A giving the same response as follows:

$$D_c = \frac{D_a}{Q + k(1 - Q)}$$

where Q is the proportion of A in the mixture and k is the ratio of the toxicities of A and B (assumed constant at all response levels).

The terms synergism and antagonism were used to describe the action of a mixture with a biological action which respectively exceeded or was less than that anticipated on the basis of experiments with the isolated constituents. Bliss's model of synergistic action was based on the assumption that a synergistic mixture behaved as if it were a single compound of greater toxicity than that of either of the components.

The concepts of two distinct types of joint action, independent and similar, together with those of synergism and antagonism, were retained by Finney (1942) in his extension of the mathematical treatment of joint action given by Bliss, but were significantly extended by Plackett and Hewlett (1952) in the four-way classification shown in Table 1.2.

Table 1.2

Interaction between toxicant	Site of action	
	same	different
Non-interactive	simple similar	independent
Interactive	complex similar	dependent

Joint action is here defined as similar or dissimilar according to whether the target sites of the two toxicants are the same or different, and as interactive or non-interactive according to whether disruption of a physiological system by one compound does or does not influence the action of the second compound at the same or different physiological site. The mathematical models developed to express the action of mixtures were essentially developments of those of Bliss, that for complex similar action being a generalisation of that for Bliss's similar action (now called simple similar action), and that for dependent action being a generalisation of that for Bliss's independent action. However, the basic distinction between independent and similar action was retained. To some extent these developments may have been due to the accumulation of data showing that pairs of insecticides (for example, organophosphorus esters)

with the same physiological target (and therefore presumed to act similarly) did not produce parallel probit regression lines in matched bioassays as would be expected on the basis of similar action as defined by Bliss, and an increasing awareness of the interdependence of the biological systems disrupted by, for example, neuroactive insecticides acting at the axon and the synapse. The lack of parallelism of probit regression lines for insecticides with the same mode of action, which implied a lack of complete positive correlation of tolerances in compounds which acted similarly, was explained in terms of the relative ability of the compounds to reach the target site and perhaps reflected an increasing awareness of the importance of penetration, metabolism and excretion in determining the toxic effects of insecticides.

In 1959 Hewlett and Plackett published the first of a series of papers (Hewlett and Plackett, 1959) in which they sought to present a unified approach to the description of joint action phenomena such that similar and independent action were interpreted as special cases of a more general model for joint toxic action. Their model included the following general assumptions:

- (a) for a response to occur, a minimum amount of a toxicant must reach a site of action in an individual (the action tolerance)
- (b) the amount of a toxicant reaching this site can be related to the

dose applied by the relationship

$$\text{amount at site} = a (\text{dose})^b$$

where a and b are constants

- (c) organisms have a bivariate normal distribution of tolerances to two toxicants with a lesser or greater degree of correlation between the tolerances for the respective toxicants.
- (d) the criterion for a response is that either the sum of the doses of the toxicants at the target site is greater than or equal to the action tolerance (similar action) or that either one or other of the doses of the toxicants exceeds the action tolerance (independent action).

The model is consistent with dosing methods in which the toxicants are applied at a single time (as in topical application) or over a prolonged period of time (as in residual bioassays) since in either case the response in an individual is triggered when the dose at the target site(s) exceeds the action tolerance.

A special case of the model is that of two toxicants acting with complete similarity, having complete positive correlation of tolerances, and for which the dose at the target site is directly proportional to the dose applied. This set of conditions gives rise to a particularly simple

relationship which can be tested for any pair of toxicants which have non-parallel probit regression lines and for which the assumption of similarity of action is not unreasonable. This relationship can be expressed

$$10(y_1 - y)/b_1 + 10(y_2 - y)/b_2 = 1$$

where y_1 and y_2 are the normal equivalent deviates (n.e.d.) of the responses of the test species to doses x_1 of toxicant 1 and x_2 of toxicant 2 when they are applied individually, b_1 and b_2 are the slopes of the $\log(\text{dose})/\text{n.e.d.}(\text{response})$ regression lines for toxicants 1 and 2, and y is the n.e.d. of the response of the test species to the doses x_1 and x_2 when they are applied jointly.

Bioassay data for pairs of toxicants applied alone and together can be tested for consistency with this special case, using a maximum likelihood technique which is essentially an extension of the standard technique of probit analysis (Finney, 1971; Plackett and Hewlett, 1963). Sawicki *et al.* (1962) showed that bioassay data for components of natural pyrethrum acting against houseflies was consistent with this special case of the model, the slopes of the probit regression lines for the individual components differing significantly in this case.

Further development of Hewlett and Plackett's model for joint action concerned interaction between the effects of jointly-applied toxicants (Hewlett and Plackett, 1964, Plackett and Hewlett, 1967).

The more complex categorisation of types of joint action proposed by Sakai (1960) is similarly based on suppositions about the various ways in which poisons might interact chemically, physiologically, and toxicologically at their target sites. However, methodologies by which the different types of interactions could be distinguished in practice were not proposed, and the system has not been used subsequently. The classification of Sakai does include the possibility of "pseudo joint action" corresponding to biological effects resulting from chemical reactions between the components of the mixture prior to reaching their respective sites of action (particularly those involving accelerated degradation), a possibility not explicitly stated in previous schemes and one which must be considered in any experiment in which the biological effects of two or more poisons applied simultaneously are analysed.

Ashford (1958, 1981) and Ashford and Smith (1964, 1965) have also derived models for interaction of drug mixtures. These are of a more general form than that of Plackett and Hewlett (1963) and cover both quantal and semi-quantal responses, single drugs acting at two or more sites of action, and the presence of two or more interacting physiological systems.

The Aims of This Project

The use of insecticide mixtures is currently of interest in stored product pest control because of the relatively few insecticides which can be used safely on stored grains and which are effective against current field strains of major pest species. Because of natural tolerance of some insect pests to individual insecticides, and because of increased tolerance to some insecticides by strains of some species, mixtures of products are more likely to achieve control of all pests at an economic and safe application rate than individual compounds. However, although mixtures have been developed for field use on an empirical basis, no methodology has been developed by which the composition and application rates of mixtures to control a given range of pests at and above some defined response level can be chosen on a rational basis.

The primary aim of this work was to develop such a methodology, and this is described in Chapter 4 and 5. If insecticide formulations can be shown to act additively, Plackett and Hewlett's model (1963, Model A) can be used to predict the response of a population of insects to any combination of two or more insecticides; linear programming can then be used to define optimal combinations of insecticides which give the cheapest control of all species at or above a defined response level.

Validation of this methodology was achieved using a number of combinations of insecticides and strains of pest species, including strains whose tolerance of individual insecticides had been greatly increased as a result of selection pressure. Dust bioassays were chosen partly on the basis of experimental convenience, but also because dust formulations are widely used for application to stored products, particularly in developing countries where small scale application is frequently practiced. However, some studies were also carried out using residual film bioassays, initially developed for resistance screening but also used widely as a convenient method of testing the response of test species to insecticides.

Since insecticides degrade following application to stored grain, the question arose as to whether a mixture which was optimised for control of a range of species at the point of application would also be the optimum mixture for control of the complex over the whole of an arbitrary storage period, given that insecticides degrade at different rates in the grain. To answer this question three insecticides were chosen on the basis of current and potential use, and the rates of degradation of both chemical residues and toxicity of those residues to two major storage pests was investigated. In seeking a mathematical relationship to describe the rate of decline of residues in the grain, it became apparent that current ideas of a simple first order breakdown process were inadequate to describe both the results obtained and also the majority of published results in this

field. An alternative model, involving diffusion of insecticide into regions of the grain where it was protected from breakdown in addition to a first order decay process, was developed. These results are described in Chapter 3.

In conclusion, an investigation was carried out into the production of progeny by adults of two species, *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) which survived a range of doses of the three insecticides chosen for residue studies. Insufficient data were obtained to extend the mixture optimisation algorithm to include this aspect of the action of insecticides on stored grain pests, and this remains an area which could be usefully explored in future work.

CHAPTER 2

Materials and Methods

2.1 Insecticides

Etrimfos (O-6-ethoxy-2-ethylpyrimidin-4-yl O,O-dimethyl phosphorothioate) was obtained from Sandoz Products Ltd., Ipswich as a technical product with an active ingredient content of 65% w/w and from Nickerson's Seeds Specialists, Kings Lynn as a dust formulation with a nominal active ingredient content of 2% w/w.

Malathion (diethyl (dimethoxythiophosphorylthioylthio)succinate) was obtained from Dow Chemical Co., Kings Lynn as a technical product with an active ingredient content of 94% w/w. A dust formulation of malathion ("Murphy Malathion Dust", no active ingredient content given) was purchased locally.

Pirimiphos methyl (O-2-diethylamino-6-methylpyrimidin-4-yl O,O-dimethyl phosphorothioate) was obtained from I.C.I. Plant Protection Division, Bracknell as a technical product with a purity of 96% v/v and as a dust formulation with a nominal active ingredient content of 2% w/w.

Deltamethrin ((S)-alpha-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromo-vinyl)-2,2-dimethylcyclopropanecarboxylate) was obtained from Wellcome Research, Berkhamsted as a technical product with a purity of 99% w/w. A dust formulation with an active ingredient content of 2% w/w was prepared using talc as carrier by the method of Section 2.2.

Gamma-HCH ((1,2,4,5/3,6)-hexachlorocyclohexane) was obtained from I.C.I. Plant Protection Division, Bracknell as a technical product and was purified by recrystallisation from hexane. It had a m.p. of 113° C. A dust formulation (2% w/w a.i.) was prepared using talc as carrier by the method of Section 2.2.

2.2 Preparation of Insecticide Dust Formulations on a Talc Carrier

Weighed samples of gamma-HCH or deltamethrin were dissolved in petroleum fraction (b.p. 60 - 80° C) and added to an amount of talc dust (BDH Ltd) sufficient to produce a 2% w/w dust formulation. The slurry was stirred and evaporated in a fume cupboard overnight. The dry dust formulations were stored in a glass stoppered bottle over silica gel in a freezer.

2.3 Preparation of Mixtures of Dust Formulations

Weighed samples of dust samples were combined in a glass bottle in a 1:1 w/w ratio and shaken thoroughly to ensure homogeneity. Binary mixtures were stored at -20°, and were allowed to defrost completely before opening the storage jar to weigh samples for bioassay experiments. The following mixtures of dust formulations were prepared:

malathion	+	etrimfos	(1:1 w/w)
malathion	+	pirimiphos methyl	(1:1 w/w)
malathion	+	gamma-HCH	(1:1 w/w)
malathion	+	deltamethrin	(1:1 w/w)
etrimfos	+	pirimiphos methyl	(1:1 w/w)
gamma-HCH	+	deltamethrin	(1:1 w/w)

2.4 Formulation Analysis

Determination of active ingredient concentration in dust samples was carried out by adding a weighed quantity of dust formulation to a 100 ml volumetric flask and adding methanol (Residue Analysis grade) to 100 ml. The flask was shaken, allowed to stand for 2 h at room temperature, reshaken, and 5 µl samples of the extract injected into the g.l.c. under

the conditions described in Sect. 2.9.2.1 Determinations were carried out in triplicate, with two sample injections carried out on each extract, and reference solutions of each insecticide injected between each sample injection.

2.5 Wheat for Bioassays and Insect Culture

Organically-grown whole wheat, free from pesticide residues, was obtained through a distributor from Doves Farm, Marlborough, Wiltshire. The wheat was sterilised prior to use in insect cultures by heating samples in glass jars for 1 h at 70° C. On cooling the wheat resorbed much of the water which had accumulated in the top part of the jar.

Moisture content of wheat samples was determined by the A.O.A.C. oven method (Horwitz, 1970). Samples (50 g) were ground in a coffee grinder for 30 - 45 s and transferred to aluminium dishes previously heated in an oven to $130 \pm 3^\circ \text{C}$, cooled in a desiccator and weighed. The weight of the wheat sample (c. 4 g) was determined, and the open dishes placed in an oven at $130 \pm 3^\circ \text{C}$ for 1 hour together with their covers. The dishes were then covered in the oven, placed in a desiccator to cool to room temperature, and then weighed. The difference in weight was expressed as a percentage of the initial sample weight. Four replicates of each sample were determined.

2.6 Insects

A susceptible strain of *Sitophilus granarius* was obtained from the MAFF Slough Laboratory. It was originally collected locally and has been maintained in the Slough Laboratory for about 10 years. It is susceptible to insecticides which have been tested against it (Prickett, 1980) and is referred to as the "Susceptible" strain.

A gamma-HCH resistant strain of *S. granarius* was collected from Lamia, Greece during the FAO global survey of insecticide resistance in stored product pests (1972-73) and its characteristics reported by Champ and Dyte (1976). The strain has been cultured at the Slough Laboratories since collection with intermittent application of selection pressure with gamma-HCH. A sub-culture of the strain was obtained from the Slough Laboratory in 1982, and selection pressure at the LC50 level applied over 25 generations to achieve an apparently homozygous resistant population (le Patourel and Salama, 1986). The culture was then maintained without further selection pressure during the present work. The strain is referred to as the "Gamma-HCH resistant" strain.

A susceptible strain of *Tribolium castaneum* was obtained from Silwood Park insectory. The culture has been maintained at Silwood for at least 20 years without exposure to insecticides, and is referred to as the

"Susceptible" strain.

A strain of *T. castaneum* with specific resistance to malathion and originating from Kano, Nigeria was obtained from Mr T.J. Binns, MAFF Laboratories, Slough in 1985. The characteristics of this specifically malathion resistant strain have been described (Dyte and Rowlands, 1968) and a resistance factor for malathion of 260 has been reported (Dyte and Blackman, 1972). The resistance factor of the Slough culture was reduced from 964 to 1 by co-administration of triphenyl phosphate in a residual film bioassay (Binns, 1986). It is referred to as the "Malathion-resistant" strain (Kano).

Susceptible strains of *Oryzaephilus surinamensis*, and *Oryzaephilus mercator* were obtained from the MAFF Slough Laboratories. The distinction between *O.surinamensis* and *O.mercator* is regarded as valid on the basis of breeding experiments, biological characteristics and food preference (Back and Cotton, 1926; Howe, 1953).

A field strain of *O. surinamensis* was obtained from Dr J.Muggleton, MAFF Slough Laboratories, in 1986. It arrived at Ellesmere Port in a container of currants from Solerno, Greece in 1985 and had been maintained at Slough under the reference number 8603. It is referred to as the "Greek" strain.

Susceptible strains of *Sitophilus oryzae* and *Rhyzopertha dominica* were obtained from Silwood Park Insectory. The susceptible strain of *Tribolium confusum* was from the MAFF Slough Laboratories insectory.

2.7 Culturing of Insect Strains

2.7.1 *Tribolium castaneum* and *Tribolium confusum*

Various methods of culturing *T. castaneum* and *T. confusum*, differing only slightly with respect to temperature and relative humidity, have been reported (Khalifa and Badawy, 1955; Howe, 1956b; Lloyd, 1973; Winks, 1973; Carter, 1975). The culture medium for both species used in the present work was a mixture of wheat middlings : whole meal wheat flour : dried yeast in the ratio 18 : 17 : 1 w/w. The medium and filter papers were sterilised by heating at 70° C for two hours. The bench top was wiped, dried and covered with paper towel to collect flour and other culture debris, and about 300 mixed adult *T. castaneum* or *T. confusum* from a previous culture introduced into 2.5 l sterilised glass culture jars containing approximately 450 g culture medium. The jars were sealed with filter paper and maintained at 25° C and 70% r.h. After 2 wks the adults were sieved out of the medium with a 25 mesh sieve, and the flour containing eggs and first instar larvae collected and resealed in a new jar together with some fresh medium. Culture jars were stored on trays of

liquid paraffin to prevent mite infestation. Emerging adults were used for bioassays, and were mixed sex populations 2 - 3 weeks old. The time from culture inoculation to use of adults for bioassay averaged 8 - 9 weeks, except in the case of the malathion-resistant strain which had a longer maturation time.

2.7.2 *Oryzaephilus surinamensis* and *Oryzaephilus mercator*

The effects of temperature, humidity, and breeding media on the development of *O. surinamensis* and *O. mercator* have been reported by Thomas and Shepard (1940); Howe (1953 and 1956a); Amos (1968); Amos and Dennler (1969); and Arbogast and Carthon (1971). Cultures have been reported to be susceptible to infestation by the protozoan *Nosema oryzaephili* n. sp (Burgess *et al.*, 1971). Culture of the insects was as for *T. castaneum*, using a medium of wheat middlings : whole meal wheat flour : rolled oats : dried yeast (18 : 17 : 5 : 1 w/w). Culture jars were painted with a broad band of fluon^R inside the jar above the level of the medium to prevent escape of the adult insects, and 4 strips of tissue paper (20 x 2 cm) placed inside the jar below the level of the fluon band to increase the surface area available for adult movement. Adult insects were sieved from the medium after 10 days, and fresh middlings and rolled oats mixed with the flour containing eggs and larvae. Cultures were maintained at 25° C and 70% r.h. and the period from culture inoculation to harvesting 2 - 3 week old adults for bioassay averaged 8 - 9 weeks.

2.7.3 *Rhyzopertha dominica*, *Sitophilus granarius* and *Sitophilus oryzae*

The effect of environmental factors on the development of *R. dominica* has been described by Potter (1935); Birch (1945); Howe (1950); Golebiowska (1962); and Khare and Agrawal (1963). Similarly, the development of *S. oryzae* and *S. granarius* has been described by Mathlein (1938); Ewer, 1945; Birch (1945); Richards (1947); Reddy (1950); Pant and Gupta (1959); Davey, (1965); Howe and Hole (1967); Russell (1968) and Singh *et al.* (1974).

The three insect species were all cultured on organically-grown whole wheat, using the techniques described in 2.5. A band of fluon was painted above the medium to prevent adults from escaping from the culture jars. After 21 days the adults were sieved out of the wheat using an 8 mesh sieve, discarded, and the wheat resealed inside the culture jars together with some fresh wheat.

The period from inoculation of the cultures to harvesting 2 - 3 week old adult beetles averaged 6 - 7 weeks for *Sitophilus* spp. and 9 - 10 weeks for *Rhyzopertha dominica*.

2.7.4 Determinations of mean body weights of populations of adult insects

Random samples of adult insects (50, mixed sex, 2-3 weeks from adult emergence) were taken from culture jars and weighed. At least 10 replicate determinations were used to find the mean body weight of the populations at this development stage. The mean weight within each genus are given in Appendix 2.1 and they were compared statistically by the multiple *F* test (Duncan, 1955).

2.8. Bioassays

2.8.1. Admixture of insecticidal dusts with whole wheat

Methods for mixing insecticidal dust formulations with whole wheat on a laboratory scale have been described by Watts and Berlin (1950), Wilber (1952) and Goodwin-Bailey and Holborn (1952), whose method was modified by Glynne-Jones and Green (1959) and used by Carter *et al.* (1975) to evaluate pyrethroid dusts. The method used in this work was the same as that described by Singh (1981) and Hose (1984).

Wheat samples (100 g) were weighed into 1 lb jam jars and equilibrated with a saturated solution of sodium chloride (75% r.h.) in an airtight plastic container at 25° C for 2 wks. Samples of insecticide dust

formulations were weighed onto aluminium foil using a calibrated micro-balance (Perkin-Elmer AD-2Z) giving four figure accuracy in the milligram range. The dust formulations were added to the wheat, the jars covered with a piece of Clingfilm[®] and a lid, and then tilted up and down (10 x) and rotated by hand for 2 minutes with brief periods of shaking at 15 s, 30 s, 45 s, 60 s, 90 s, and 120 s. The jars were kept closed for a few minutes after shaking to allow any free dust to settle.

2.8.2 Bioassay methods using dusts mixed with wheat

Adult insects 2-3 weeks from emergence were used for all bioassays. The beetles were removed from culture jars and randomly divided into populations of 50, then added to the treated wheat in the 1 lb jars and incubated in air-tight plastic containers over saturated sodium chloride solutions (75% r.h.) at 25° C for either 1, 2 or 7 days. The wheat was then tipped onto a tray with fluon-coated sides and the insects separated. They were categorised as alive or dead (brittle, and showing no movement over a 5 min observation period).

Probit(mortality)/log (dose) regression equations for the insecticides and their 1:1 w/w mixtures were calculated using the maximum likelihood algorithm described by Finney (1952) adapted as a BASIC computer program (le Patourel, unpublished). LC50 and LC95's and associated fiducial

limits were calculated by the methods described by Finney, and goodness of fit of the experimental data to the regression equation was estimated by Pearson's chi squared.

2.8.3 Residual Film Bioassays

The residual film bioassay technique has been described by Champ and Campbell-Brown (1970a) and (1970b) and is recommended by the FAO (1970) as a rapid screening method for insecticide resistance.

Technical insecticides were dissolved in a solvent mixture consisting of petroleum fraction (b.p. 60 - 80°C) : acetone : Risella EL (Shell Ltd) as a 3 : 1 : 1 v/v mixture. Whatman No 1 filter papers (9 cm dia) supported on drawing pins were impregnated with insecticide solution (0.85 ml) using a graduated 1 ml pipette. The papers were stored at room temperature overnight to allow the volatile solvents to evaporate and then placed on glass squares with a fluon-coated metal ring (5.2 cm dia.) covering them. 30 adult insects were released into the rings and maintained in a constant temperature room at 25°C and 70% r.h. for 48 hours. Mortality determination and statistical analysis of the data was as in Sect. 2.8.2

2.9 Degradation of malathion, etrimfos and pirimiphos methyl applied to wheat as dust formulations and stored at 25°C/14.9% m.c. or 30°C/10.1% m.c.

2.9.1 Admixture of dust formulations with wheat

Dust formulations of the insecticides mixed with wheat as described in Sect. 2.8.1. After admixture the jars were kept closed until the dust had settled, and then incubated in sealed plastic containers at either 25°C or 30°C. Application rates of formulations to wheat samples used for chemical analysis were: 500 mg/kg (etrimfos and pirimiphos methyl) and 400 mg/kg (malathion). These rates corresponded to 10 mg a.i./kg etrimfos, 10 mg a.i./kg pirimiphos methyl, and 16 mg a.i./kg malathion, calculated from the experimental a.i. content of the dust formulations. The wheat sample incubated at 25°C had an initial moisture content of 14.7% and a final moisture content of 15.0%, and that incubated at 30°C had an initial and final moisture content of 10.1%.

2.9.2 Residue analysis

2.9.2.1 *Analytical Procedure*

Extraction of the organophosphorus insecticides from the wheat samples was carried out by a modification of the method recommended by the Working Group of the Committee for Analytical Methods for Residues of Pesticides and Veterinary Products in Foodstuffs and the Working Party on Pesticide Residues of MAFF (Anon, 1973 and 1980). Wheat samples (100 g) were divided into two 50 g aliquots, each of which was then ground to flour for 20 seconds in a sample grinder. A sample (20 g) was taken from each flour batch and homogenised at high speed with 40 ml methanol (residue analysis grade, BDH) for 2 min. The homogenate was filtered through a celite support on a sintered glass Buchner funnel under partial vacuum, the residue being re-homogenised with two portions of methanol (2 x 30 ml) for 30 s, with re-filtration after each homogenisation. The volume of the combined filtrates was adjusted to 100 ml with methanol and 5 μ l samples injected into the gas liquid chromatograph without further clean-up. Reference solutions of each insecticide (in acetone) used to prepare calibration curves for the quantification of residue levels in the extracts.

Wheat extracts were stored at -20°C pending analysis.

Gas liquid chromatographic conditions:

Detector:	flame photometric
Column:	3% OV 225 on Chromosorb W HP (80-100 mesh) length 1 m and i.d. 4 mm.
Injection temperature	220° C
Column	180° C
Detector	350° C
Nitrogen (carrier)	40 ml/min

Under these conditions retention times were as follows:

etrimfos	2.3 min
pirimiphos methyl	4.0 min
malathion	7.0 min

2.9.2.2 Solvent effect on Detector Response

The possibility of different detector response to samples in methanol and acetone was tested by direct comparison of peak heights from standard samples of pirimiphos methyl prepared in methanol, acetone and methanol : acetone (1:1 v/v). The results, shown in Appendix 2.2 , indicate a small

difference in the response at a concentration (2 mg/l) which gave full scale deflection under the detector conditions used. No correction was applied to the results for this effect.

2.9.2.3 *Recovery of Insecticide and Storage Stability of Extracts*

Samples of wheat which had been maintained under the two storage conditions for a period of 2-3 weeks were treated with the three insecticides at the same application rates as were used for the residue experiments. The samples were then extracted with methanol as described above. Extracts were stored at -5°C, together with the reference solutions of the insecticides (in acetone), and both extracts and standards periodically analysed by g.l.c. in order to assess the efficiency of the procedure and the stability of the extracts on storage.

2.10 Bioassay of *S. granarius* and *R. dominica* on wheat treated with pirimiphos methyl, etrimfos and malathion dust formulations and stored at 25°C/14.9% m.c. or 30°C/10.1% m.c.

Wheat (14.9% m.c.) was treated with dust formulations of the insecticides at the following rates (mg a.i./kg wheat): malathion (8, 16, 17.1), pirimiphos methyl (5, 10, 13.6), and etrimfos (5, 6.3, 10). Mixtures (1:1

w/w) of dust formulations were similarly mixed at the following rates: etrimfos : malathion (3.1 : 6.2), pirimiphos methyl : malathion (4.7 : 9.4), etrimfos : pirimiphos methyl (3.7 : 3.7). Samples were stored in batches of 100 g at 25°C in sealed containers over saturated sodium chloride solutions.

Replicate samples of the wheat treated at the lowest application rate of single insecticides was bioassayed against *S. granarius* at time intervals varying from 7 to 14 days depending on the estimated rate of degradation of the insecticides. Bioassay was by the procedure described in Section 2.8.2 .

Wheat treated at the higher two application rates of pirimiphos methyl (13.6 mg a.i./kg) and malathion (17.1 mg a.i./kg), 6.3 mg a.i. /kg of etrimfos and also the previous described mixtures of insecticides were similarly bioassayed against *R. dominica* at intervals varying from 5 - 7 days for a period of 40 days. Bioassays were conducted at 25°C.

Samples of wheat (100 g, 10.1% m.c.) were treated with dust formulations of the insecticides at the following rates (mg a.i./kg wheat): malathion (8,16), pirimiphos methyl (5,10), etrimfos (5,10), and stored at 30°C in sealed containers over saturated potassium carbonate solutions. Replicate samples of the wheat treated at the higher concentration of each insecticide were bioassayed against *R. dominica* and

replicate samples of the wheat treated at the lower concentration bioassayed against *S. granarius* at intervals varying from 1 - 4 weeks for a period of 20 weeks. Bioassays were conducted at 30°C. All the batches were examined after 48 hr exposure.

2.11 Effects of etrimfos, malathion, and pirimiphos methyl dust formulations and their 1:1 mixtures on progeny production and feeding of *S. granarius* and *R. dominica*

LC5, LC10 and LC20 concentrations were interpolated from 7 day bioassay data for *S. granarius* and *R. dominica* when bioassayed in wheat treated with insecticidal dust formulations. Wheat was admixed with insecticidal dusts at these concentrations using the method of 2.8.1.

Two samples of 2-3 week old adults of each species were collected from culture jars and divided randomly into batches of 50 insects. These batches were weighed, added to insecticide-treated wheat or untreated wheat in 1 lb jam jars, and incubated for 7 days at 25°C and 70% r.h.. The adults were sieved from the wheat and the wheat samples incubated at 25°C in sealed containers over saturated sodium chloride solutions for 9 weeks.

Newly-emerged adults (live and dead) were separated from the wheat and counted, and wheat remaining in the culture jar weighed. Live adults were anaesthetised with diethyl ether, counted and weighed on a microbalance in batches of 10 insects.

2.12 Data Analysis

Probit analysis was performed using an implementation of the algorithm described by Finney (1952) written in BBC BASIC (le Patourel, unpublished).

Analysis of data from bioassays with mixtures of insecticides were tested for additivity of action using an implementation of the algorithm described by Plackett and Hewlett (1963) written in BBC BASIC (le Patourel, unpublished). A description of the program and a listing is given in the Appendix 4.1.

Linear programming was carried out using a commercial program "LINPROG", Unisoft Ltd., London.

CHAPTER 3

Degradation of Malathion, Pirimiphos methyl and Etrimfos on Wheat under Two Storage Conditions

3.1 Introduction

Insecticides are generally applied to wheat as aqueous emulsions or dusts. A survey carried out by MAFF in the UK in 1982 indicated that of those commercial grain stores which routinely treated incoming grain with insecticide, primiphos methyl was the compound most frequently used, and dust formulations were preferred to emulsions (Wilkin, 1985). Preferences for formulations and insecticides most probably reflect their local availability, frequency of application, existing machinery, and appreciation of the resistance status of pest species.

There have been suprisingly few studies on the decline of insecticide residues resulting from treatment with dust formulations of insecticides used for stored grain protection, given their widespread use. There are likewise few studies in which the relative efficacy of insecticidal dust and emulsion formulations have been directly assessed. There is some evidence from early work on pyrethrum that the insecticidal action of dust formulations lasts longer than that of emulsions (Floyd and Smith, 1953); comparisons between other formulation types (emulsions, solutions in

organic solvents, wettable powders, microcapsules) have generally showed no great difference between either residue decay or insecticidal effect over time (Strong *et al.*, 1961; LaHue and Kadoun, 1979). However, dust formulations differ from those in which the insecticide is applied in a liquid carrier in both the rate and extent to which the chemical is absorbed by the grain (Rowlands, 1971). The phenomenon of progressive biological inactivation of residues of a number of insecticides during storage (Hargreaves *et al.*, 1982; Samson *et al.* 1987), which is thought to result from progressive accumulation of insecticide in the bran fraction of the grain, indicates that while determination of chemical residues in wheat may be used to indicate whether or not a batch may be traded or consumed with safety, it does not necessarily indicate the toxicity of the residue to insect pests.

This Chapter describes an investigation into the decline of both chemical residues and insecticidal activity of three insecticides frequently used as dust formulations in stored grain.

Temperatures and grain moisture contents were chosen to simulate the warm moist conditions (25° C, 15% wheat moisture content) and hot dry conditions (30° C, 10% moisture content) found in the northern and southern regions of countries bordering the Southern Mediterranean. Application rates for insecticides were higher than those recommended in the U.K., to allow for an enhanced rate of degradation under these

conditions. The rates for etrimfos and pirimiphos methyl corresponded to the current MRL's of these compounds in whole wheat (Anon, 1982), while that of malathion was twice the MRL, this compound being known to undergo fairly rapid degradation on wheat.

3.2 Loss of active ingredient from wheat treated with malathion, pirimiphos methyl and etrimfos dust formulations

3.2.1 Active ingredient content of dust formulations of malathion, pirimiphos methyl, and etrimfos.

Determination of active ingredient content of the dust formulations by gas chromatography gave the following results: pirimiphos methyl 2.0%, etrimfos 2.0%, malathion 4.0%.

3.2.2 Stability of insecticides in methanolic extracts of wheat

The results of this experiment are shown in Figures 3.1 a and b. The extracts containing etrimfos and malathion showed no loss of insecticide when stored at -5° C for up to 20 weeks to within the error of determination, whereas there appeared to be some loss of pirimiphos methyl, amounting to about 10%, after this period of storage. The

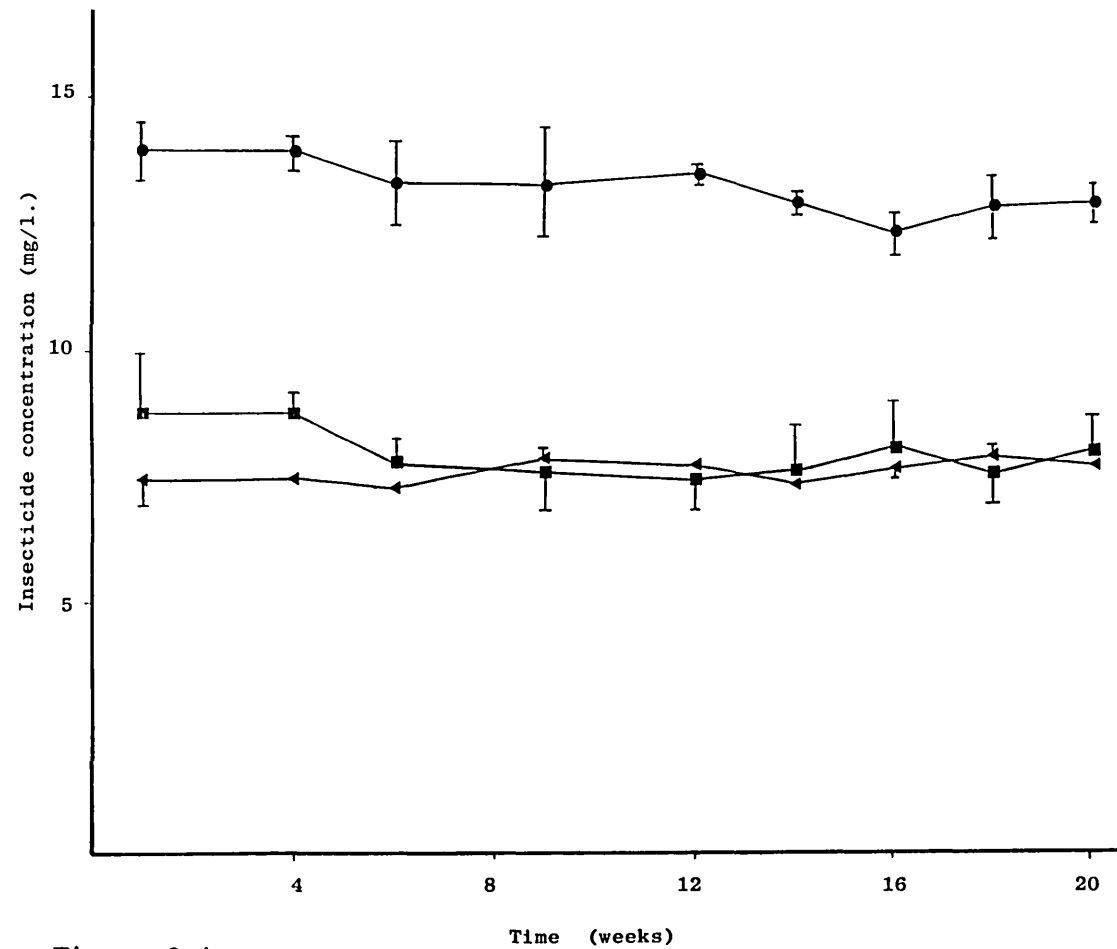


Figure 3.1a

Stability of insecticides in methanolic extracts of wheat (14.9% moisture content) stored at -5°C .

● malathion

■ pirimiphos-methyl

◄ etrimfos

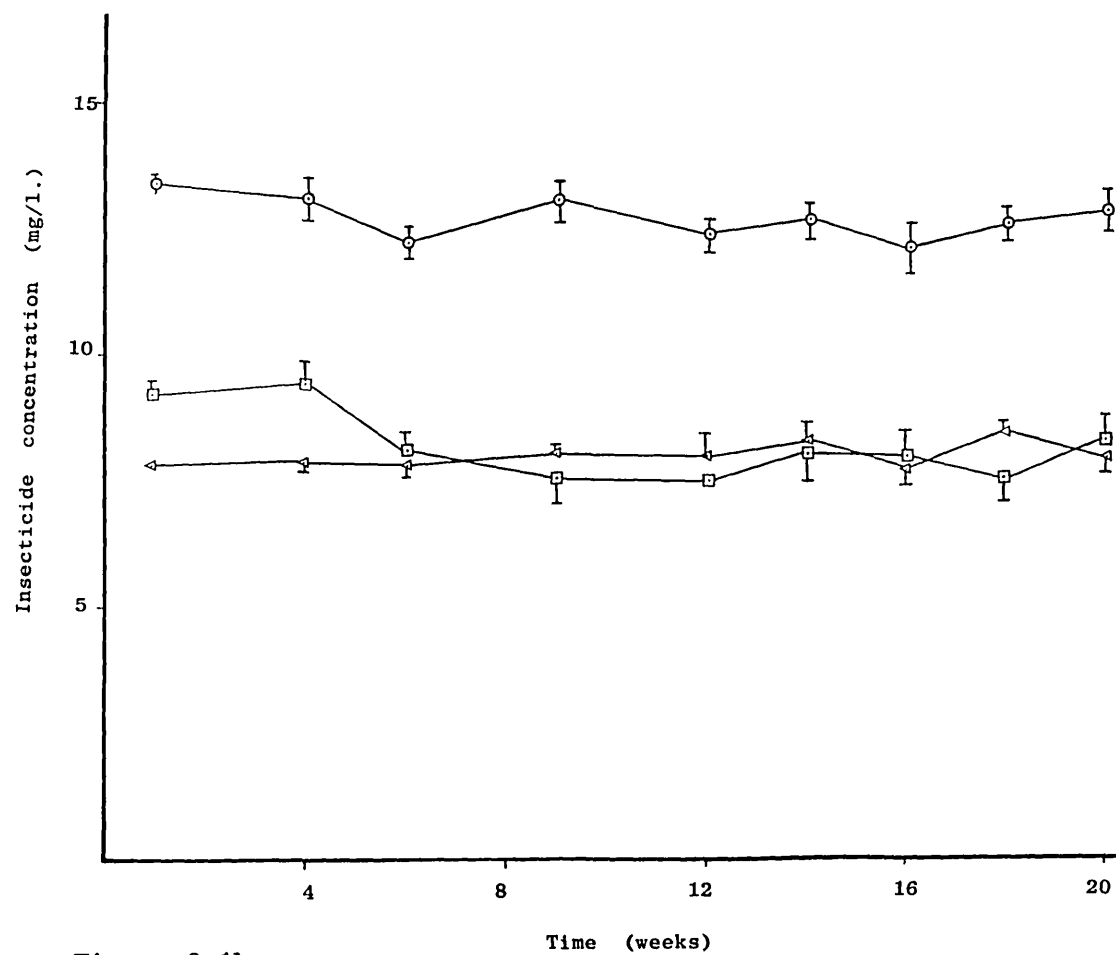


Figure 3.1b

Stability of insecticides in methanolic extracts of wheat (10.1% moisture content) stored at -5°C .

○ malathion

□ pirimiphos-methyl

△ etrimfos

analytical results are tabulated in Appendix 3.1. This data was obtained so that correction factors could be applied to analytical results from samples stored for periods up to 20 weeks as methanolic extracts, but in view of the results and the fact that no samples were kept for more than 5 days prior to analysis, no correction factors were used.

3.2.3 Recovery of insecticide residues from treated wheat

Mean percentage recoveries of insecticide from dust-treated wheat, calculated on the basis of the weight of formulation applied to the wheat were: pirimiphos methyl 90% (range 77 - 98%); etrimfos 76% (range 67 - 81%); malathion 85% (range 81 - 92%).

3.2.4 Loss of insecticides from treated wheat over 20 week storage period

Results of analyses of treated wheat samples for malathion, pirimiphos methyl and etrimfos under the two storage conditions are shown in Figures 3.2 a, b, and c. At 25° C and a wheat moisture content of 14.9% loss of malathion was rapid, residues declining to 5% of the initial concentration after 20 weeks, whereas at 30° C and a moisture content of 10.1% the stability of the insecticide was considerably greater, declining to 31% of the same initial concentration over the same period.

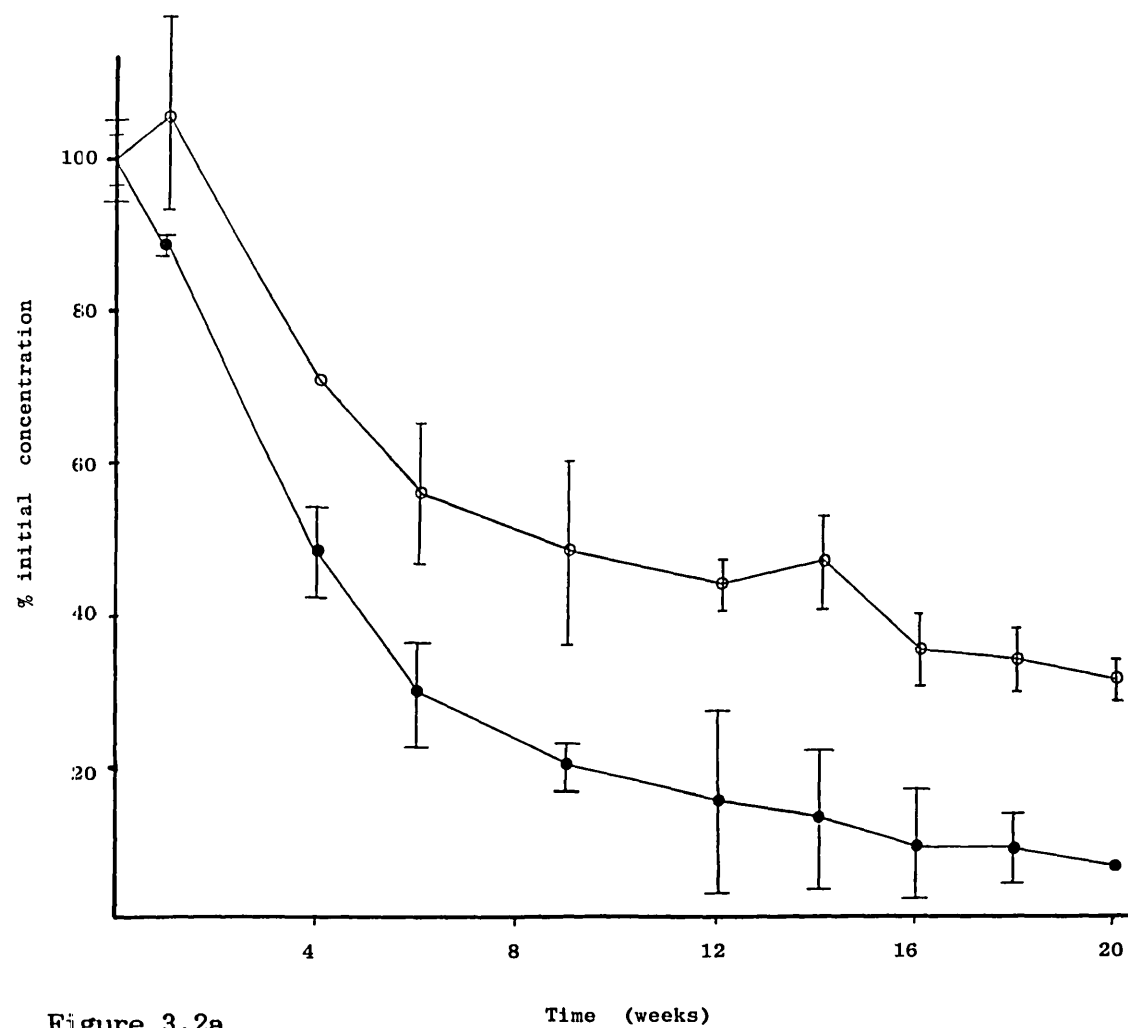


Figure 3.2a

Decline in extractable residues of malathion applied at 16 mg a.i/kg as a 4% w/w dust formulation to whole wheat

- storage temperature 30° C, wheat moisture content 10.1%
- storage temperature 25° C, wheat moisture content 14.9%

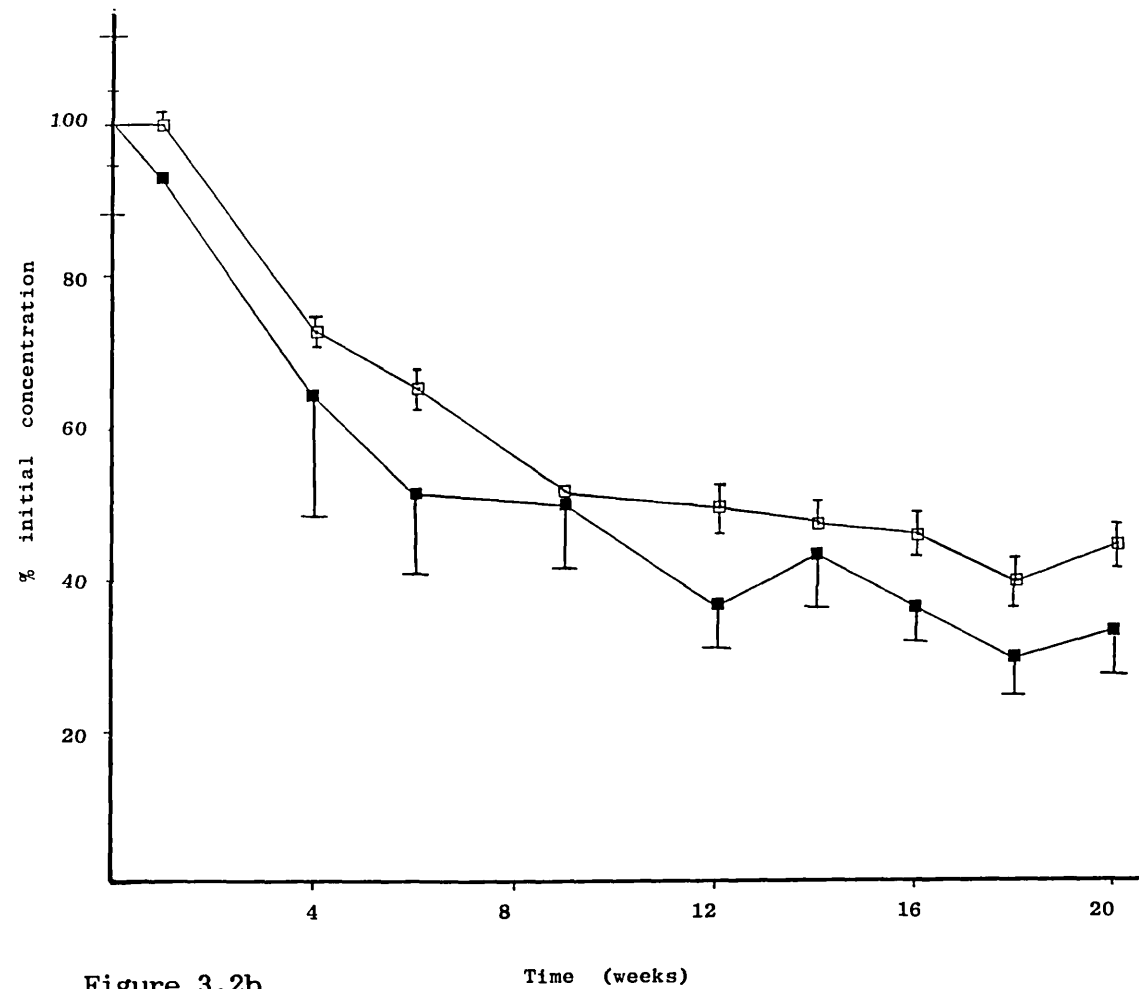


Figure 3.2b

Decline in extractable residues of pirimiphos methyl applied at 10 mg a.i/kg as a 2% w/w dust formulation to whole wheat

- storage temperature 30° C, wheat moisture content 10.1%
- storage temperature 25° C, wheat moisture content 14.9%

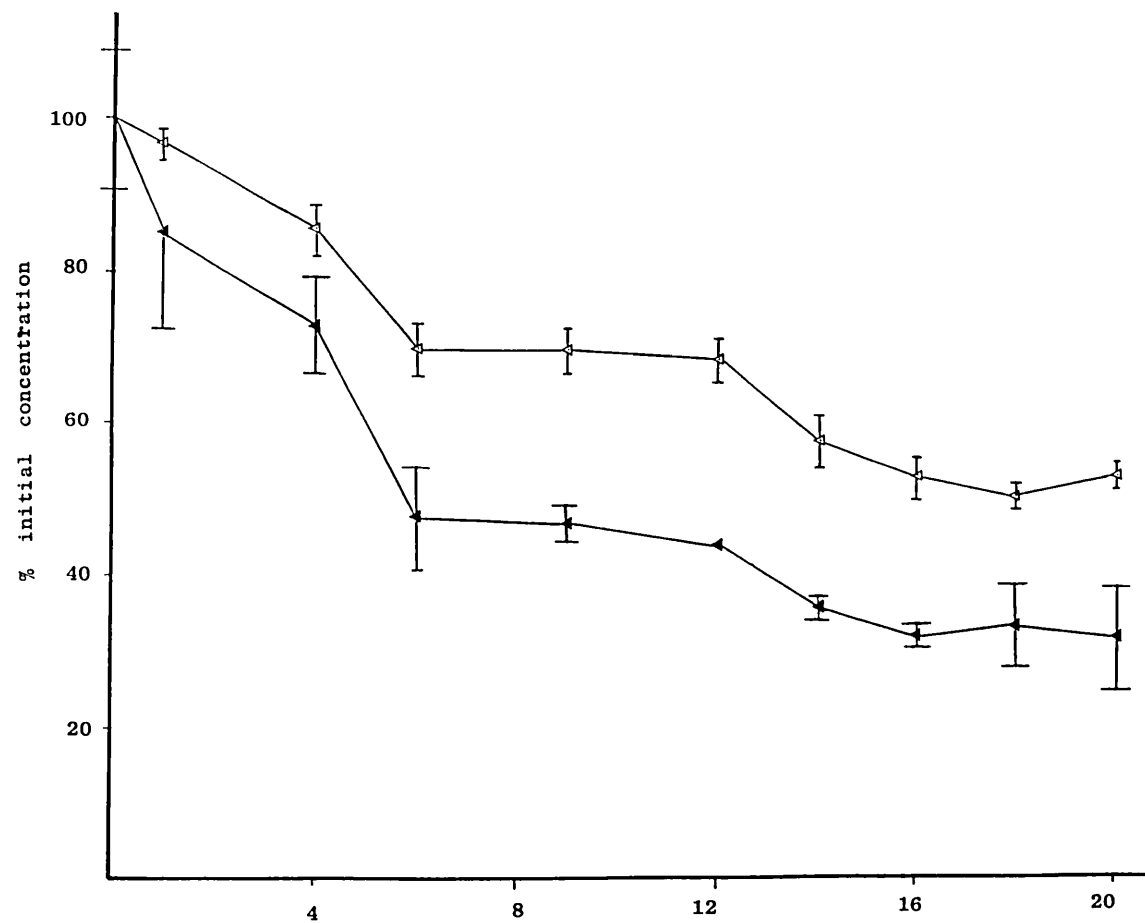


Figure 3.2c

Time (weeks)

Decline in extractable residues of etrimfos applied at 10 mg a.i./kg as a 2% w/w dust formulation to whole wheat

- ◁ storage temperature 30° C, wheat moisture content 10.1%
- ◼ storage temperature 25° C, wheat moisture content 14.9%

The stability of pirimiphos methyl showed less variation between the high and low humidity storage conditions, with 32% and 44% respectively of the initial concentration (10 mg a.i./kg calculated) remaining after 20 weeks.

Etrimfos, which was the most stable compound under either storage condition, declined to 53% of the initial concentration after 20 weeks at 30° C and 10.1% wheat moisture content, and to 31% after storage for the same time at 25° C and 14.9% m.c.

Analytical data for mean pesticide residues remaining at different times are tabulated in Appendix 3.2.

3.2.5 Loss of insecticides from treated wheat as a first order process

The general pattern of the degradation curves was similar to that reported for the decline of insecticide residues in wheat following application of insecticides as emulsions. Particularly under high temperature storage conditions when the wheat had a low moisture content it appeared that the residue concentration approached a limiting value after 20 week storage period. This effect was particularly noticable in the case of pirimiphos methyl and etrimfos under both storage conditions

and in the case of malathion stored at the higher temperature and lower moisture content.

The form of the decay curves was consistent with a two-compartment first order rate model in which it is assumed that the insecticide decays by first order kinetics but also diffuses into a conceptual compartment in which it is metabolically stable. The model is represented diagrammatically as follows:

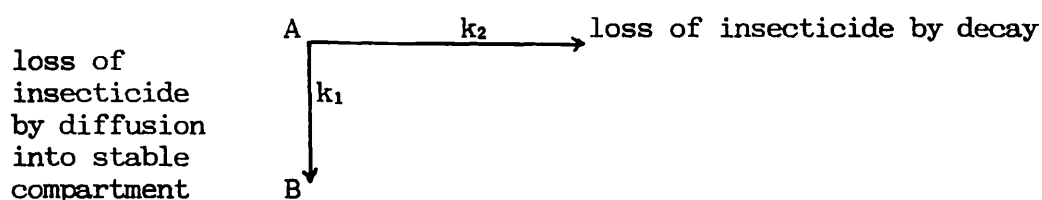


Figure 3.3

Here, k_1 and k_2 are first order rate constants for the diffusion and decay processes respectively. The residue determined in the grain represents the sum of the concentrations of insecticide in compartments A and B. $[A]$ is the concentration of residues in region where it is metabolised and $[B]$ is the concentration of residues in region where it is metabolically inert.

From the assumption of first order rate processes:

$$\frac{d[A]}{dt} = -(k_2 + k_1)[A] \qquad \frac{dB}{dt} = k_1 [A]$$

giving

$$\begin{aligned}
[A] &= [A_0] \exp[-(k_2 + k_1) t] \\
[B] &= \frac{[A_0] k_1 (1 - \exp[-(k_2 + k_1) t])}{(k_2 + k_1)} \\
[A+B] &= \frac{[A_0] (k_1 + k_2 \exp[-(k_2 + k_1) t])}{(k_2 + k_1)} \quad (i)
\end{aligned}$$

The data was fitted to equation (i) using a computer program designed to determine the least-squares best fit. Corresponding values of k_2 (first order rate constant for loss of insecticide by decay) and k_1 (first order rate constant for diffusion of insecticide into metabolically-stable areas of wheat) are shown in Table 3.1 together with the sum of the squares of the residuals for the fitted equation.

3.2.6 Comparison of one- and two-compartment models for decay of insecticides on stored grain - an analysis of published data.

The two compartment model is likely to show clear differences from the assumption of a simple first order decay process under conditions in which the rate constant for decay is of a similar magnitude to the rate constant for metabolic inactivation of the insecticide. Such conditions are likely to be those where low grain moisture content slows down the rate of decay of insecticide, and where an intermediate to high temperature permits ready diffusion of insecticide into metabolically inert regions of the grain seeds. A comparison of the goodness-of-fit of the the two

Table 3.1

Comparison of one- and two-compartment models for decay of malathion (4% w/w), pirimiphos methyl and etrimfos (2% w/w) dust formulations on wheat

insecticide	storage conditions		2-compartment model		sum of squares	1-compartment model	
	temp. (°C)	m.c. (%)	k ₁ (days ⁻¹)	k ₂ (days ⁻¹)		k ₁ (days ⁻¹)	sum of squares
malathion	25	14.9	0.00189	0.0272	1.154	0.0239	2.636
	30	10.1	0.00454	0.0125	5.083	0.00930	7.488
etrimfos	25	14.9	0.00668	0.0164	0.925	0.0106	2.755
	30	10.1	0.00530	0.00729	0.690	0.00538	1.041
pirimiphos methyl	25	14.9	0.00686	0.0158	0.945	0.0102	3.712
	30	10.1	0.00770	0.0127	1.182	0.00790	3.624

Table 3.2

Comparison of one- and two-compartment models for decay of insecticides on stored grain - an analysis of published data

grain (insecticide)	grain moisture content	temp. (°C)	2-compartment model		sum of squares	1-compartment model		reference
			k_2	k_1		k_2	sum of squares	
			(days ⁻¹)	(days ⁻¹)		(days ⁻¹)		
sorghum (mal.)	14	26.7	0.0122	0.00156	0.109	0.0105	0.764	1
sterilized sorghum (mal.)	14	26.7	0.00668	0.00278	0.0294	0.00482	1.264	1
wheat (mal.)	12.5	26.7	0.0134	0.00412	1.575	0.00705	5.051	2
wheat (chlor.)	12.5	26.7	0.00570	0.00147	0.120	0.00442	0.403	2
wheat (fen.)	12.5	26.7	0.00681	0.00154	0.173	0.00522	0.551	2
wheat (pir.)	12.5	26.7	0.00147	0.00593	0.762	0.000686	0.895	2
wheat (mal. dust)	12.5	26.7	0.00816	0.00182	0.477	0.00452	1.204	2
wheat (mal. dust)	12.5	26.7	0.00579	0.00159	0.122	0.00441	0.816	2
wheat (mal. dust)	12.5	26.7	0.00609	0.00190	0.174	0.00441	1.044	2
rice (pir.)	-	25-35	0.00775	0.00000	0.635	0.00775	0.635	3
rice (pir.)	-	25-35	0.00693	0.00000	1.082	0.00693	1.082	3
rice (pir. dust)	-	25-35	0.0147	0.00000	0.012	0.0147	0.012	3

Table 3.2 (Cont.)

Comparison of one- and two-compartment models for decay of insecticides on stored grain - an analysis of published data

grain (insecticide)	grain moisture content	temp. (°C)	2-compartment model		sum of squares	1-compartment model		reference
			k_2 (days ⁻¹)	k_1		k_2 (days ⁻¹)	sum of squares	
rice (pir. dust)	-	25-35	0.0115	0.00000	0.166	0.0115	0.166	3
rice (pir. dust)	-	25-35	0.00937	0.00000	0.926	0.00937	0.926	3
wheat (pir.)	12.5	26	0.00130	0.00204	0.051	0.000959	0.113	4
wheat (pir.)	12.5	26	0.00204	0.00305	0.783	0.00130	1.620	4
maize (mal.)	10	27	0.00497	0.00270	1.660	0.00339	3.543	5
maize (mal.)	12	27	0.00634	0.00258	2.409	0.00434	4.442	5
maize (mal.)	14	27	0.0167	0.00364	1.775	0.0114	4.587	5
maize (mal.)	16	27	0.00849	0.000855	0.167	0.00759	0.300	5
wheat (mal.)	10	27	0.00484	0.00387	0.776	0.00287	3.448	5
wheat (mal.)	12	27	0.00706	0.00224	1.054	0.00517	3.220	5
wheat (mal.)	14	27	0.0159	0.00319	2.299	0.0112	4.824	5
wheat (mal.)	16	27	0.0120	0.00127	0.380	0.0104	0.873	5

Table 3.2 (Cont.)

Comparison of one- and two-compartment models for decay of insecticides on stored grain - an analysis of published data

grain (insecticide)	grain moisture content	temp. (°C)	2-compartment model		sum of squares	1-compartment model		reference
			k_2 (days ⁻¹)	k_1 (days ⁻¹)		k_1 (days ⁻¹)	sum of squares	
wheat (mal.)	12	20-23	0.0103	0.00306	1.619	0.00829	2.077	6
wheat (mal.)	16	20-30	0.0175	0.00233	1.576	0.0152	1.977	6
wheat (mal.)	12	20-23	0.00617	0.000356	0.652	0.00600	0.662	6
wheat (mal.)	16	20-23	0.0107	0.000000	0.972	0.0107	0.972	6
wheat (pir.)	12	20-23	0.00247	0.000235	0.002	0.002	0.007	6
wheat (pir.)	16	20-23	0.00227	0.00000	0.045	0.00227	0.045	6
wheat (pir.)	12	20-23	0.00277	0.00180	0.045	0.00242	0.054	6
wheat (pir.)	16	20-23	0.00401	0.00787	0.078	0.00238	0.203	6
wheat (mal.)	11.7	20	0.00634	0.00000	2.011	0.00634	2.011	7
wheat (mal.)	11.7	30	0.0130	0.00000	2.441	0.0130	2.441	7
wheat (mal.)	16.8	20	0.0146	0.00000	1.906	0.0146	1.906	7
wheat (mal.)	16.8	30	0.0305	0.00000	0.747	0.0305	0.747	7

Table 3.2 (Cont.)

Comparison of one- and two-compartment models for decay of insecticides on stored grain - an analysis of published data

grain (insecticide)	grain moisture content	temp. (°C)	2-compartment model		sum of squares	1-compartment model		reference
			k_2 (days ⁻¹)	k_1		k_1 (days ⁻¹)	sum of squares	
wheat (fen.)	12.5	20	0.0116	0.00204	0.991	0.00855	3.263	8
wheat (fen.)	12.5	27	0.0195	0.00197	0.853	0.0168	2.402	8
maize (fen.)	14	25	0.00573	0.00549	0.693	0.00426	0.928	9
maize (fen.)	20	25	0.0215	0.00000	1.345	0.0215	1.345	9

- 1 Kadoun and LaHue (1972) 2 LaHue (1974) 3 Seth (1974)
 4 Kadoun et al. (1978) 5 Kadoun and LaHue (1979)
 6 Mensah and Watters (1979) 7 Watters and Mensah (1979)
 8 Abdel-Kader et al. (1982) 9 Samson et al. (1988)

mal. malathion
 fen. fenitrothion
 pir. pirimiphos-methyl
 chlor. chlorpyrifos-methyl

models to published data is shown in Table 3.2. Goodness-of-fit is measured by the minimised residual sum of squares of the deviations of predicted values of insecticide residues in the grain.

3.3 Loss of insecticidal activity from wheat treated with dust formulations of malathion, pirimiphos methyl and etrimfos

3.3.1 *Rhyzopertha dominica* as test species

3.3.1.1 Initial application rates of insecticides as for residue experiments

Jars containing wheat treated with malathion dust (16 mg a.i./kg), pirimiphos methyl dust (10 mg a.i./kg), or etrimfos (10 mg a.i./kg) were taken from the controlled temperature room at intervals over the storage period and residual insecticidal activity of the treatments determined by bioassay against *Rhyzopertha dominica*. Figures 3.4 a, b, and c show the loss of insecticidal activity with time for wheat stored at either 25° C and 14.9% moisture content, or at 30° C and 10.1% moisture content. Each data point is the mean of two replicate bioassays. Complete results for the bioassays are tabulated in Appendices 3.3 and 3.4.

There was a considerable difference between the persistence of

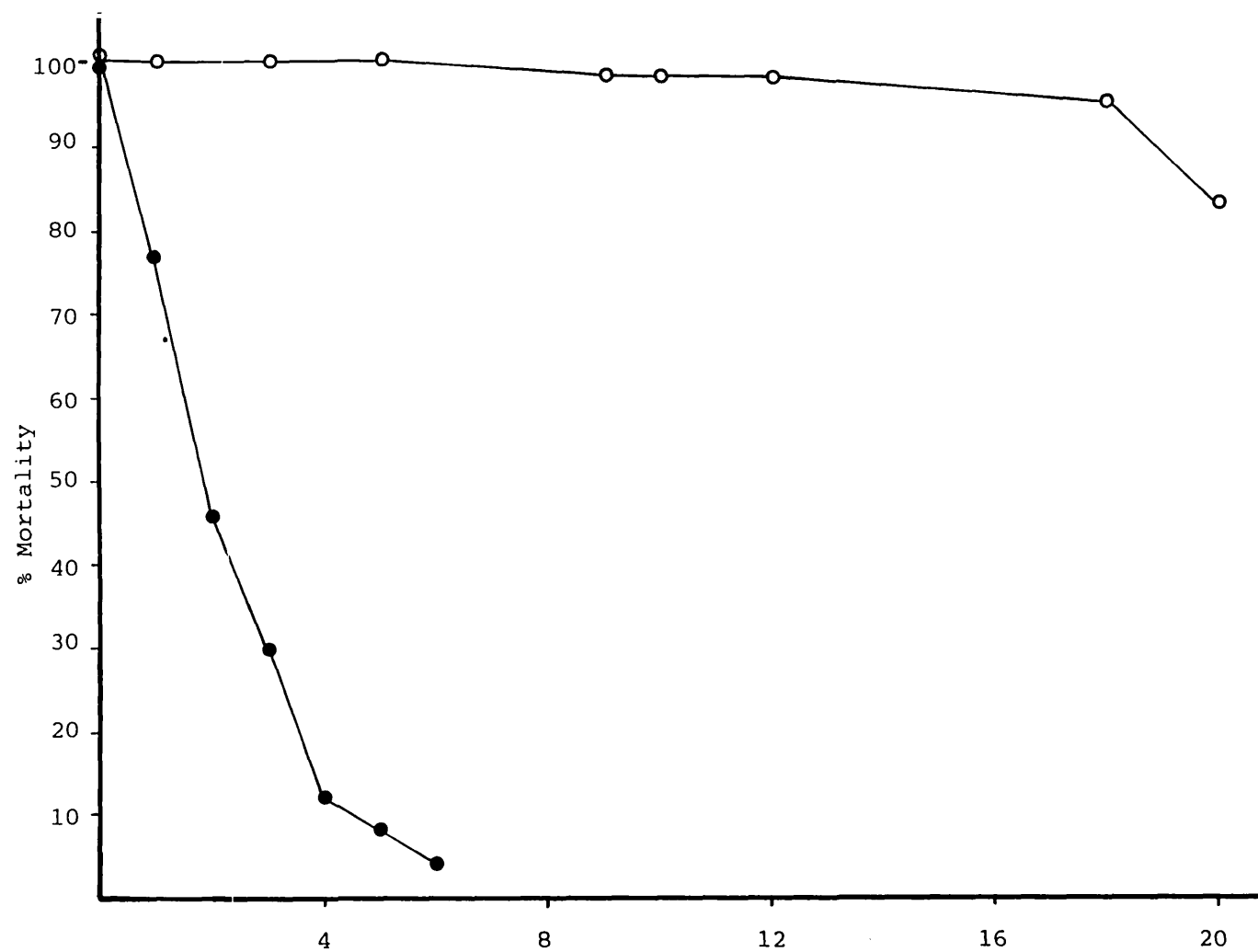


Figure 3.4a

Time (weeks)

Insecticidal activity of malathion applied at 16 mg a.i./kg to *R. dominica* in stored wheat

- storage temperature 30° C, wheat moisture content 10.1%
- storage temperature 25° C, wheat moisture content 14.9%

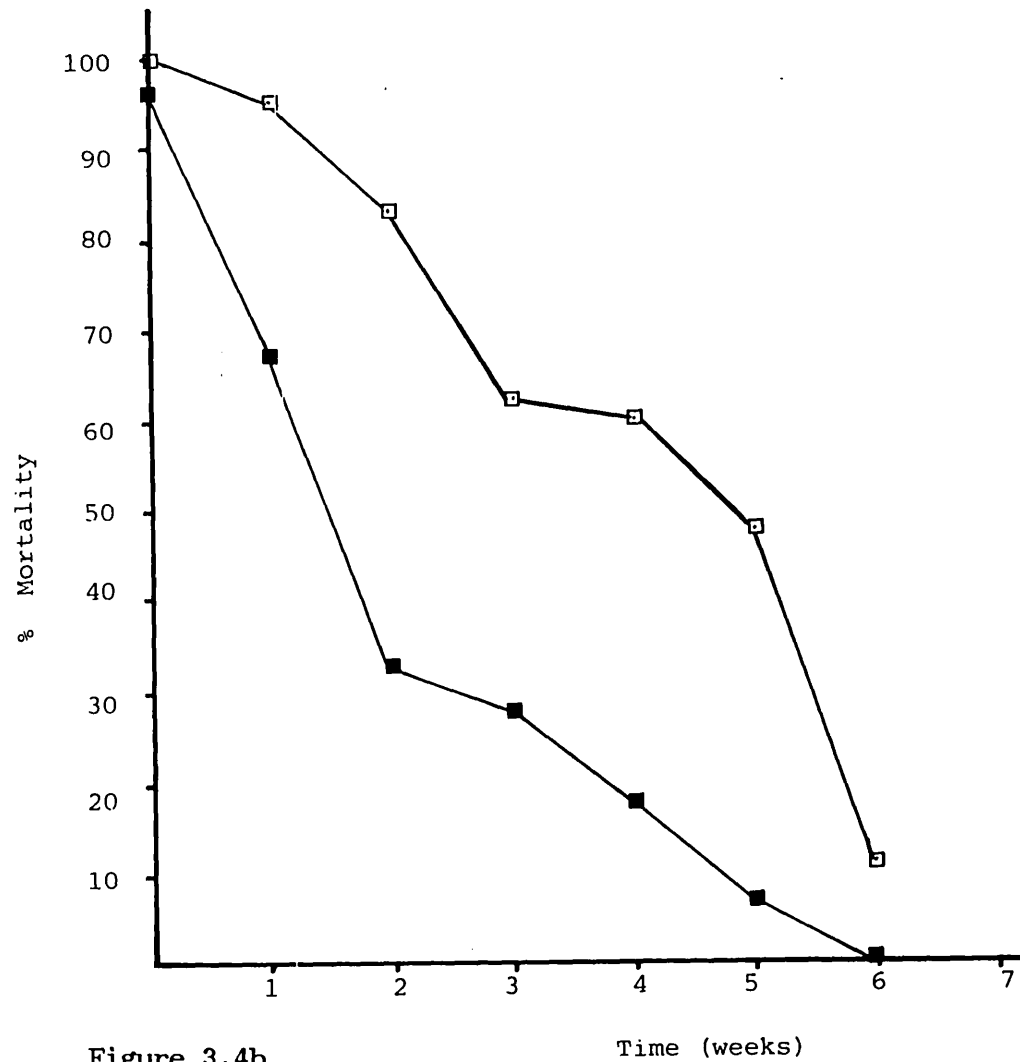


Figure 3.4b

Insecticidal activity of pirimiphos-methyl applied at 10 mg a.i./kg to *R. dominica* in stored wheat

- storage temperature 30° C, wheat moisture content 10.1%
- storage temperature 25° C, wheat moisture content 14.9%

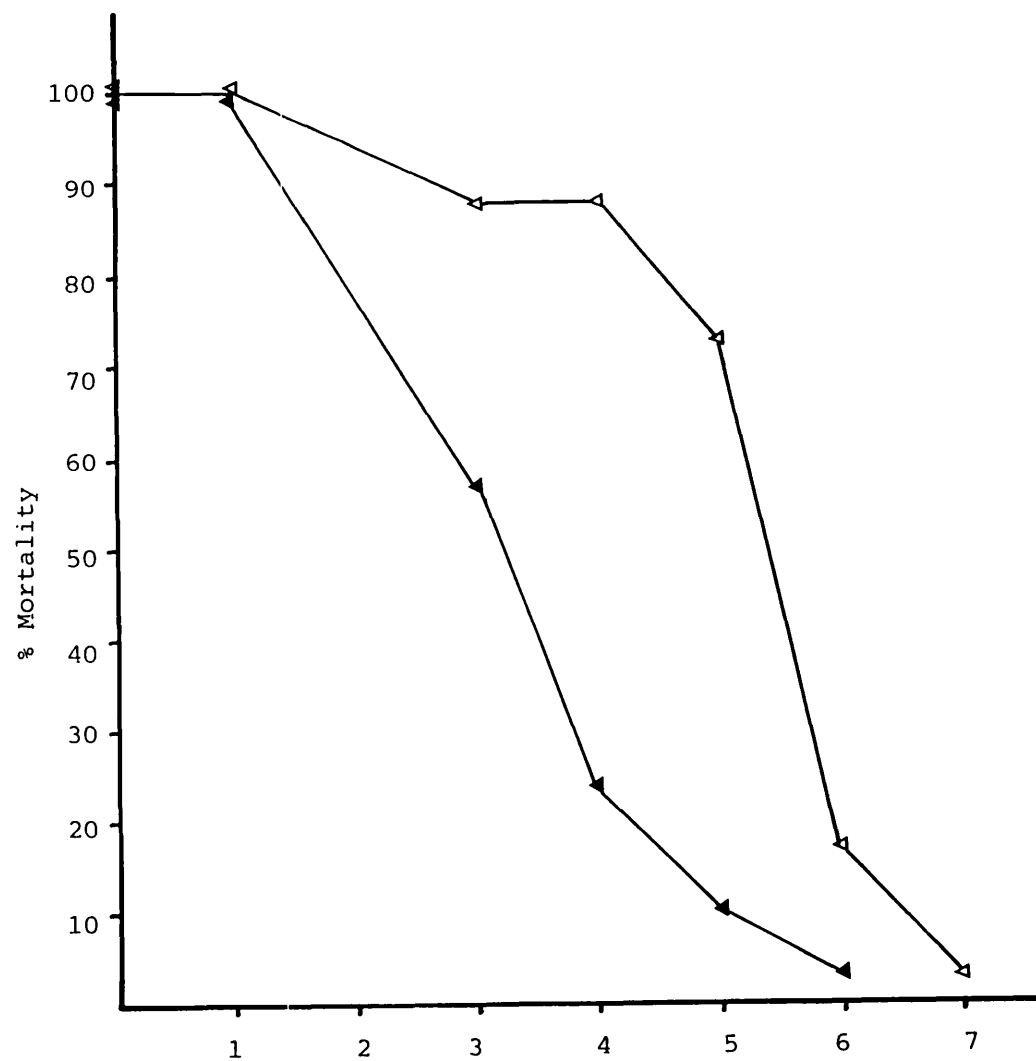


Figure 3.4c

Time (weeks)

Insecticidal activity of etrimfos applied at 10 mg a.i./kg to *R. dominica* in stored wheat

◁ storage temperature 30° C, wheat moisture content 10.1%

◀ storage temperature 25° C, wheat moisture content 14.9%

insecticidal activity of malathion dust on the treated wheat towards *R. dominica* under the two conditions of storage. At 30° C and a moisture content of 10.1% the mortality was greater than 95% in all bioassays up to and including 18 weeks post treatment. In contrast, the malathion dust applied to wheat stored at 25° C and with a moisture content of 14.9% showed a rapid loss of insecticidal activity, such that the bioassay conducted at 6 weeks post treatment gave a mortality of less than 5%.

Rapid loss of insecticidal activity was also found using *R. dominica* as test organism for both pirimiphos methyl and etrimfos dust treatments (both at 10 mg a.i./kg), and in both cases the lower temperature/high moisture content storage conditions resulted in a faster rate of loss of insecticidal activity. However, the differences between the persistence of activity under the two storage conditions was relatively small compared to the dramatic difference observed in the case of malathion. Mortality in the bioassays had declined to less than 10% after 6 weeks for both storage conditions in the case of pirimiphos methyl, and after 7 weeks under both storage conditions in the case of etrimfos.

The experiment with malathion was repeated, the results being tabulated in Appendix 3.4.

Log dose/probit response regression lines for *R. dominica* bioassayed against the three insecticides (48 hr bioassay) are shown in Appendices

3.5 a, b and c; these may be used to transform insect mortality between 0 and 100% to equivalent insecticide concentrations on the supposition that the insecticide was as available to the insects following wheat storage as when freshly applied.

3.3.1.2 Initial application rates calculated as values greater than LC99 for *R. dominica*

Wheat grain (14.9% m.c) treated with malathion 4% w/w dust (17.1 mg a.i/kg), pirimiphos methyl 2% w/w dust (13.6 mg/kg), etrimfos 2% w/w (6.3 mg/kg) and stored at 25°C was used to determine the residual insecticidal activity by bioassay against *R. dominica*. The treatment levels were chosen so as to give 100% mortality for insects bioassayed at the start of the storage period and corresponded to LC99.3 values for pirimiphos methyl, LC99.5 values etrimfos dusts, and an LC99.4 value for malathion dust (Tables 4.4 and 4.5). In terms of active ingredient to give >99% kill of *R. dominica* adults within 48 hr bioassay, the most efficient insecticide was etrimfos (6.3 mg a.i/kg) followed by pirimiphos methyl (13.6) and malathion (17.1)

The insecticidal activity of the treated wheat was bioassayed at 5 days intervals using 50 *R. dominica* adults (48 hr bioassay). Differences appeared in the performance of the insecticides or their mixtures in terms

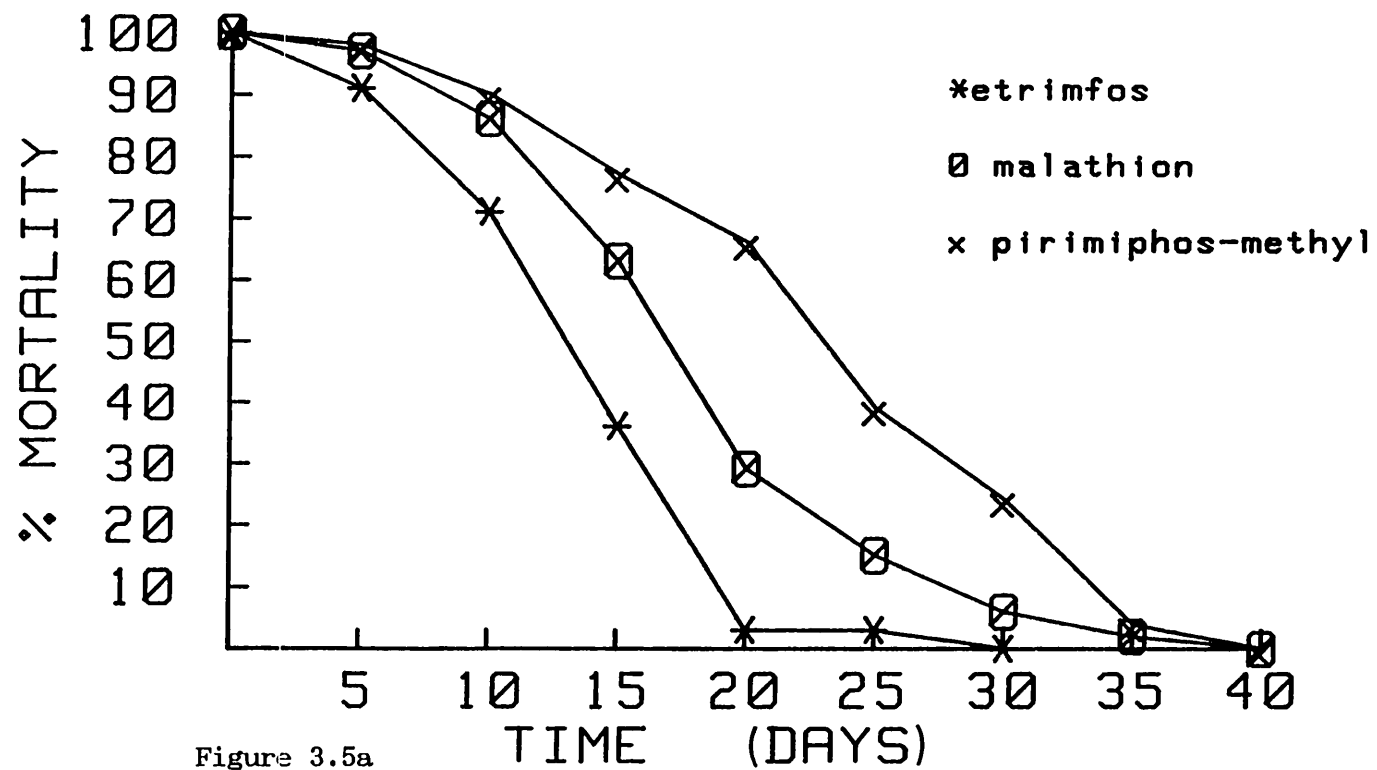


Figure 3.5a

Residual insecticidal activity to *R. dominica* of etrimfos applied at 6.3 mg a.i./kg, pirimiphos-methyl applied at 13.6 mg a.i./kg, and malathion applied at 17.1 mg a.i./kg on wheat (14.9% m.c.) and stored at 25°C over a 40 day period

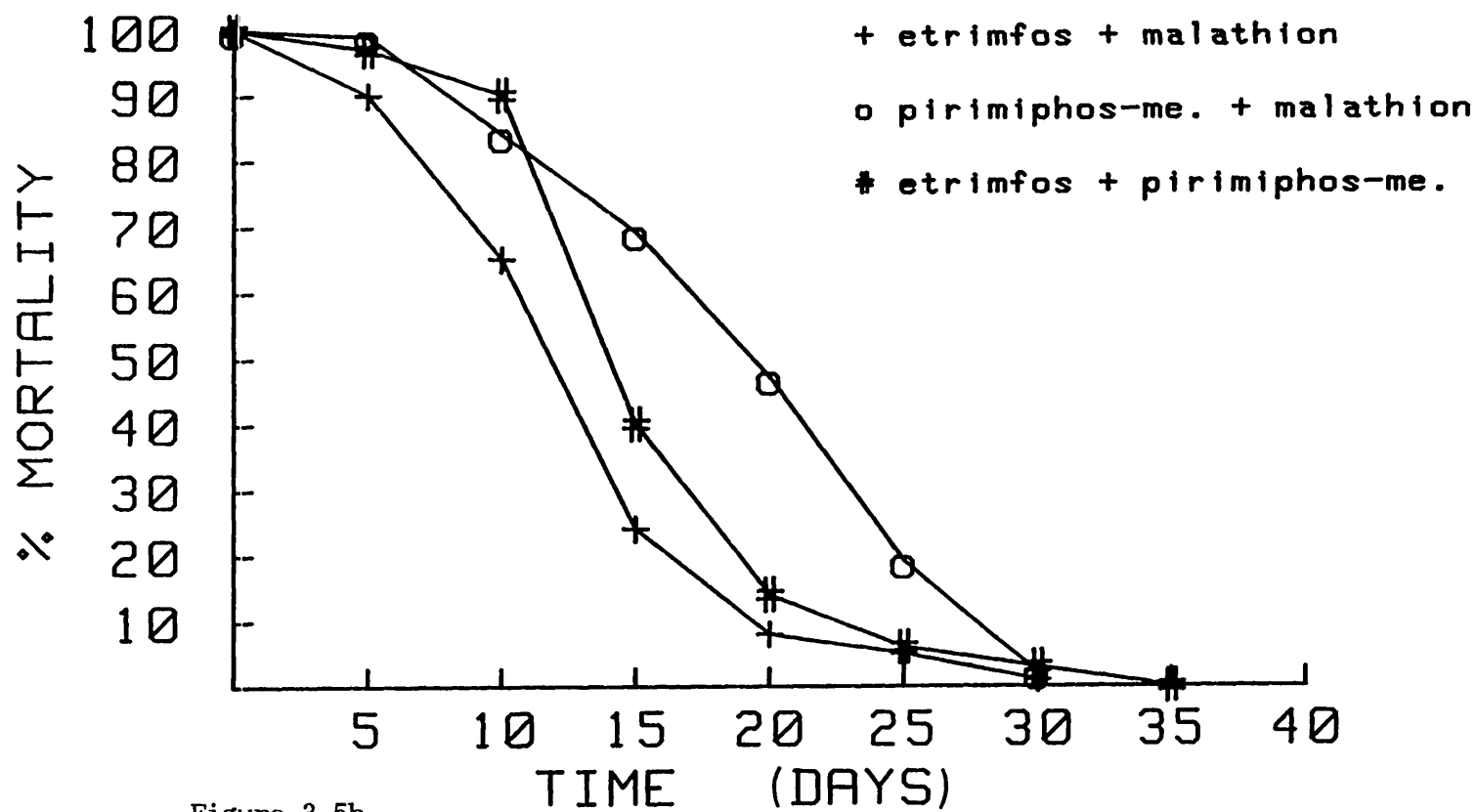


Figure 3.5b

Residual insecticidal activity to *R. dominica* of mixtures of etrimfos+malathion (3.1 + 6.2 mg a.i/kg), pirimiphos-methyl + malathion (4.7 + 9.4 mg a.i/kg) and etrimfos + pirimiphos-methyl (3.7 + 3.7 mg a.i/kg) on wheat (14.9% m.c.) and stored at 25°C over a 40 day period

of per cent kill of R. dominica adults. The results are shown in Figures 3.5 a and b; each data point is the mean of two replicates. Full bioassay data from this experiment are shown in Appendix 3.6. The experiments were terminated when bioassays gave less than 3% mortality.

Mortality decreased with the length of the storage period. All treatments admixed with high moisture content wheat (14.9%) and stored at 25° C declined rapidly and failed to give effective control of R. dominica or to persist more than 30 days. Etrinfos concentration applied to wheat was about one half of that of pirimiphos methyl and one third of that of malathion. However, etrimfos showed the same pattern of activity to *R. dominica* at this dose level as the high rates of the malathion and pirimiphos methyl.

3.3.2 *Sitophilus granarius* as test species

The greater sensitivity of *S. granarius* to the three insecticides permitted the determination of decline of insecticide activity with time to be carried out at initial application rates one half of those used for the experiments with *R. dominica*, viz. 8 mg a.i./kg for malathion and 5 mg a.i./kg for pirimiphos methyl and etrimfos. The variation of insecticidal activity of the treated wheat with time using *S. granarius* as bioassay organism is shown in Figures 3.6 a, b, and c for the three insecticides

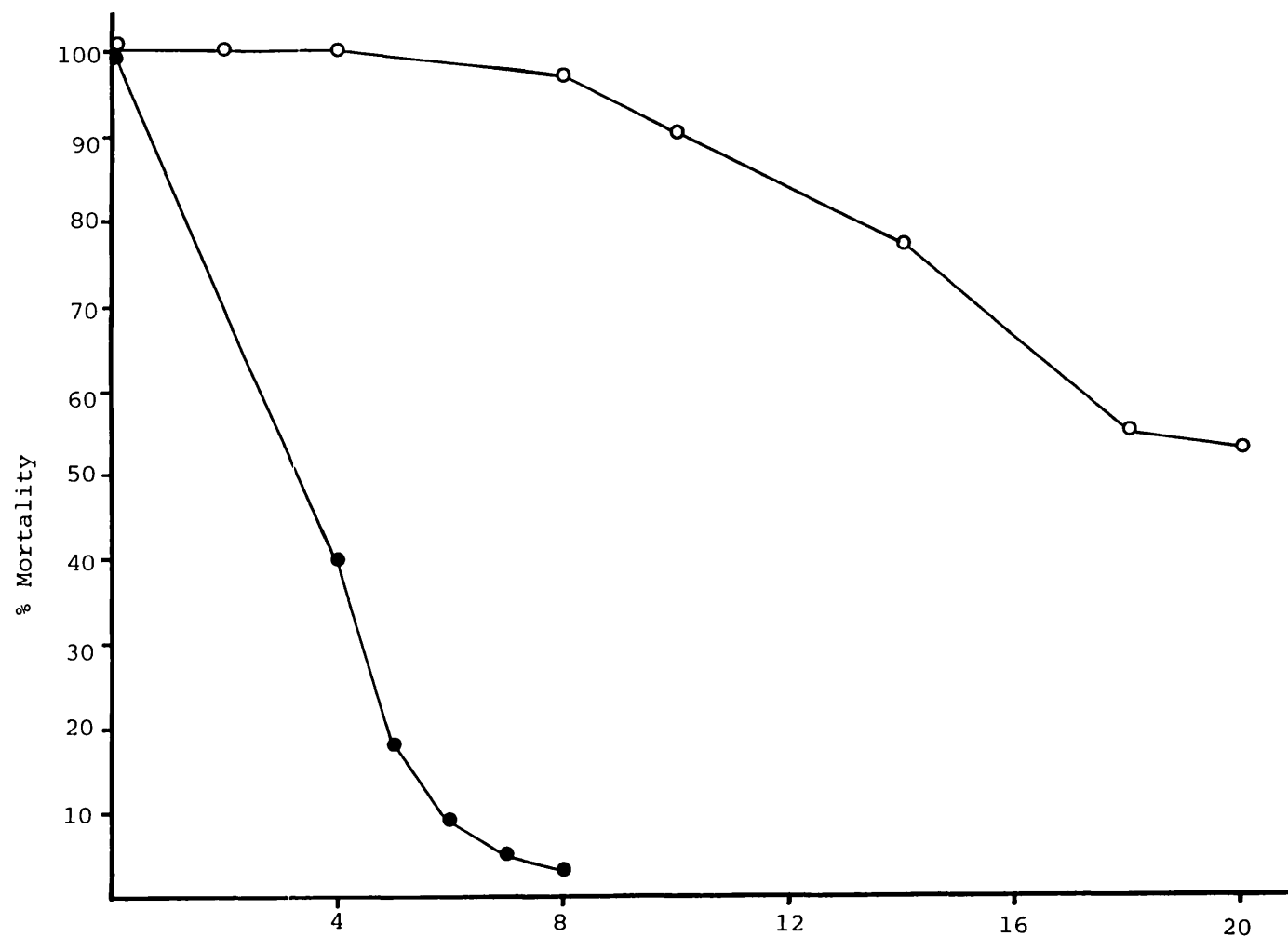


Figure 3.6a

Time (weeks)

Insecticidal activity of malathion applied at 8 mg a.i/kg to *S. granarius* in stored wheat

- storage temperature 30° C, wheat moisture content 10.1%
- storage temperature 25° C, wheat moisture content 14.9%

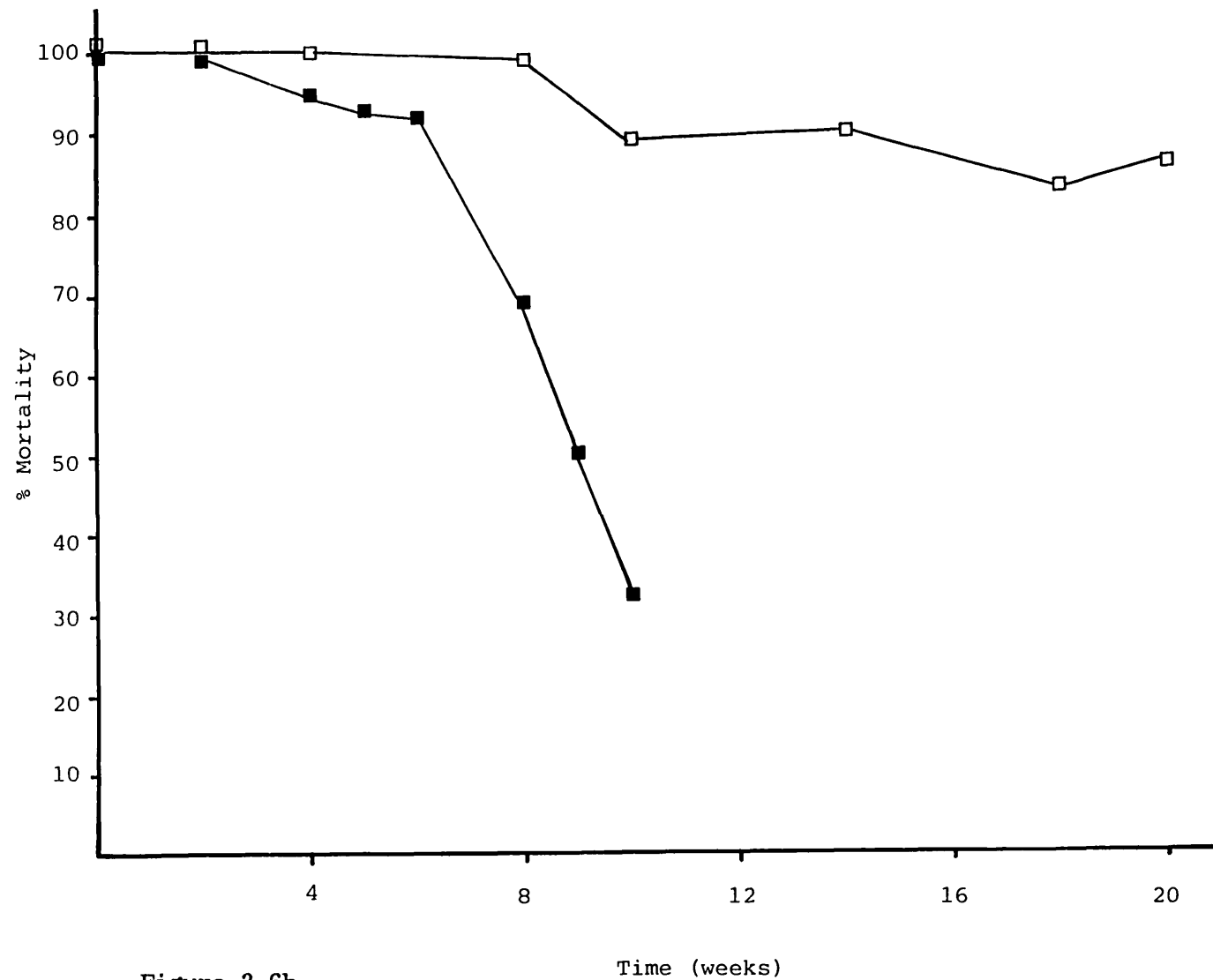


Figure 3.6b

Insecticidal activity of pirimiphos-methyl applied at 5 mg a.i/kg to *S. granarius* in stored wheat

- storage temperature 30° C, wheat moisture content 10.1%
- storage temperature 25° C, wheat moisture content 14.9%

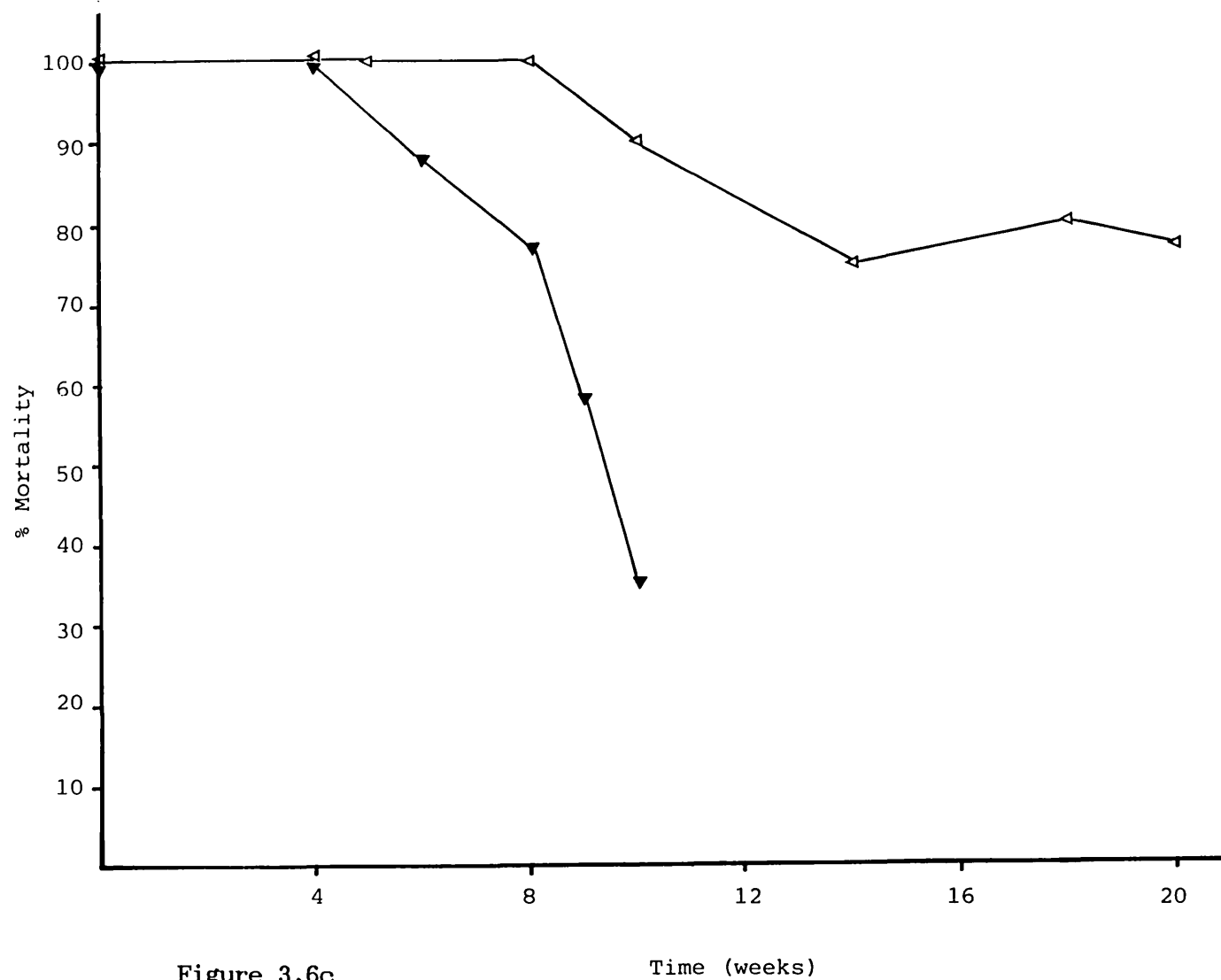


Figure 3.6c

Insecticidal activity of etrimfos applied at 5 mg a.i./kg to *S. granarius* in stored wheat

- ◁ storage temperature 30° C, wheat moisture content 10.1%
- ◀ storage temperature 25° C, wheat moisture content 14.9%

under the two storage conditions. Data points are the mean of two replicate bioassays; results from these experiments are tabulated in Appendices 3.7 and 3.8.

The rapid loss of insecticidal activity from the malathion-treated wheat stored at 25° C and with a moisture content of 14.9% was similar to that observed in the *R. dominica* bioassays when the wheat was treated at 16 mg a.i./kg. Insecticidal activity had virtually disappeared after 7 weeks storage. At the higher temperature and low wheat moisture content the wheat continued to give greater than 95% mortality in the bioassay up to 8 weeks post-treatment, and thereafter declined relatively slowly, a mean of 53% mortality being obtained at 20 weeks.

Pirimiphos methyl and etrimfos showed similar patterns of loss of insecticidal activity (Figures 3.6 b and c). In both cases a rapid decline in the bioassay mortality was observed after 6 weeks storage at 25° C with a wheat moisture content of 14.9%, whereas at the higher temperature and lower wheat moisture content the insecticidal activity was considerably more persistent. In both cases the experiment was terminated after 20 weeks, at which time the 48 h mortality of *S. granarius* was 86% in the case of pirimiphos methyl and 77% in the case of etrimfos at 30° C/10.1 m.c.

Bioassays were also carried out with *S. granarius* using wheat treated

at 16 mg a.i./kg (malathion dust) and 10 mg a.i./kg (pirimiphos methyl and etrimfos dusts) and stored for 20 weeks at either 25° C (14.9% m.c.) or 30° C (10.1%). The results of these bioassays are shown in Table 3.3. The loss of insecticidal activity in wheat with low moisture content stored at 30° C was not sufficient to discriminate between the three treatments over this storage period using *S. granarius* as test species, whereas a greater persistence of insecticidal activity in the pirimiphos methyl-treated wheat compared with that in wheat treated with malathion or etrimfos was evident in the wheat with a m.c. of 14.9% and stored at 25° C.

Log dose/probit response data and regression lines for *S. granarius* in wheat treated with the three insecticides are shown in Appendices 3.9 a, b, and c can be used to transform percentage mortality to insecticide concentration in the wheat under the assumption that the insecticide present was equally available to the insects at all times after treatment.

3.4 Discussion

Degradation of malathion applied as a dilute emulsion to stored wheat has been investigated as a function of temperature and moisture content by a number of workers (Strong and Sbur, 1960; Minnett *et al.*, 1968; Kadoum

Table 3.3

Average percentage mortality of *R. dominica* (50 adults) and *S. granarius* (50 adults) exposed to wheat treated with pirimiphos-methyl, malathion, and etrimfos dust formulations following 20 weeks storage (48 hour bioassay)

pesticide (dose mg a.i./kg)	conditions	species	
		<i>S. granarius</i>	<i>R. dominica</i>
etrimfos (10mg/kg)	25°C/14.9% m.c	16*	0
	30°C/10.1% m.c	100	1
malathion (16mg/kg)	25°C/14.9% m.c	0	0
	30°C/10.1% m.c	100	83
pirimiphos- methyl (10mg/kg)	25°C/14.9% m.c	91	0
	30°C/10.1% m.c	100	0

* Mean of 2 replicates

and LaHue, 1969; Kadoum *et al.*, 1978, Quinlan *et al.*, 1979, Kadoum and LaHue, 1979; ; Mensah and Watters, 1979; Watters and Mensah, 1979; Abdel-Kader *et al.*, 1980). Malathion is relatively unstable on wheat, especially as the moisture content increases to the range 14 - 16% and at temperatures above 20°. The storage conditions most closely resembling the ones reported in this work were those of Kadoum and LaHue (1979) for an unstated wheat variety, in which a malathion emulsion was applied at 10 mg/kg and wheat samples maintained at 27°C with moisture contents of 10 and 14%. These workers found 56 and 16% of the original malathion concentration after 24 weeks storage and the malathion degradation curves were qualitatively similar to those shown in Figure 3.2a.

Pirimiphos methyl degradation on stored wheat has not been as intensively studied as that of malathion. Kadoum *et al.* (1978) reported pirimiphos methyl residues in wheat and milled fractions of wheat following treatment with emulsions at 7.3 and 14.6 mg/kg and storage at 12.5% wheat moisture content and 26° C, and Mensah and Watters (1979) compared residues as a function of time in wheat of two different moisture contents (12% and 16%) (see also Mensah *et al.*, 1979). The stability of this insecticide when applied to wheat as an emulsion is reported to be greater than that of malathion, particularly at higher moisture contents, and the results of the current work with dust formulations are consistent with this conclusion.

Etrimfos is a comparatively recently introduced insecticide for stored grain protection, and the only published data relating to its decomposition on wheat is that of Thomas *et al.* (Thomas *et al.*, 1987). These workers compared the effectiveness of chlorpyrifos-methyl, etrimfos, fenitrothion and pirimiphos methyl emulsions when applied to wheat under bin storage conditions in the U.K. and at mean temperatures considerably lower than those reported in the present study. Residues of pirimiphos methyl and etrimfos were found to remain substantially constant and close to the initial application rate under these conditions throughout the 36 month storage period. However, the present study indicates that chemical residues of etrimfos decline at approximately the same rate as those of pirimiphos methyl when applied as dust formulations to wheat at both intermediate temperature /high moisture content and high temperature/low moisture content storage conditions.

The results of the present study indicate that residue decline curves for malathion and pirimiphos methyl applied as dusts follow the same general pattern as those previously reported for emulsion application. This is perhaps suprising as insecticides might not be expected to diffuse as rapidly to metabolically-active regions of wheat grains from mineral dust particles as when the insecticide is applied in an organic solvent directly onto the wheat surface, and suggests that much of the decay of the insecticides occurs prior to diffusion into the grain. The recommended application rate of 9 mg/kg for malathion dust to wheat in the U.K. may be

insufficient to provide a useful period of protection from insect attack under storage conditions found in the Southern Mediterranean countries, but the results reported here suggest that a dust application of twice this rate would decline to the current MRL of 8 mg/kg (whole wheat) within a period of about 16 weeks under the high temperature/low moisture content storage conditions, and in considerably less time for the intermediate temperature/high moisture content conditions.

There was little correlation between residue concentrations in wheat and the insecticidal activity of the residue to different insect species, partly because of the varied feeding habits of pests of stored wheat, and partly because residues can become increasingly inactivated during storage, a phenomenon thought to be due to diffusion of insecticide into grain fractions inaccessible to the insect (Desmarchelier, 1978b; Hargreaves *et al.*, 1982; Samson *et al.*, 1987, 1988). Consequently, the decay curves for malathion, pirimiphos methyl and etrimfos do not provide any indication of the relative period of protection against insect attack afforded by these treatments.

First order kinetics were used by Desmarchelier (1978b) to describe the decay of three insecticides in stored wheat and to relate the residues remaining at various times after treatment to the biological effect they produced. Desmarchelier and Bengston (1979) calculated first order rate constants for organophosphorus insecticides at a number of temperatures

and grain moisture contents. Other authors have adopted a similar approach to the loss of pyrethroids from stored grain (Hargreaves *et al.*, 1982; Noble and Hamilton, 1985).

However, examination of data in the published literature on decline of residues in grain does not lend general support to the concept of a simple, first order decay process, deviations being particularly marked in grain of low moisture contents. Under these conditions, the data of Kadoum *et al.* (1978), Kadoum and LaHue (1979) (malathion, maize, 27°C /10% m.c.) and Samson *et al.* (1988) (fenitrothion, maize, 25°C/14% m.c.) shows residues declining to an apparent limiting value, a situation which could be explained if the insecticide had become sequestered in some part of the grain where it was considerably more metabolically stable. The data of Rowlands (1971) and others suggests that this region might be the pericarp of the grain seed. The first order rate model developed in this section incorporates two concurrent processes: the metabolic degradation of the insecticide and its diffusion into an inert region of the grain. The estimated residue extracted from the wheat at any time represents the sum of the residues in the inert region and elsewhere. The fit of the data obtained in this work for residue decay of malathion, etrimfos and pirimiphos methyl to this model is closer than to a simple first order decay model, as is all the published data examined. The two-compartment model includes the simple first order decay model as a limiting case when the rate of diffusion into the inert compartment is set equal to zero.

Although the two compartment model may be more useful predictively, there is no obvious method by which to estimate the values of the two kinetic constants independently. The least squares method adopted in this work varies both parameters within a "grid" of points and identifies the pair giving the best fit to the data. The "mesh" of the grid is then decreased and the process repeated until sufficient accuracy has been achieved.

CHAPTER 4

Additivity of Action of Mixtures of Some Insecticides Used for Stored Product Pest Control : The Use of Plackett and Hewlett's Model for Joint Toxic Action

4.1 Introduction

Mixtures of insecticides may be useful for the protection of stored grain against insect attack when one pest species is either resistant or naturally tolerant to an insecticide which effectively controls other species. The tolerance of *R. dominica* to organophosphorus insecticides approved for grain mixture in Australia has resulted in recommendations for grain treatment using mixtures of certain organophosphorus compounds with bioresmethrin, pyrethrins or carbaryl (Anon, 1985). The use of insecticide mixtures is likely to increase in importance as a result of the limited number of compounds approved for grain admixture and the growing number of populations resistant to one or more of them.

Considerations of cost and of residues of insecticides in grain after storage indicate that the minimum amount of each component of a mixture should be applied to provide adequate control of all potential pest species over the proposed storage period. The composition and application

rate of mixtures of insecticides which will produce a particular response in populations of each of a number of pest species under laboratory conditions can be calculated from a knowledge of the dose/response relationship of the species to each insecticide together with a model describing the way the toxicities of the components interact.

This Chapter investigates the use of a particular model to describe the interaction of toxicities of dust formulations of three insecticide formulations used to control stored product pests following wheat admixture.

4.2 The additive model for joint toxic action of insecticides

Hewlett and Plackett (1959) proposed equation (i) to describe the response of a population of organisms to a mixture of two insecticides when their tolerance to the two chemicals is completely and positively correlated, the amount of either chemical reaching the target site is proportional to the dose applied, and the insecticidal action is similar

$$10(y_1 - y)/b_1 + 10(y_2 - y)/b_2 = 1 \quad (i)$$

Here, y_1 and y_2 are the normal equivalent deviates (n.e.d.) of the responses of the population to doses of two toxicants applied separately,

y is the n.e.d. of the response of the population to the same toxicants applied simultaneously as a mixture, and b_1 and b_2 are the slopes of the n.e.d. (response)/log dose regression lines for toxicants 1 and 2 (corresponding to the reciprocal of the variance of the log(tolerance) distribution of the populations to toxicant 1 and 2).

Equation (i) can obviously be extended to mixtures of three or more chemicals.

If the regression equations for the response of the population to toxicants 1 and 2 are written

$$y_i = a_i + b_i \log x_i \quad (i = 1, 2) \quad (ii)$$

then (i) can be written in the form

$$k_1 x_1 + k_2 x_2 = 1 \quad (iii)$$

$$\text{where } k_i = 10^{(a_i - y)/b_i} \quad (i = 1, 2) \quad (iv)$$

for the i^{th} toxicant at a dose producing a response with an n.e.d of y .

The form of equation (iii) makes clear the linear relation between pairs of doses x_1 and x_2 of toxicants which combine to produce a particular response in the target organism.

4.3 Testing bioassay data for consistency with the additive joint action model

Plackett and Hewlett (1963) provided methods for testing bioassay data obtained by applying two insecticides singly and jointly to a test species for consistency with the above model for joint action. Two limiting cases were analysed in their paper: (a) the variance of the log (tolerance) distribution for the test species was the same for both insecticides, implying that the slopes of the $\log(\text{dose})/\text{n.e.d.}(\text{response})$ lines to the insecticides were parallel, and (b) the variances were not identical (non-parallel $\log(\text{dose})/\text{n.e.d. response}$ lines). In the present work, only case (b) was considered, since there was no reason to suppose that the variances of the log (tolerance) distributions for any of the species considered would be identical, even for insecticides of the same structural type. The pharmacokinetics of penetration, metabolism, storage, and other modifiers of insecticide toxicity can differ widely even for compounds which are closely chemically related.

Estimates of the $\log(\text{dose})/\text{n.e.d.}(\text{response})$ parameters a_i and b_i for individual insecticide dust formulations applied to populations of a species of insect were obtained using a standard implementation of the probit method (Finney, 1971). The goodness of fit of the data to the regression line was tested by a chi-square test with $n-2$ degrees of

freedom, where n data points were included in the analysis.

Bioassays using individual insecticides and their 1:1 w/w mixtures were generally run concurrently, using samples of insects allotted randomly to the three bioassays from one or two culture jars.

Data from these bioassays were input into the computer program MIX, based on the maximum likelihood method of determining the parameters a_1 , b_1 , and a_2 , b_2 described by Plackett and Hewlett (1963).

This is done by making initial maximum likelihood estimates α_1 , β_1 , α_2 , β_2 for the insecticides applied alone, and then deriving corrections for these estimates.

From equation (i)

$$\frac{dy}{da_1} = \frac{b_2}{(b_2 - b_1 + b_1 \cdot 10^{(y-y_1)/b_2})}$$

$$\frac{dy}{db_1} = \frac{dy}{da_1} (y - a_1) / b_1$$

with similar equations for dy/da_2 and dy/db_2 .

The corrections to the parameters are then given by equations comparable to equations (II, 13) of Finney (1952, p 250), namely

$$\begin{aligned} \delta\alpha_1 \left\{ \sum_1 W + \sum_3 W \left(\frac{\partial x}{\partial \alpha_1} \right)^2 \right\} + \delta\beta_1 \left\{ \sum_1 W \log z_1 + \sum_3 W \left(\frac{\partial x}{\partial \alpha_1} \right) \left(\frac{\partial x}{\partial \beta_1} \right) \right\} \\ = \sum_1 W \left(\frac{p^* - p}{Z} \right) + \sum_3 W \left(\frac{p^* - p}{Z} \right) \left(\frac{\partial x}{\partial \alpha_1} \right), \\ \delta\alpha_1 \left\{ \sum_1 W \log z_1 + \sum_3 W \left(\frac{\partial x}{\partial \alpha_1} \right) \left(\frac{\partial x}{\partial \beta_1} \right) \right\} \\ + \delta\beta_1 \left\{ \sum_1 W (\log z_1)^2 + \sum_3 W \left(\frac{\partial x}{\partial \beta_1} \right)^2 \right\} \\ = \sum_1 W \left(\frac{p^* - p}{Z} \right) \log z_1 + \sum_3 W \left(\frac{p^* - p}{Z} \right) \left(\frac{\partial x}{\partial \beta_1} \right), \end{aligned}$$

A computer program MIX was written to iteratively determine corrections to the parameters using these equations until all four corrections $\delta\alpha_1$, $\delta\alpha_2$, $\delta\beta_1$, $\delta\beta_2$ are numerically equal to or less than 0.001.

The expected response (y) to an insecticide mixture consisting of the pair of doses x_1 and x_2 of insecticides 1 and 2 is calculated in the program from the current estimates of the parameters a_1 , b_1 , a_2 , and b_2 using a bisection routine to locate the root of

$$1 - 10^{(y_1 - y)/b_1} - 10^{(y_2 - y)/b_2} = 0 \quad (v)$$

in place of the linear interpolation technique described by Plackett and

Hewlett (1963).

The overall fit of the experimental data to the two regression lines is tested by calculating the chi squared with $n-4$ degrees of freedom where n is the number of data points included in the analysis, a non-significant result being taken as not contradicting the hypothesis that the data fitted the model for similar joint action with complete correlation of tolerances.

A description and listing of the MIX program, written in BBC BASIC, is given in Appendix 4.1.

4.4 Calculation of LC50 and LC95 for a 1:1 w/w mixture of toxicants from the best fit regression parameters for the individual insecticides

For 1:1 mixtures of toxicants 1 and 2, equation (iii) gives

$$x_1 = x_2 = (k_1 + k_2)^{-1} \quad (\text{vi})$$

Doses x_1 and x_2 which combine to produce a particular response can be calculated by substituting the appropriate value of y into equation (v).

Thus, for an LD50, $y = 0$ and

$$x_1 = x_2 = (10^{a_1/b_1} + 10^{a_2/b_2})^{-1} = (LC50(1) + LC50(2))^{-1}$$

and for an LC95, $y = 1.645$ and

$$\begin{aligned} x_1 = x_2 &= (10^{(a_1 - 1.645)/b_1} + 10^{(a_2 - 1.645)/b_2})^{-1} \\ &= (LC95(1) + LC95(2))^{-1} \end{aligned}$$

LC values for mixtures of toxicants combined in other proportions can be calculated in a similar way.

4.5 Testing for goodness of fit of data to model of additive joint action

The criterion for an adequate fit to the model depends on the nature of the bioassay data and on the purpose for which the model is to be used. Plackett and Hewlett (1963) suggested the use of a chi-square test applied to predicted and observed responses for individual compounds and mixtures of them to test the hypothesis of no significant difference between the data and the results predicted on the basis of their additive model, and this approach has also been used in the present work.

However, this is a stringent test, requiring good bioassay data produced under carefully controlled conditions. The only published studies on the applicability of Plackett and Hewlett's model for joint toxic action have involved the dosing techniques using residual film or topical application techniques applied to specially reared and homogeneous populations of insects (Turner, 1955 - data analysed in Plackett and Hewlett, 1963; Sawicki *et al*, 1962; Hewlett, 1963a, Hewlett 1963b). The use of dust admixture bioassays which rely on the insect dosing itself by moving through and feeding on wheat might be expected to introduce additional variability into the response of the population to the treatment and this may be reflected in a greater heterogeneity of response than is obtained using residual film bioassay. However, a bioassay method which exposes the insects to the insecticide mixture in a form similar to that which will be encountered in a field test will give results which may serve as better guide to optimal mixture compositions for field application (Chapter 5).

Consequently, in cases for which $p < 0.05$ for a chi-square test on the combined bioassay data, the predicted dose/response relationships for the individual compounds and the 1 : 1 mixture of dusts were plotted manually and compared with the experimental data to establish whether the deviations from predicted values for any of the three sets of data appeared subjectively to be random or showed a trend indicating that the additive model was inappropriate. Discrepancy between predicted and

experimental values for responses to particular doses of the mixture is indicated in the Tables by showing values for the LC50 and LC95 doses (i) calculated from the experimental regression lines for the two mixture components bioassayed by themselves, using the method described in Section 4.4, (ii) calculated from the regression line for the mixture of components, and (iii) calculated from the modified regression lines for the individual components after combination with the bioassay data for the mixture and using Plackett and Hewlett's model for additive joint action. The joint action model for additive action does not predict that a straight line relationship necessarily exists between the log of the sum of the doses and the n.e.d. response to binary mixtures, but in the great majority of cases such linear regressions gave an adequate fit to the data ($p > 0.05$).

4.6 Fit of additive model to bioassay data for susceptible strains of *O. surinamensis*, *T. castaneum*, and *S. granarius* using mixtures of dust formulations of malathion, etrimfos and pirimiphos methyl and 24 h bioassay data

Estimated values for the parameters of the n.e.d.(response)/log (dose) regressions for dust formulations of malathion, etrimphos, and pirimiphos methyl applied individually and as 1:1 w/w mixtures against the susceptible strains of *O. surinamensis*, *T. castaneum*, and *S. granarius*

using a 24 h bioassay are shown in Table 4.1 (*O. surinamensis*), 4.2 (*T. castaneum*) and 4.3 (*S. granarius*), together with probabilities corresponding to chi squared values for fit of the data to the regression lines.

Modified values of these parameters were then estimated on the assumption that pairs of insecticides acted additively, using the program MIX and the three sets of bioassay data corresponding to the two insecticides applied singly and as a 1:1 w/w mixture. These parameters, together with probabilities corresponding to chi-square for fit of the data set to the two regression lines, are also shown in Table 4.1 (*O. surinamensis*), 4.2 (*T. castaneum*), and 4.3 (*S. granarius*).

In general, the fit of the data to the model for additive joint action was good for all three species. Three out of the nine chi-square values for goodness of fit of the combined bioassay data to the model were significant, but only in one case (the malathion/pirimiphos methyl mixture applied against *T. castaneum*) was this the result of a significant trend of the experimental results away from the predicted dose/response line, as indicated by the discrepancies between experimental and predicted LC50 and LC95 values. The corresponding regression lines are shown for this mixture in Fig. 4.1 and Table 4.2. In this case, the homogeneity of the regression lines for the individual compounds and the 1 : 1 w/w mixture was good ($p = 0.87, 0.72, \text{ and } 0.67$ respectively), and the result therefore may indicate

Table 4.1

Response of *O. surinamensis* to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) dusts applied to wheat either alone or as 1:1 (w/w) mixtures and analysis of bioassay data for fit to model for additive action (24 hour bioassays at 25°C)

insecticide	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate) (data based on concentrations at mg a.i./kg wheat)	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate including mixture data)	LC50 (mg a.i./kg) (95 % fiducial limits)	LC 95 (mg a.i./kg)
etrimfos	$y = 16.4 + 14.4 x$ $p = 0.49$		0.072 0.068-0.075	0.094 0.085-0.103
malathion	$y = 4.6 + 5.8 x$ $p = 0.38$		0.160 0.143-0.180	0.308 0.237-0.399
pirimiphos methyl	$y = 12.6 + 17.4 x$ $p = 0.51$		0.189 0.184-0.194	0.235 0.218-0.253
etrimfos + malathion (1:1)	$y = 8.2 + 8.5 x$ $p = 0.75$	$y = 16.4 + 14.4 x$ $y = 5.2 + 5.9 x$ $p = 0.59$	calculated (1) 0.114 calculated (2) 0.114 experimental 0.110 0.099-0.121	0.174 0.171 0.172 0.137-0.214
pirimiphos met malathion (1:1)	$y = 6.2 + 8.4 x$ $p = 0.33$	$y = 12.5 + 17.2 x$ $y = 4.7 + 6.1 x$ $p = 0.41$	calculated (1) 0.168 calculated (2) 0.147 experimental 0.180 0.166-0.195	0.279 0.267 0.283 0.236-0.340
pirimiphos met etrimfos (1:1)	$y = 11.8 + 12.5 x$ $p = 0.67$	$y = 11.5 + 16.0 x$ $y = 13.2 + 11.7 x$ $p = 0.01$	calculated (1) 0.104 calculated (2) 0.108 experimental 0.114 0.107-0.121	0.134 0.144 0.155 0.139-0.171

(1) calculated values based on regression lines for two compounds bioassayed separately.

(2) calculated values based on regression lines calculated from compounds bioassayed separately and as 1:1 mixture.

p probability corresponding to chi-square.

Table 4.2

Response of *T. castaneum* to malathion (4%), pirimiphos methyl (2%), and etrimfos (2%) dusts applied to wheat either alone or as 1:1 (w/w) mixtures and analysis of bioassay data for fit to model for additive action (24 hour bioassays at 25°C)

insecticide	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate) (data based on concentrations at mg a.i./kg wheat)	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate including mixture data)	LC50 (mg a.i./kg) (95 % fiducial limits)	LC 95 (mg a.i./kg)
etrimfos	$y = 10.6 + 12.6 x$ $p = 0.10$		0.142 0.130-0.156	0.193 0.173-0.214
malathion	$y = 2.5 + 8.3 x$ $p = 0.87$		0.503 0.477-0.529	0.794 0.729-0.864
pirimiphos methyl	$y = 8.2 + 10.6 x$ $p = 0.72$		0.171 0.163-0.179	0.244 0.216-0.275
etrimfos + malathion (1:1)	$y = 6.9 + 11.8 x$ $p = 0.99$	$y = 9.8 + 11.3 x$ $y = 2.5 + 8.4 x$ $p = 0.63$	calculated (1) 0.273 calculated (2) 0.264 experimental 0.261 0.253-0.268	0.387 0.384 0.360 0.333-0.389
pirimiphos met + malathion (1:1)	$y = 7.8 + 13.5 x$ $p = 0.67$	$y = 8.1 + 10.2 x$ $y = 2.3 + 7.2 x$ $p = <0.001$	calculated (1) 0.306 calculated (2) 0.289 experimental 0.261 0.252-0.271	0.453 0.445 0.346 0.321-0.372
pirimiphos met + etrimfos (1:1)	$y = 6.4 + 7.8 x$ $p = 0.17$	$y = 7.3 + 9.8 x$ $y = 9.1 + 9.9 x$ $p = 0.18$	calculated (1) 0.156 calculated (2) 0.152 experimental 0.152 0.142-0.162	0.214 0.224 0.247 0.199-0.305

(1) calculated values based on regression lines for two compounds bioassayed separately.

(2) calculated values based on regression lines calculated from compounds bioassayed separately and as 1:1 mixture.

p probability corresponding to chi-square.

Table 4.3

Response of *S. granarius* to malathion (4%), pirimiphos methyl (2%), and etrimfos (2%) dusts applied to wheat either alone or as 1:1 (w/w) mixtures and analysis of bioassay data for fit to model for additive action (24 hour bioassays at 25°C)

insecticide	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate)	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate including mixture data)	LC50 (mg a.i./kg) LC 95 (mg a.i./kg) (95 % fiducial limits)	
	(data based on concentrations at mg a.i./kg wheat)			
etrimfos	$y = 6.7 + 9.9 x$ $p = 0.39$		0.209 0.201-0.217	0.306 0.282-0.330
malathion	$y = 1.5 + 8.9 x$ $p = 0.76$		0.688 0.661-0.715	1.054 0.955-1.169
pirimiphos methyl	$y = 5.6 + 15.7 x$ $p = 0.51$		0.442 0.432-0.453	0.562 0.539-0.589
etrimfos + malathion (1:1)	$y = 3.8 + 10.5 x$ $p = 0.55$	$y = 6.3 + 9.5 x$ $y = 1.4 + 9.1 x$ $p = 0.002$	calculated (1) 0.390 calculated (2) 0.405 experimental 0.433 0.420-0.477	0.582 0.609 0.622 0.575-0.671
pirimiphos met malathion (1:1)	$y = 3.2 + 11.7 x$ $p = 0.73$	$y = 5.5 + 15.4 x$ $y = 1.5 + 8.4 x$ $p = 0.01$	calculated (1) 0.582 calculated (2) 0.558 experimental 0.533 0.509-0.557	0.816 0.795 0.734 0.673-0.808
pirimiphos met etrimfos (1:1)	$y = 6.6 + 11.9 x$ $p = 0.69$	$y = 5.6 + 15.8 x$ $y = 6.9 + 10.0 x$ $p = 0.70$	calculated (1) 0.284 calculated (2) 0.283 experimental 0.281 0.270-0.293	0.396 0.398 0.387 0.351-0.427

(1) calculated(1) values based on regression lines for two compounds bioassayed separately.

(2) calculated(2) values based on regression lines calculated from compounds bioassayed separately and as 1:1 mixture.

p probability corresponding to chi-square.

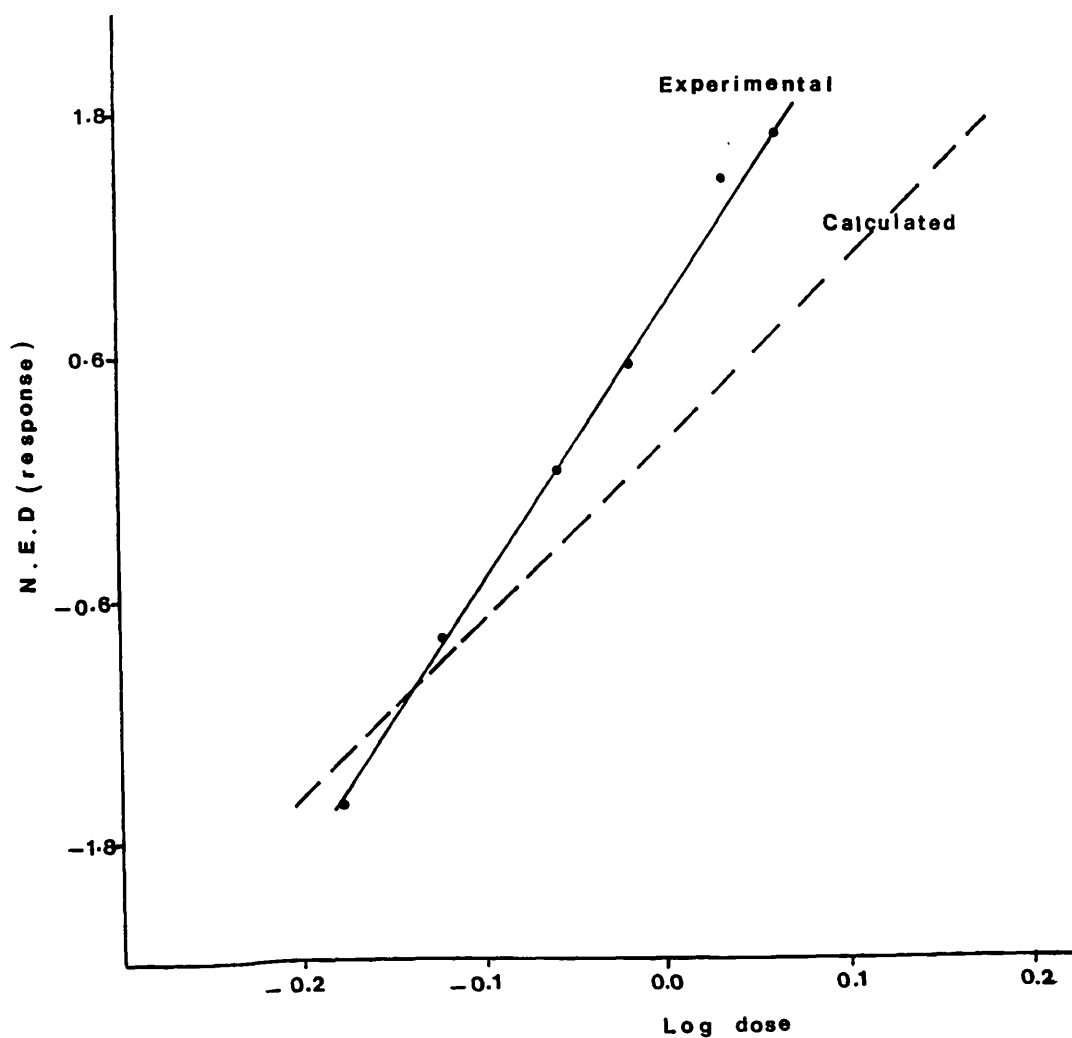


Figure 4.1

Experimental log (dose)/n.e.d. (response) regression for *T. castaneum* bioassayed against a 1:1 (w/w) mixture of pirimiphos methyl (2% a.i.) and malathion (4% a.i.), and corresponding regression calculated from the experimental log (dose)/n.e.d. (response) regressions for the compounds applied separately, using Plackett and Hewlett's method. (24 hr dust bioassay data)

genuine synergism between these two formulations against this species under the bioassay conditions used. The result would need to be checked at a number of different ratios of the dust formulations for synergism at this level to be established with any degree of confidence, however.

Etrimfos was the most effective insecticide against all three species of insect at response levels between 5% and 95% mortality.

4.7 Fit of additive model to bioassay data for susceptible strains of a number of stored product pests and resistant strains of *T. castaneum* and *S. granarius* using mixtures of dust formulations of malathion, etrimfos and pirimiphos methyl (48 hr bioassay data)

A number of stored grain pest species were bioassayed against malathion, pirimiphos methyl, and etrimfos dusts both alone and as 1:1 w/w formulation mixtures. The pest species included a strain of *T. castaneum* specifically resistant to malathion, designated the "Kano" strain (Dyte and Blackman, 1972; Binns, 1986), and a strain of *S. granarius* resistant to gamma-HCH (Champ and Dyte, 1976; le Patourel and Salama, 1986) with a slight cross-resistance to organophosphorus insecticides.

The results of the bioassays are given in Table 4.4 for insecticides applied individually and Table 4.5 for insecticides applied as 1:1 w/w

Table 4.4

Response of seven species of stored product insects to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) dust formulations admixed with whole wheat (48 hour bioassays at 25°C)

species	insecticide dust	N.E.D.(response)/ log (dose) regression [*]	LC50 (mg a.i./kg wheat)	95% fiducial limits		goodness of-fit p
				lower	upper	
<i>T. castaneum</i> (susceptible)	malathion	y= 6.3 + 15.0 x	0.379	0.367	0.392	0.35
	pirimiphos methyl	y= 9.2 + 11.4 x	0.155	0.149	0.161	0.30
	etrimfos	y= 12.4 + 14.1 x	0.133	0.128	0.137	0.54
<i>T. castaneum</i> (malathion resistant)	malathion	y= -7.6 + 4.9 x	36.629	29.643	45.212	0.11
	pirimiphos methyl	y= 4.1 + 6.4 x	0.230	0.098	0.567	0.85
	etrimfos	y= 3.9 + 6.0 x	0.222	0.180	0.275	0.44
<i>T. confusum</i>	malathion	y= -1.4 + 4.6 x	2.008	1.876	2.149	0.92
	pirimiphos methyl	y= 0.2 + 6.9 x	0.929	0.866	0.987	0.86
	etrimfos	y= 1.7 + 6.6 x	0.561	0.531	0.592	0.64
<i>O. surinamensis</i>	malathion	y= 6.2 + 7.3 x	0.160	0.147	0.176	0.91
	pirimiphos methyl	y= 11.5 + 13.8 x	0.147	0.142	0.152	0.98
	etrimfos	y= 23.6 + 19.2 x	0.059	0.046	0.076	0.43
<i>O. surinamensis</i> (Greek strain)	malathion	y= 6.7 + 7.2 x	0.117	0.110	0.125	0.78
	pirimiphos methyl	y= 6.3 + 8.1 x	0.169	0.161	0.176	0.91
	etrimfos	y= 6.8 + 5.6 x	0.092	0.058	0.067	0.98

* Data based on concentration units of mg a.i./kg wheat.

Table 4.4 (Cont.)

Response of seven species of stored product insects to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) dust formulations admixed with whole wheat (48 hour bioassays at 25°C)

species	insecticide dust	N.B.D.(response)/ log (dose) regression*	LC50 (mg a.i./kg wheat)	95% fiducial limits		goodness of-fit p
				lower	upper	
<i>S. granarius</i> (susceptible)	malathion	y= 3.6 + 13.7 x	0.547	0.527	0.569	0.11
	pirimiphos methyl	y= 8.7 + 14.4 x	0.249	0.241	0.256	0.36
	etrimfos	y= 8.4 + 9.7 x	0.137	0.131	0.144	0.29
<i>S. granarius</i> (gamma-HCH resistant)	malathion	y= -0.1 + 5.2 x	1.058	0.979	1.134	0.96
	pirimiphos methyl	y= 2.7 + 5.6 x	0.327	0.300	0.356	0.21
	etrimfos	y= 4.2 + 6.3 x	0.218	0.204	0.234	0.26
<i>O. mercator</i>	malathion	y= 6.7 + 7.9 x	0.143	0.135	0.151	0.93
	pirimiphos methyl	y= 9.2 + 11.9 x	0.168	0.163	0.174	0.97
	etrimfos	y= 11.0 + 9.3 x	0.064	0.061	0.068	0.69
<i>S. oryzae</i>	malathion	y= 2.1 + 6.6 x	0.499	0.463	0.536	0.28
	pirimiphos methyl	y= 5.5 + 9.3 x	0.255	0.242	0.268	0.96
	etrimfos	y= 5.3 + 6.0 x	0.132	0.122	0.144	0.06
<i>B. dominica</i>	malathion	y= -2.0 + 3.6 x	3.517	3.190	3.884	0.58
	pirimiphos methyl	y= -3.2 + 5.0 x	4.339	4.105	4.587	0.35
	etrimfos	y= -1.1 + 4.6 x	1.701	1.587	1.822	0.11

* Data based on concentration units at mg a.i./kg wheat .

Table 4.5

Response of seven species of stored product insects to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) as 1:1 (w/w) dust mixtures and admixed with whole wheat (48 hour bioassays at 25°C)

species	1:1 (w/w) dust formulation mixtures	N.B.D.(response)/ log (dose) regressions for mixtures [*]	LC50 (mg a.i./kg wheat)	95 % fiducial limits lower	upper	goodness of-fit p
<i>T. castaneum</i> (susceptible)	malathion pirimiphos methyl	y= 6.6 + 11.2 x	0.258	0.243	0.274	0.61
	malathion etrimfos	y= 7.0 + 10.6 x	0.220	0.211	0.230	0.51
	etrimfos pirimiphos methyl	y= 7.4 + 8.5 x	0.134	0.126	0.143	0.20
<i>T. castaneum</i> (resistant)	malathion pirimiphos methyl	y= 0.5 + 5.9 x	0.837	0.445	1.783	0.93
	malathion etrimfos	y= 1.7 + 8.7 x	0.636	0.510	0.804	0.36
	etrimfos pirimiphos methyl	y= 3.5 + 5.8 x	0.251	0.057	1.198	0.77
<i>T. confusum</i>	malathion pirimiphos methyl	y= -0.7 + 5.1 x	1.367	1.267	1.473	0.08
	malathion etrimfos	y= -0.3 + 7.0 x	1.093	1.030	1.155	0.26
	etrimfos pirimiphos methyl	y= 0.6 + 6.1 x	0.797	0.749	0.846	0.23
<i>O. surinamensis</i>	malathion pirimiphos methyl	y= 12.6 + 16.5 x	0.172	0.162	0.181	0.76
	malathion etrimfos	y= 8.3 + 8.0 x	0.091	0.056	0.149	0.93
	etrimfos pirimiphos methyl	y= 17.5 + 17.0 x	0.093	0.090	0.096	0.47

* Data based on concentration units at mg a.i./kg wheat.

Table 4.5 (Cont.)

Response of seven species of stored insects to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) as 1:1 (w/w) dust mixtures and admixed with whole wheat (48 hour bioassays at 25°C)

species	1:1 (w/w) dust formulation mixtures	N.B.D.(response)/ log (dose) regressions for mixtures [†]	LC50 (mg a.i./kg wheat)	95 % fiducial limits lower upper	goodness of-fit p
<i>O. surinamensis</i> (Greek strain)	malathion pirimiphos methyl	y= 4.1 + 5.2 x	0.164	0.152 0.178	0.98
	malathion etrimfos	y= 7.8 + 7.2 x	0.083	0.077 0.089	0.91
	etrimfos pirimiphos methyl	y= 8.9 + 8.3 x	0.086	0.080 0.092	0.92
<i>S. granarius</i> (susceptible)	malathion pirimiphos methyl	y= 5.9 + 13.6 x	0.366	0.355 0.377	0.49
	malathion etrimfos	y= 5.9 + 10.1 x	0.261	0.252 0.271	0.58
	etrimfos pirimiphos methyl	y= 8.7 + 11.1 x	0.165	0.159 0.171	0.24
<i>S. granarius</i> (gamma-HCH resistant)	malathion pirimiphos methyl	y= 1.4 + 8.3 x	0.685	0.658 0.714	0.71
	malathion etrimfos	y= 1.9 + 6.4 x	0.514	0.486 0.543	0.83
	etrimfos pirimiphos methyl	y= 2.3 + 4.5 x	0.303	0.273 0.338	0.91
<i>O. mercator</i>	malathion pirimiphos methyl	y= 5.5 + 6.7 x	0.150	0.140 0.161	0.98
	malathion etrimfos	y= 5.9 + 6.2 x	0.111	0.104 0.118	0.38
	etrimfos pirimiphos methyl	y= 5.8 + 5.9 x	0.103	0.095 0.111	0.77

[†] Data based on concentration units at mg a.i./kg wheat.

Table 4.5 (Cont.)

Response of seven species of stored insects to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) as 1:1 (w/w) dust mixtures and admixed with whole wheat (48 hour bioassays at 25°C.)

species	1:1 (w/w) dust formulation mixtures	N.E.D.(response)/ log (dose) regressions for mixtures [*]	LC50 (mg a.i./kg wheat)	95 % fiducial limits		goodness of-fit p
				lower	upper	
<i>S. oryzae</i>	malathion pirimiphos methyl	$y = 2.7 + 6.1 x$	0.371	0.348	0.395	0.16
	malathion etrimfos	$y = 3.3 + 6.4 x$	0.305	0.284	0.326	0.09
	etrimfos pirimiphos methyl	$y = 4.1 + 5.1 x$	0.155	0.142	0.169	0.40
<i>E. dominica</i>	malathion pirimiphos methyl	$y = -2.4 + 4.2 x$	3.662	3.381	3.964	0.98
	malathion etrimfos	$y = -1.9 + 4.7 x$	2.594	2.405	2.797	0.42
	etrimfos pirimiphos methyl	$y = -1.7 + 4.9 x$	2.204	2.058	2.359	0.07

* Data based on concentration units at mg a.i./kg wheat.

formulation mixtures. The malathion resistant strain of *T. castaneum* had resistance factors of 96 (LC50) and 164 (LC95) to malathion relative to the susceptible Silwood strain, with negligible cross-resistance to pirimiphos methyl and etrimfos. Etrimfos dust was again the most effective insecticide formulation against all seven insect species at the LC50 level. *Rhyzopertha dominica* showed the greatest tolerance to all three dust formulations, with the exception of malathion applied against the resistant strain of *T. castaneum*.

The fit of the combined bioassay data for pairs of insecticides applied singly and together to the additive model for joint action is shown in Table 4.6. In cases where a significant chi-square value was obtained, LC50 and LC95 values for the 1:1 w/w formulation mixture calculated from the maximum likelihood values of the parameters a_1 , a_2 , b_1 , b_2 obtained by applying the insecticides by themselves were compared with those obtained experimentally from the 1:1 w/w formulation mixtures.

In 14 cases out of the 30 investigated, the chi-square test for goodness of fit was significant, but the predicted LC50 and LC95 values were close to those obtained experimentally. Similarly, the modified values for the slope and intercept parameters obtained by including the bioassay data for the mixtures were in all cases close to those obtained using the data for the insecticides applied alone. It was concluded from comparison of plots of the dose/response relationships predicted on the

Table 4.6

Analysis of bioassay data for insecticides applied alone and as 1:1 mixtures to test for fit to model for additive action

species	1:1(w/w) dust formulation mixtures	modified N.E.D.(response)/ log(dose) regression for component insecticides	calculated LC50 for 1:1 w/w mixture of dusts(mg a.i/kg wheat) calc.1 ¹ calc. 2 ²		goodness of-fit p
<i>T. castaneum</i> (susceptible)	malathion	y= 6.2 + 14.7 x	0.255	0.255	0.52
	pirimiphos methyl	y= 8.7 + 10.7 x			
	malathion etrimfos	y= 5.0 + 11.4 x y= 10.4 + 11.7 x	0.234	0.228	0.01
<i>T. castaneum</i> (malathion resistant)	etrimfos pirimiphos methyl	y= 11.9 + 13.5 x y= 6.0 + 7.3 x	0.143	0.139	0.01
	malathion	y= -7.7 + 4.9 x	0.681	0.738	0.10
	pirimiphos methyl	y= 4.0 + 6.5 x			
<i>T. confusum</i>	malathion	y= -7.6 + 4.9 x	0.660	0.660	0.31
	etrimfos	y= 4.3 + 6.5 x			
	etrimfos pirimiphos methyl	y= 3.9 + 5.9 x y= 3.7 + 6.1 x	0.226	0.234	0.62
<i>O. surinamensis</i>	malathion	y= -1.3 + 4.4 x	1.446	1.422	0.59
	pirimiphos methyl	y= 0.3 + 6.8 x			
	malathion	y= -1.5 + 4.9 x	1.077	1.077	0.71
<i>O. surinamensis</i>	etrimfos	y= 1.8 + 7.0 x			
	etrimfos	y= 1.3 + 6.0 x	0.699	0.736	0.01
	pirimiphos methyl	y= 0.1 + 6.8 x			
<i>O. surinamensis</i>	malathion	y= 6.7 + 8.7 x	0.159	0.156	0.003
	pirimiphos methyl	y= 12.2 + 14.8 x			
	malathion	y= 4.3 + 5.3 x	0.102	0.099	0.05
<i>O. surinamensis</i>	etrimfos	y= 21.9 + 17.7 x			
	etrimfos	y= 19.9 + 16.6 x	0.083	0.088	0.003
	pirimiphos methyl	y= 11.0 + 13.3 x			

(1) Calculated LC50 values based on regression lines for two compounds bioassayed separately.

(2) Calculated LC50 values based on modified estimates of regression parameters for the component insecticides, using the equation for additive action.

Table 4.6 (Cont.)

Analysis of bioassay data for insecticides applied alone and as 1:1 mixtures to test for fit to model for additive action.

species	1:1(w/w) dust formulation mixtures	modified N.E.D.(response)/ log(dose) regression for component insecticides	calculated LC50 for 1:1 w/w mixture of dusts(ug a.i/kg wheat) calc. 1 ¹ calc. 2 ²		goodness of-fit p
<i>O. surinamensis</i> (Greek strain)	malathion	y= 4.9 + 5.4 x	0.132	0.141	< 0.001
	pirimiphos methyl	y= 5.7 + 7.3 x			
	malathion	y= 6.9 + 7.3 x	0.090	0.087	0.80
	etrimfos	y= 7.0 + 5.8 x			
	etrimfos	y= 7.4 + 6.1 x	0.091	0.089	0.67
	pirimiphos methyl	y= 6.6 + 8.4 x			
<i>S. granarius</i> (susceptible)	malathion	y= 3.8 + 13.4 x	0.390	0.381	< 0.001
	pirimiphos methyl	y= 8.4 + 13.4 x			
	malathion	y= 3.6 + 13.6 x	0.276	0.267	0.11
	etrimfos	y= 7.8 + 8.9 x			
	etrimfos	y= 8.1 + 9.2 x	0.177	0.172	0.03
	pirimiphos methyl	y= 8.7 + 14.3 x			
<i>S. granarius</i> (gamma-HCH resistant)	malathion	y= -0.3 + 5.7 x	0.606	0.645	0.002
	pirimiphos methyl	y= 2.7 + 5.9 x			
	malathion	y= -0.2 + 5.3 x	0.465	0.486	0.44
	etrimfos	y= 4.2 + 6.6 x			
	etrimfos	y= 4.0 + 6.2 x	0.262	0.273	0.06
	pirimiphos methyl	y= 2.2 + 4.8 x			

(1) Calculated LC50 values based on regression lines for two compounds bioassayed separately.

(2) Calculated LC50 values based on modified estimates of regression parameters for the component insecticides, using the equation for additive action.

Table 4.6 (Cont.)

Analysis of bioassay data for insecticides applied alone and as 1:1 mixtures to test for fit to model for additive action.

species	1:1(w/w) dust formulation mixtures	modified N.E.D.(response)/ log(dose) regression for component insecticides	calculated LC50 for 1:1 w/w mixture of dusts(mg a.i/kg wheat) calc. 1 ¹ calc. 2 ²		goodness of-fit p
<i>O. mercator</i>	malathion pirimiphos methyl	y= 5.7 + 6.7 x y= 8.8 + 11.4 x	0.150	0.150	0.91
	malathion etrimfos	y= 5.6 + 6.7 x y= 9.9 + 8.4 x	0.102	0.105	0.01
	etrimfos pirimiphos methyl	y= 8.0 + 6.8 x y= 8.0 + 10.4 x	0.093	0.096	< 0.001
<i>S. oryzae</i>	malathion pirimiphos methyl	y= 1.9 + 5.9 x y= 5.2 + 8.8 x	0.381	0.462	0.17
	malathion etrimfos	y= 2.0 + 6.5 x y= 4.8 + 5.8 x	0.336	0.276	0.001
	etrimfos pirimiphos methyl	y= 4.5 + 5.0 x y= 5.5 + 9.1 x	0.174	0.167	0.01
<i>E. dominica</i>	malathion pirimiphos methyl	y= -2.1 + 3.8 x y= -3.2 + 5.0 x	3.753	3.705	0.82
	malathion etrimfos	y= -2.1 + 3.9 x y= -1.1 + 4.8 x	2.595	2.586	0.28
	etrimfos pirimiphos methyl	y= -1.0 + 4.7 x y= -3.2 + 5.0 x	2.444	2.349	0.01

(1) Calculated LC50 values based on regression lines for two compounds bioassayed separately.

(2) Calculated LC50 values based on modified estimates of regression parameters for the component insecticides, using the equation for additive action.

basis of the additive model with the experimental data that the significance of the chi-square values resulted from a greater than expected scatter of points about the regression lines rather than a systematic trend of the data which might indicate non-additivity of action between the insecticides.

4.8 Fit of additive model to bioassay data for susceptible and resistant strains of *T. castaneum* and *S. granarius* using mixtures of dust formulations of malathion, gamma-HCH and deltamethrin (7 day bioassay data)

The effect of deltamethrin on beetle pests of stored grain has been described by Williams *et al.* (1983) and is typical of that of the earlier synthetic pyrethroids which have been used for stored grain treatment. Knock-down is rapid at typical application rates, but an end-point mortality is not reached until several days after the beetles are exposed to treated wheat. In the present work a bioassay time of 7 days was chosen for mixtures comprising pirimiphos methyl with deltamethrin and gamma-HCH with deltamethrin.

N.e.d. (response)/log(dose) regression lines for malathion (4% a.i.) dust, gamma-HCH (2% a.i.) dust and deltamethrin (2% a.i.) dust formulations and their 1:1 (w/w formulation) mixtures against both the

susceptible and Kano strain of *Tribolium castaneum* and a susceptible and a gamma-HCH resistant strain of *S. granarius* are shown in Tables 4.7 and 4.8. The Kano strain of *Tribolium castaneum* was cultured through 8 generations without selection pressure between the bioassays described in this section and those of the previous two sections, and during this time the resistance factor dropped considerably relative to the susceptible strain, giving a value of 26.4 for the 7 day dust admixture bioassay. The gamma-HCH strain of *S. granarius* had a resistance factor of 16.8 relative to the Windsor susceptible strain. Cross-resistance of the resistant strains to the other insecticides was low ($RF < 2$) apart from the cross-resistance of the Kano strain of *T. castaneum* to gamma-HCH which, at 4.8, was much higher than would have been expected on the basis of a resistance mechanism involving greater carboxyesterase activity.

Table 4.9 shows the fit of bioassay data for individual insecticides combined with bioassay data for 1:1 w/w formulation mixtures to the model for additive joint action. For the susceptible strains of *S. granarius* and *T. castaneum* there was a good fit between experimental and predicted LC50 values. In 4 cases out of the 12 investigated the test for goodness of fit was not significant.

Table 4.7

Response of susceptible and resistant strains of *T. castaneum* and *S. granarius* to malathion (4%), deltamethrin (2%), and gamma-HCH (2%) formulated as dusts and admixed with 14.5% m.c. whole wheat (7 days bioassays at 25°C)

species	insecticide dust formulation	N.E.D (response)/ log (dose) regression	LC50 (mg a.i./kg wheat)	95% fiducial limits		goodness of fit p	resistance factor
				lower	upper		
<i>T. castaneum</i> (susceptible)	malathion	$y = 3.1 + 7.9 x$	0.402	0.261	0.596	0.001	-
	deltamethrin	$y = 1.4 + 2.2 x$	0.216	0.183	0.256	0.43	-
	gamma-HCH	$y = -0.2 + 4.2 x$	1.100	1.025	1.189	0.01	-
<i>T. castaneum</i> (malathion resistant)	malathion	$y = -4.7 + 4.6 x$	10.612	9.608	11.718	0.10	26.40
	deltamethrin	$y = 1.3 + 2.6 x$	0.325	0.286	0.37	0.91	1.51
	gamma-HCH	$y = -2.6 + 3.6 x$	5.306	4.812	5.847	0.68	4.82
<i>S. granarius</i> (susceptible)	malathion	$y = 4.2 + 9.1 x$	0.350	0.246	0.493	0.01	-
	deltamethrin	$y = 3.3 + 3.4 x$	0.106	0.091	0.122	0.31	-
	gamma-HCH	$y = 2.4 + 4.0 x$	0.261	0.212	0.322	0.10	-
<i>S. granarius</i> (gamma-HCH resistance)	malathion	$y = 1.7 + 8.0 x$	0.611	0.584	0.639	0.40	1.75
	deltamethrin	$y = 2.4 + 3.0 x$	0.155	0.140	0.173	0.70	1.46
	gamma-HCH	$y = -2.1 + 3.2 x$	4.378	3.978	4.829	0.57	16.77

Table 4.8

Response of susceptible and resistant strains of *T. castaneum* and *S. granarius* to malathion (4%), deltamethrin (2%) and gamma-HCH (2%) formulated as 1:1 w/w mixtures dust and admixed with 14.5% m.c. whole wheat (7day bioassays at 25°C)

species	1:1(w/w) formulation mixtures (2% dusts)	N.B.D response/log (dose) regression for 1:1(w/w)mixture [*]	LC50 (mg a.i./kg wheat)	95% fiducial limits		goodness of fit p
				lower	upper	
<i>T. castaneum</i> (susceptible)	gamma-HCH malathion	$y = 1.3 + 3.0 x$	0.363	0.284	0.442	0.51
	deltamethrin malathion	$y = 3.0 + 4.7 x$	0.237	0.217	0.259	0.14
	deltamethrin gamma-HCH	$y = 1.9 + 2.9 x$	0.227	0.190	0.272	0.33
<i>T. castaneum</i> (malathion resistant)	gamma-HCH malathion	$y = -4.1 + 4.2 x$	9.801	8.965	10.711	0.53
	deltamethrin malathion	$y = 0.4 + 3.4 x$	0.757	0.661	0.842	0.79
	deltamethrin gamma-HCH	$y = 0.6 + 2.8 x$	0.620	0.544	0.700	0.67
<i>S. granarius</i> (susceptible)	gamma-HCH malathion	$y = 4.7 + 9.6 x$	0.325	0.309	0.342	0.50
	deltamethrin malathion	$y = 2.6 + 3.8 x$	0.207	0.183	0.232	0.37
	deltamethrin gamma-HCH	$y = 3.1 + 3.9 x$	0.157	0.143	0.173	0.29
<i>S. granarius</i> (gamma-HCH resistant)	gamma-HCH malathion	$y = 1.2 + 6.3 x$	0.652	0.610	0.696	0.29
	deltamethrin malathion	$y = 3.4 + 7.8 x$	0.362	0.338	0.387	0.14
	deltamethrin gamma-HCH	$y = 1.7 + 4.4 x$	0.420	0.384	0.460	0.92

* Data based on concentration units at mg a.i./kg wheat.

Table 4.9

Analysis of bioassay data for insecticides applied alone and as 1:1 w/w mixtures to test for fit to model for additive action (7 day bioassay)

species	1:1 (w/w) mixtures of dust formulation	modified N.E.D(response)/ log(dose) regressions for component insecticides (data based on concentrations at mg a.i/kg)	LC50 of 1:1 w/w mixture (mg a.i/kg wheat)		goodness of fit p
			calculated (1) ^a	calculated (2) ^a	
<i>T. castaneum</i> (susceptible)	gamma-HCH malathion	y= -0.2 + 4.1 x y= 1.9 + 6.1 x	0.510	0.504	< 0.001
	deltamethrin malathion	y= 2.1 + 2.6 x y= 3.6 + 6.7 x	0.312	0.282	< 0.001
	deltamethrin gamma-HCH	y= 1.6 + 2.1 x y= -0.2 + 4.2 x	0.362	0.311	< 0.001
<i>T. castaneum</i> (malathion resistant)	gamma-HCH malathion	y= -2.4 + 3.5 x y= -4.8 + 4.6 x	7.959	8.526	< 0.001
	deltamethrin malathion	y= 0.9 + 3.1 x y= -4.7 + 4.6 x	0.918	0.825	0.02
	deltamethrin gamma-HCH	y= 1.3 + 2.7 x y= -2.6 + 3.6 x	0.613	0.615	0.96
<i>S. granarius</i> (susceptible)	gamma-HCH malathion	y= 2.7 + 4.7 x y= 4.6 + 10.4 x	0.315	0.321	< 0.001
	deltamethrin malathion	y= 3.0 + 3.1 x y= 4.0 + 8.8 x	0.198	0.201	0.06
	deltamethrin gamma-HCH	y= 3.4 + 3.5 x y= 2.4 + 4.1 x	0.153	0.154	0.20
<i>S. granarius</i> (gamma-HCH resistant)	gamma-HCH malathion	y= -2.0 + 3.1 x y= 1.8 + 6.9 x	0.867	0.777	< 0.001
	deltamethrin malathion	y= 2.7 + 3.5 x y= 1.8 + 8.4 x	0.309	0.324	< 0.001
	deltamethrin gamma-HCH	y= 2.4 + 3.2 x y= -2.1 + 3.2 x	0.300	0.354	0.001

(1) Calculated LC50 values based on regression lines for two compounds bioassayed separately.

(2) Calculated LC50 values based on modified estimates of regression parameters for the component, using equation for additive action

4.9 Additivity of action of pirimiphos methyl/deltamethrin and malathion/deltamethrin mixtures against *T. castaneum* (susceptible), *S. granarius*, *O. surinamensis*, and *R. dominica* using 48 h residual film bioassays

Mixtures of deltamethrin with either malathion or pirimiphos methyl were investigated for the control of a group of stored grain pests which included *Rhyzopertha dominica*. *R. dominica* is known to be naturally tolerant to many organophosphorus insecticides but reasonably susceptible to pyrethroid insecticides (Desmarchelier, 1977; Ardley and Desmarchelier, 1978) and consequently mixtures of a cheap organophosphorus compound together with a more expensive pyrethroid might be expected, under suitable conditions, to give a cheaper method of control at or above some chosen level than either of the compounds used separately.

The results of bioassays of the three insecticides alone and as 1:1 w/w a.i. mixtures against susceptible strains of *O. surinamensis*, *R. dominica*, *T. castaneum*, and *S. granarius* are shown in Table 4.10. At the LC50 level, deltamethrin was most effective insecticide against *R. dominica* and the least effective against *T. castaneum*, while pirimiphos methyl was the least effective against *R. dominica* and the most effective against *T. castaneum*.

Table 4.10

Toxicity of two organophosphorous insecticides, a pyrethroid insecticide and their mixtures against some storage insects (48 hour residual film bioassay)

insecticides	insect species	Regression of N.E.D. response(y) on log dose(x) (maximum likelihood estimate)	LC50 mg/ml.*	95% fiducial limits mg/ml.	P
deltamethrin					
	<i>O. surinamensis</i>	y= 2.3 + 1.2 x	0.015	(0.012-0.019)	0.07
	<i>B. dominica</i>	y= 0.7 + 1.8 x	0.414	(0.325-0.534)	0.90
	<i>T. castaneum</i>	y= 2.3 + 2.8 x	0.160	(0.142-0.180)	0.53
	<i>S. granarius</i>	y= 3.1 + 1.9 x	0.025	(0.021-0.030)	0.70
malathion					
	<i>O. surinamensis</i>	y= 6.5 + 4.3 x	0.030	(0.026-0.030)	0.23
	<i>B. dominica</i>	y= 0.7 + 4.3 x	0.686	(0.630-0.749)	0.81
	<i>T. castaneum</i>	y= 5.9 + 6.7 x	0.131	(0.124-0.139)	0.78
	<i>S. granarius</i>	y= 8.2 + 7.4 x	0.080	(0.076-0.084)	0.63
pirimiphos-methyl					
	<i>O. surinamensis</i>	y= 4.4 + 3.3 x	0.047	(0.036-0.060)	0.43
	<i>B. dominica</i>	y= 0.2 + 4.4 x	0.909	(0.844-0.985)	0.81
	<i>T. castaneum</i>	y= 7.3 + 5.6 x	0.051	(0.044-0.059)	0.70
	<i>S. granarius</i>	y= 5.5 + 4.7 x	0.070	(0.054-0.090)	0.96
deltamethrin + malathion					
	<i>O. surinamensis</i>	y= 2.8 + 1.8 x	0.026	(0.020-0.033)	0.48
	<i>B. dominica</i>	y= 0.6 + 3.0 x	0.634	(0.575-0.700)	0.90
	<i>T. castaneum</i>	y= 3.4 + 5.2 x	0.218	(0.205-0.231)	0.96
	<i>S. granarius</i>	y= 3.7 + 2.3 x	0.025	(0.021-0.028)	0.91
deltamethrin + pirimiphos-me.					
	<i>O. surinamensis</i>	y= 2.1 + 1.4 x	0.033	(0.024-0.047)	0.23
	<i>B. dominica</i>	y= 0.5 + 2.1 x	0.599	(0.520-0.688)	0.63
	<i>T. castaneum</i>	y= 5.4 + 4.6 x	0.065	(0.060-0.071)	0.22
	<i>S. granarius</i>	y= 2.9 + 2.1 x	0.037	(0.032-0.042)	0.94
malathion + pirimiphos-me.					
	<i>O. surinamensis</i>	y= 6.1 + 4.4 x	0.040	(0.035-0.046)	0.78
	<i>B. dominica</i>	y= 0.5 + 3.3 x	0.730	(0.644-0.808)	0.92
	<i>T. castaneum</i>	y= 7.3 + 6.9 x	0.087	(0.082-0.093)	0.94
	<i>S. granarius</i>	y= 7.5 + 7.0 x	0.085	(0.079-0.090)	0.26

* Solution in petroleum fraction : acetone : Bisella BL as a 3 : 1 : 1 v/v mixture.

Table 4.11

Analysis of bioassay data for insecticides applied alone and as 1:1 solution mixtures to test for fit to model for additive action.

species	1:1 formulation mixture	modified log(dose)/N.E.D. response regressions for component insecticides	experimental LC50 of 1:1 mixture (mg/ml.) [*]		experimental LC95 of 1:1 mixture mg/ml.		goodness of fit p
			calc.1 calc.2	(95% fiducial limits)	calc.1 calc.2	(95% fiducial limits)	
<i>O. surinamensis</i>	deltamethrin	$y = 2.3 + 1.3 x$	0.020	0.026	0.117	0.217	0.05
	malathion	$y = 6.3 + 4.1 x$	0.022	0.020-0.033	0.123	0.129-0.369	
	deltamethrin	$y = 2.2 + 1.2 x$	0.023	0.033	0.200	0.488	0.02
	pirimiphos-me	$y = 4.1 + 3.1 x$	0.025	0.024-0.047	0.225	0.186-1.319	
<i>E. dominica</i>	deltamethrin	$y = 0.5 + 1.8 x$	0.516	0.634	2.238	2.258	0.85
	malathion	$y = 0.7 + 4.5 x$	0.583	0.575-0.700	2.341	1.806-2.918	
	deltamethrin	$y = 0.5 + 1.6 x$	0.568	0.599	2.635	3.828	0.99
	pirimiphos-me	$y = 0.2 + 4.6 x$	0.613	0.520-0.688	2.906	2.825-5.554	
<i>T. castaneus</i>	deltamethrin	$y = 1.4 + 2.2 x$	0.144	0.218	0.336	0.454	< 0.001
	malathion	$y = 4.3 + 5.1 x$	0.177	0.205-0.231	0.491	0.379-0.542	
	deltamethrin	$y = 2.3 + 2.8 x$	0.077	0.065	0.172	0.150	0.28
	pirimiphos-me	$y = 7.4 + 5.5 x$	0.071	0.060-0.071	0.158	0.121-0.184	
<i>S. granarius</i>	deltamethrin	$y = 3.1 + 1.8 x$	0.038	0.025	0.154	0.128	0.30
	malathion	$y = 8.3 + 7.5 x$	0.032	0.021-0.028	0.142	0.103-0.157	
	deltamethrin	$y = 2.9 + 1.8 x$	0.037	0.037	0.169	0.233	0.98
	pirimiphos-me	$y = 5.4 + 4.7 x$	0.038	0.032-0.042	0.178	0.173-0.314	

- Calculated LC50 and LC95 values are based on modified estimates of regression parameters for the component insecticides, using the equation for additive action.

- Calculated 1. based on regression lines for two compounds bioassayed separately.

- Calculated 2. based on regression lines calculated from bioassay data for separate compounds together with bioassay data for 1:1 mixture.

- Experimental estimates are based on bioassay data for tests with 1:1 solution mixtures.

* solution in petroleum fraction : acetone : Bisella EL as a 3 : 1 : 1 v/v mixture.

The results of tests for additive action between the 1:1 w/w a.i. mixtures of deltamethrin with malathion and of deltamethrin with pirimiphos methyl are shown in Table 4.11. In general, the fit of the combined data to the model for additive action was good. Of the 8 cases investigated, 2 gave a significant chi-square value, but the agreement between observed and predicted LC50 and LC95 values was reasonable, suggesting greater than expected scatter of points rather than a systematic trend away from the experimental data.

4.10 Discussion

The general model of Hewlett and Plackett has not been widely used to test data for the action of two or more insecticides applied jointly. Champ et al (1969) used the method of Finney (1952) to test data from 4.5 hr bioassays of dichlorvos with fenitrothion, malathion or diazinon against *R. dominica* and *S. oryzae* for additive action. Finney's method includes a statistical test for fit of the data to the model, but requires the slopes of the probit lines of the component insecticides to be parallel, a condition which will only be met fortuitously. Most tests for additive action use the cotoxicity coefficient of Sun and Johnson (1960) (Wickham et al, 1974; Wickham 1976; Chapman and Penman, 1980; Kay, 1981). This method compares an experimental LD(response) figure for the insecticides applied jointly with the harmonic mean of the corresponding

LD(response) figures for the components applied singly. While having the advantage of simplicity of calculation, cototoxicity coefficients only test for additive action at one response level, may vary with the ratio of toxicants in the test mixture (Hewlett, 1963b), and do not readily lend themselves to statistical analysis. The estimation of maximum likelihood parameters for the n.e.d.(response)/log(dose) regressions of components of mixtures under the hypothesis of additive action, and the testing for overall fit of the bioassay data to these regressions, appeared to provide the most appropriate technique for testing the hypothesis of additive joint action and was accordingly used in this work.

Tests to determine the most appropriate model of joint action by which to describe the effects of two compounds on a target organism require a bioassay period which is sufficiently short to minimise the effects of different rates of decomposition or evaporation of the compounds but which is long enough to allow the full expression of the toxic effects of the components of the mixture. In the present case, only the toxic effects of the compounds tested on the adult insects were considered, although it could be argued that long term effects of residual compounds on breeding of survivors of treatment and on progeny development might also be relevant toxicological characteristics of the test compounds. Such sub-lethal effects are considered in Chapter 6, although the data could not be subjected to the statistical analysis described in this Chapter.

Dust bioassays have not been widely used to estimate the response of insects to insecticides in stored grain. Champ and Campbell-Brown (1970a) considered the method unsatisfactory because of difficulty in reproducing those characteristics (flow and adsorption) of dust formulations which vary with the mineral or organic carrier, the difficulty of reproducing a material with a constant particle distribution, and the possibility of chemical breakdown of materials adsorbed on mineral carriers. There is, however, no evidence available which suggests that insects are particularly sensitive to small changes in the characteristics of carriers, and the use of deactivators and careful screening of dust carriers ensures that good commercial dust formulations have shelf lives at least as long as those of other formulation types. For convenience of mixing dusts the grain sample was kept small (100 g), which necessitated the use of a micro-balance to weigh dust samples to the required accuracy. However, with such a balance it was found that a dust bioassay was no less convenient than a residual film bioassay. The slopes of the $n.e.d.(response)/\log(dose)$ regressions tended to be larger than those of residual film bioassays, reflecting a smaller variance in the distribution of log tolerances in the population; this may be an additional advantage of dust bioassays. Dust admixture has been used for stored grain insect bioassays by Goodwin-Bailey and Holborn (1952), Parkin (1965) and Carter *et al.* (1975).

The 24 h residual film bioassay is a convenient and rapid method of testing insecticides against stored product beetle pests and forms the basis of the recommended FAO method for testing for resistance to malathion in *T. castaneum* (FAO , 1970; Champ, 1968; Champ & Cambell-Brown, 1970a and 1970b ; Dyte & Forster, 1973). Although there is no direct method of extrapolating from the results of such tests to the performance of the insecticides in the field, its convenience and the large number of published results from different strains of insects were considered a suitable justification for testing to see if the insecticides of interest in the present context acted additively in mixtures using this bioassay technique. The bioassay time of 48 h is shorter than that normally used in pyrethroid bioassays, and the full toxicity of these compounds to the adult insects may have been underestimated. Bioassays of pyrethroids admixed with grain have used exposure times varying from 6 days (Carter et al., 1975) to 28 days (Makundi, 1986).

Attempts to demonstrate additivity of action of insecticides to control stored grain pests under field conditions are complicated by the different rates of disappearance of the components over prolonged storage. Champ *et al.* (1969) found the action of mixtures of dichlorvos with malathion, diazinon, and fenitrothion to be non-additive over a storage period of 3 months when tested against *R. dominica* and *S. oryzae*, but these results were undoubtedly affected by the high volatility of dichlorvos relative to that of the residual compounds. The authors suggested that one

organophosphorus ester might inhibit the esterases responsible for the decomposition of another, leading to apparent synergism between the components when their effects were tested over a prolonged storage period.

The results described in this Chapter indicate that in the great majority of the cases considered here the additive model for joint toxic action described by Hewlett and Plackett (1959) and Plackett and Hewlett (1963) provides an adequate description for the interaction of toxicities of dust formulations of malathion, etrimfos, pirimiphos methyl, deltamethrin, and gamma-HCH applied to wheat against a number of target pest species, including two resistant strains.

CHAPTER 5

The use of linear programming to optimise utility functions based on the additive toxic action of insecticide mixtures to stored grain insect pests.

5.1 Introduction

5.1.1 Linear Programming

Linear programming is a technique widely used in the field of economics to optimise a linear utility function, such as cost of production or return on capital, where the utility (cost, or return) is subject to a number of constraints which can be expressed mathematically in the form of linear inequalities (Chatval, 1983). Such problems can not generally be solved analytically, but because they occur so frequently in commerce much effort has gone into devising efficient algorithms for their numerical solution. Fast digital computers are essential to find solutions to problems which involve many variables in the utility function and many constraint inequalities which limit the possible values which the function may take. In such situations, an algorithm known as the "Revised Simplex" method (Chatval, 1983) is usually employed. However, it is possible

to solve small problems - those involving two variables and two or more constraint equations - graphically.

5.1.2 Applications to Design of Mixtures to Control of Stored Product Pests

The rational design of mixtures of insecticides for stored grain protection can be simplified if appropriate bioassay data for mixtures of insecticides is shown to be consistent with the model described in Chapter 4 for additive joint toxic action. Such an interaction of toxic effects demonstrated between pairs of insecticides when tested against a number of different target species makes it possible to optimise utility functions, such as cost of application of a unit of an insecticide mixture whose cost is equal to the sum of the unit cost of each component times its proportion in the mixture. The requirement that a mixture is regarded as feasible is that there should exist an application rate such that it will control all the target species at or above some target mortality level. The linear programming problem is to find the composition of the feasible mixture which has the lowest cost of application.

Preliminary screening of commercial mixtures may involve selection of a number of key target pests and bioassays of many combinations of trial products against them; the design of an integrated package for processing bioassay results and estimating the relative costs of such optimised

mixtures could save a considerable amount of time.

Mixtures of insecticides to control pests of stored products may be useful to protect the commodity against attack by a complex of pest species, one or more of which may be either naturally tolerant to, or have acquired resistance to, a product which would otherwise be chosen because of cost, efficacy, or other considerations. In this Thesis two such situations are highlighted: the use of mixtures including malathion against pest complexes containing a species which has acquired resistance to malathion; and, the use of mixtures including an organophosphorus insecticide against pest complexes including a species which is naturally tolerant to organophosphorus insecticides.

5.2 The use of linear programming to identify minimum cost mixtures producing responses greater than or equal to a chosen level

Suppose that the dose/response relationships of a number of insect species to a number of insecticides can all be represented by the relationship

$$y = a + b x$$

where y is the n.e.d. of the mortality and x is the log dose.

If the response/log(dose) parameters a_{ij} and b_{ij} are established experimentally for a number of insect species each bioassayed against each of a number of toxicants, the requirement that species i should have a response whose n.e.d. is greater than y to mixtures of doses x_j of n toxicants is

$$\sum_{i=1}^m \sum_{j=1}^n k_{ij} x_j > 1, \quad x_j > 0 \quad (\text{vii})$$

Also, if the cost C of applying a mixture of n insecticides simultaneously is taken as the sum of the products of the unit cost of each component and the amount of each component used in the application, then the composition of the mixture to be used in a minimum cost application designed to produce a response whose n.e.d. is greater than y in each of the species is given by minimising c in the equation

$$C = \sum_{j=1}^n c_j x_j \quad (\text{viii})$$

where m is the number of species tested.

For example, the linear programming problem which describes the minimisation of cost of control using mixtures of some or all of three insecticides to control three species at or above some arbitrary response level is given as:

$$\text{minimise } C = c_1 x_1 + c_2 x_2 + c_3 x_3$$

$$\text{subject to } k_{11} x_1 + k_{12} x_2 + k_{13} x_3 > 1$$

$$k_{21} x_1 + k_{22} x_2 + k_{23} x_3 > 1$$

$$k_{31} x_1 + k_{32} x_2 + k_{33} x_3 > 1$$

Such linear programming problems can be solved graphically for mixtures of two insecticides to control two or more insect species at or above some arbitrarily chosen response level, or by using an implementation of the Simplex algorithm (Chvatal, 1983) for mixtures of more than two insecticides applied to control two or more species.

Mixtures of more than three insecticides are unlikely to be used in practice, but in a screening program in which a large number of insecticides are tested against three or more pest species, minimum cost binary or ternary mixtures giving greater than some chosen response level can be identified using this technique.

5.3 Illustration of possible outcomes in the case of a binary insecticide mixture used to control two insect pests.

Suppose insecticides 1 and 2 give the following n.e.d.(response)/log

dose regression lines when applied against species A and B:

	species A	species B
insecticide 1	$y = a_1 + b_1 x_1$	$y = a_2 + b_2 x_1$
insecticide 2	$y = a_3 + b_3 x_2$	$y = a_4 + b_4 x_2$

The linear programming problem is to minimise the cost of applying insecticide

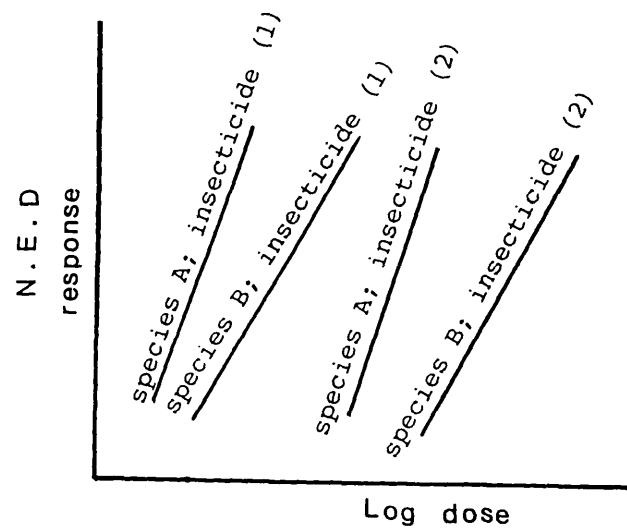
$$C = c_1 x_1 + c_2 x_2$$

subject to

$$10(a_1 - y)/b_1 x_1 + 10(a_3 - y)/b_1 x_2 > 1$$

$$10(a_2 - y)/b_2 x_1 + 10(a_4 - y)/b_4 x_2 > 1$$

Case 1: Insecticide 1 is more effective than insecticide 2 against both species at all response levels (Fig 5.1).



Dose of insecticide 2 (x_2)
to give a chosen response
level

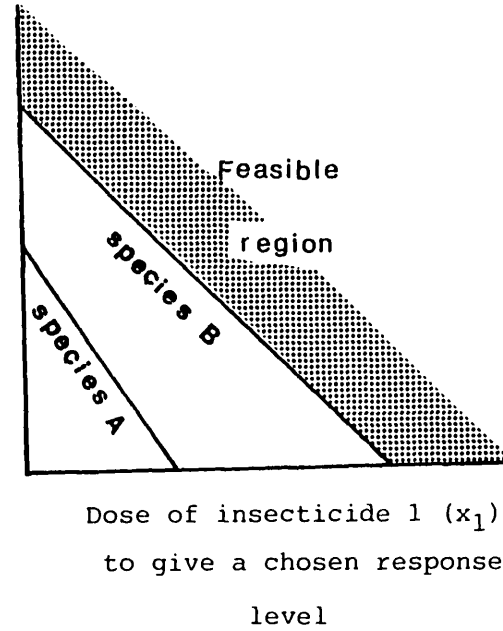


Figure 5.1

* Feasible region defining pairs of doses of insecticides 1 and 2 giving greater than the chosen response level.

Both species can be controlled at any response level by taking the smaller of $c_1 x_1$, ($x_2 = 0$) and $c_2 x_2$, ($x_1 = 0$). A mixture of the two insecticides will not give a cheaper control.

Case 2: Insecticide 1 is more effective against species A and insecticide 2 is more effective against species B at the required response level (Fig. 5.2)

Depending on the ratio of the costs of the two insecticides, the cost of control at the required level of response can be minimised by using either dose x_1 of insecticide 1, dose x_2 of insecticide 2, or the mixture of insecticides 1 and 2 whose composition is defined by the intersection of the two isoboles in Figure 5.3. This mixture will be cheaper than the use of either of the two individual components if the gradient of the cost function lies between those of the isoboles A and B.

5.4 Simulation of effect of increasing resistance to malathion in a population of *T. castaneum*

This section simulates the effect of increasing specific resistance to malathion in a population of *T. castaneum*, on the composition and rate of application of a minimum cost mixtures intended to protect stored grain from infestation by any combination of *T. castaneum*, *S. granarius*, and *O.*

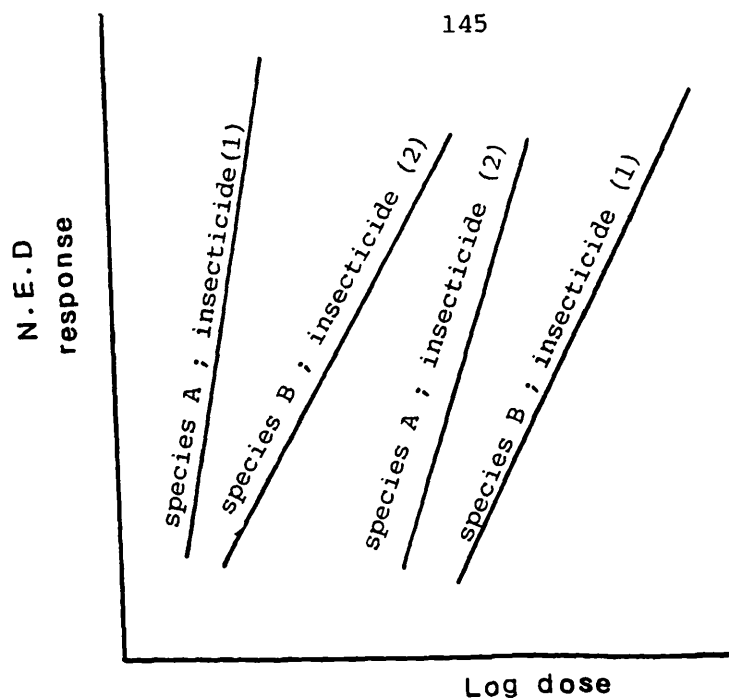


Figure 5.2

Log (dose)/n.e.d. (response) regression for two species to insecticide 1 and insecticide 2 applied separately.

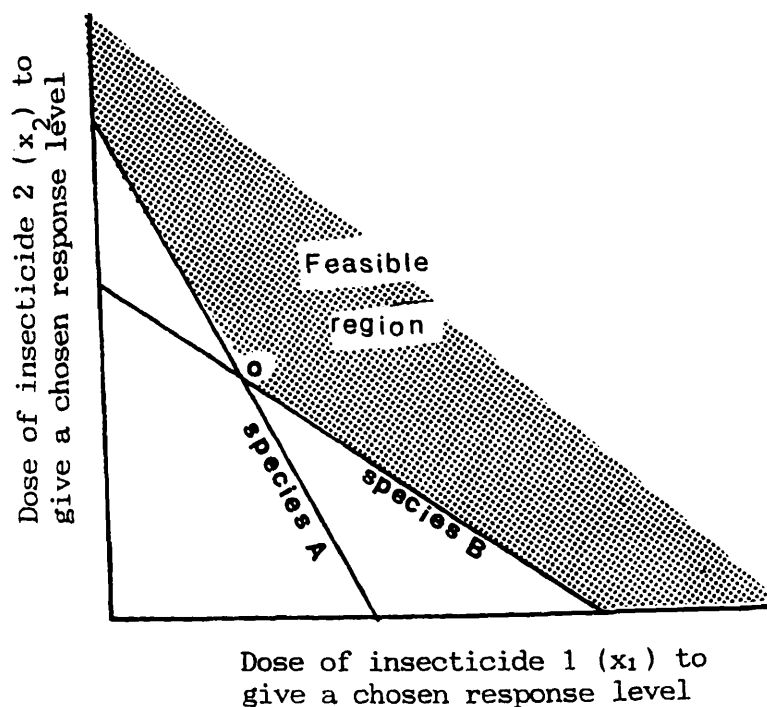


Figure 5.3

* Feasible region defining pairs of doses of insecticides 1 and 2 giving greater than the chosen response level for two species of insects.

surinamensis. The variables considered are the intended response of the species to the mixture, and the level of resistance of *T. castaneum* to malathion. The two insecticides chosen for mixture with malathion were pirimiphos methyl and etrimfos, and the response parameters of all three species to these two insecticides, and of *S. granarius* and *O. surinamensis* to malathion are taken to be those of section 4.6.

Increasing resistance is reflected in the value of

$$k_i = 10^{(a - 1.645)/b} = \frac{1}{LC_{95}} \quad \text{for the } i^{\text{th}} \text{ toxicant}$$

$$\text{Define} \quad K_2 = (LC_2)^{-1}$$

Values of k_i corresponding to 95% and to 99% mortality in the population of each species were considered, and it was assumed that for economic resistance in the *T. castaneum* population the value of k_i at these two response levels would decrease as resistance increased.

Table 5.1 shows values of K_{95} and K_{99} calculated for the three insect species when bioassayed against malathion, pirimiphos methyl, and etrimfos, using regression parameters a and b shown in Tables 4.1 - 4.3 for insecticides applied individually against each species.

Etrimfos is more toxic to all three species at the 95% and 99% response levels, so the analysis of Case 1, (Section 5.3) is applicable. No mixture of malathion and etrimfos or of malathion and pirimiphos methyl

Table 5.1

Values of reciprocal of LC95 ($K_{0.5}$) and LC99 ($K_{0.9}$) calculated for three insect species when bioassayed against malathion (4%), pirimiphos methyl (2%), and etrimfos (2%) dust formulations

species	insecticide	$K_{0.5}$	$K_{0.9}$
<i>T. castaneum</i>	malathion	1.26	1.05
	pirimiphos methyl	4.10	3.54
	etrimfos	5.20	4.59
<i>S. granarius</i>	malathion	0.95	0.80
	pirimiphos methyl	1.78	1.61
	etrimfos	3.27	2.79
<i>O. surinamensis</i>	malathion	3.26	2.49
	pirimiphos methyl	4.26	3.89
	etrimfos	10.70	9.59

will give cheaper control at either of the two response levels than the use of single insecticides, regardless of the ratio of costs of insecticides in the mixture.

The K_{95} and K_{99} for *T. castaneum* and malathion were decreased from the values for the susceptible population of 1.26 and 1.05, simulating a population of *T. castaneum* becoming increasingly specifically resistant to malathion. The linear programming problem considered was thus:

minimise cost function $C = c_1 x_1 + c_2 x_2 + c_3 x_3$

subject to $3.26 x_1 + 4.26 x_2 + 10.70 x_3 > 1$

$K_{95} x_1 + 4.10 x_2 + 5.20 x_3 > 1$

$0.95 x_1 + 1.78 x_2 + 3.27 x_3 > 1$

Solutions were obtained using the LINPROG program and are presented in Table 5.2 a and b in which costs per unit of concentration (mg a.i./kg wheat) for etrimfos and pirimiphos methyl are expressed relative to that of malathion and were varied between 1 and 10 times that of malathion. The critical cost ratio at which mixtures became cheaper than malathion applied alone decreased as the level of malathion-resistance in *T. castaneum* increased.

Table 5.2a

Minimum cost mixtures calculated from 24 hour bioassay data (dust formulation) with projections for increasing specific resistance to malathion in *Tribolium castaneum*.

cost ratio		RF**	K ₉₅	X ₁ (malathion)*	X ₂ (etrimfos)*	Cost C
mala.	etrim.					
1.0	1.0	1	1.26	0	0.306	0.306
		15	0.084	0	0.306	0.306
		20	0.063	0	0.306	0.306
		30	0.042	0	0.306	0.306
		40	0.031	0	0.306	0.306
		50	0.025	0	0.306	0.306
		100	0.013	0	0.306	0.306
1.0	3.4	1	1.26	0	0.306	1.040
		15	0.084	0	0.306	1.040
		20	0.063	0	0.306	1.040
		30	0.042	0	0.306	1.040
		40	0.031	0	0.306	1.040
		50	0.025	0	0.306	1.040
		100	0.013	0	0.306	1.040
1.0	3.5	1	1.26	1.053	0	1.053
		15	0.084	0.414	0.186	1.063
		20	0.063	0.408	0.187	1.064
		30	0.042	0.402	0.189	1.064
		40	0.031	0.399	0.190	1.064
		50	0.025	0.397	0.190	1.064
		100	0.013	0.394	0.191	1.064
1.0	10.0	1	1.26	1.053	0	1.053
		15	0.084	0.414	0.186	2.270
		20	0.063	0.408	0.187	2.281
		30	0.042	0.402	0.189	2.292
		40	0.031	0.399	0.190	2.298
		50	0.025	0.397	0.190	2.301
		100	0.013	0.394	0.191	2.307

* Concentrations of components of mixtures are in mg a.i./kg wheat.

** Resistance factor for resistance to malathion (cross-resistance to other organophosphorus insecticides is assumed to be zero).

Table 5.2b

Minimum cost mixtures calculated from 24 hour bioassay data (dust formulation) with projections for increasing specific resistance to malathion in *Tribolium castaneum*.

cost ratio		RF**	K ₉₅	X ₁ (malathion)*	X ₂ (pirimipho-me.)*	Cost C
mala.	pirim.					
1.0	1.0	1	1.26	0	0.562	0.562
		15	0.084	0	0.562	0.562
		20	0.063	0	0.562	0.562
		30	0.042	0	0.562	0.562
		40	0.031	0	0.562	0.562
		50	0.025	0	0.562	0.562
		100	0.013	0	0.562	0.562
1.0	1.8	1	1.26	0	0.562	1.011
		15	0.084	0	0.562	1.011
		20	0.063	0	0.562	1.011
		30	0.042	0	0.562	1.011
		40	0.031	0	0.562	1.011
		50	0.025	0	0.562	1.011
		100	0.013	0	0.562	1.011
1.0	1.9	1	1.26	1.053	0	1.053
		15	0.084	0.619	0.231	1.059
		20	0.063	0.613	0.234	1.059
		30	0.042	0.607	0.238	1.059
		40	0.031	0.604	0.239	1.059
		50	0.025	0.603	0.240	1.059
		100	0.013	0.599	0.242	1.059
1.0	10.0	1	1.26	1.053	0	1.053
		15	0.084	0.619	0.231	2.932
		20	0.063	0.613	0.234	2.958
		30	0.042	0.607	0.238	2.984
		40	0.031	0.604	0.239	2.998
		50	0.025	0.603	0.240	3.005
		100	0.013	0.599	0.242	3.019

* Concentrations of components of mixtures are in mg a.i./kg wheat.

** Resistance factor resistance to malathion (cross-resistance to other organophosphorus insecticides is assumed to be zero).

5.5 Calculation of minimum cost mixtures of malathion + etrimfos and malathion + pirimiphos methyl for protection of wheat against attack by seven insect species including the Kano (specifically malathion-resistant) strain of *Tribolium castaneum* using 48 h dust admixture bioassays

Coefficients k_{ij} (equation iv - Section 4.2) were calculated from the bioassay data using an n.e.d. value for y of 1.645, corresponding to 95% mortality in each of the 7 species tested and the values of the regression parameters shown in Table 4.4. The linear program for calculation of a minimum cost mixture is shown in Table 5.3.

Solutions were obtained using the LINPROG program and are presented in Tables 5.4a (for K_{95} values) and 5.4b (for K_{99} values) in which costs per unit of concentration (mg a.i./kg wheat) for etrimfos and pirimiphos methyl are expressed relative to that of malathion and were each varied between 1 and 10 times that of malathion. No investigation was carried out to identify ternary mixtures of the insecticides by varying the cost ratios of both pirimiphos methyl and etrimfos simultaneously as such mixtures were considered to be of little practical interest. Consequently, the problem of finding minimum cost mixtures was reduced to finding combinations which controlled *Rhyzopertha dominica* and the *T. castaneum* malathion-resistant strain at the required response level and at the minimum cost. This is illustrated graphically in Figure 5.4 for malathion : pirimiphos methyl mixtures and in Figure 5.5 for malathion :

Table 5.3

Constraint coefficients (k_1 (LC95, $y=1.645$) and k_2 (LC99, $y=2.326$) for malathion (4%), etrimfos (2%), and pirimiphos-methyl (2%) expressed in the form of inequalities defining insecticide mixtures giving greater than 95% and 99% kill of species tested (48 hour bioassay) .

species	constraint equation coefficient for					
		malathion		pirimiphos-methyl		etrimfos
<i>T. castaneum</i> (susceptible)	k_1	2.05	$x_1 +$	4.63	$x_2 +$	5.77 $x_3 > 1$
	k_2	1.84	$x_1 +$	4.03	$x_2 +$	5.16 $x_3 > 1$
<i>T. castaneum</i> (malathion- resistant)	k_1	0.01	$x_1 +$	2.40	$x_2 +$	2.38 $x_3 > 1$
	k_2	0.009	$x_1 +$	1.87	$x_2 +$	1.83 $x_3 > 1$
<i>T. confusum</i>	k_1	0.22	$x_1 +$	0.62	$x_2 +$	1.00 $x_3 > 1$
	k_2	0.16	$x_1 +$	0.50	$x_2 +$	0.79 $x_3 > 1$
<i>O. surinamensis</i>	k_1	3.85	$x_1 +$	5.18	$x_2 +$	13.87 $x_3 > 1$
	k_2	3.15	$x_1 +$	4.62	$x_2 +$	12.78 $x_3 > 1$
<i>O. surinamensis</i> (Greek strain)	k_1	5.03	$x_1 +$	3.72	$x_2 +$	8.16 $x_3 > 1$
	k_2	4.04	$x_1 +$	3.07	$x_2 +$	6.17 $x_3 > 1$
<i>O. mercator</i>	k_1	4.34	$x_1 +$	4.32	$x_2 +$	10.33 $x_3 > 1$
	k_2	3.56	$x_1 +$	3.79	$x_2 +$	8.72 $x_3 > 1$
<i>S. granarius</i> (susceptible)	k_1	1.39	$x_1 +$	3.09	$x_2 +$	4.93 $x_3 > 1$
	k_2	1.24	$x_1 +$	2.77	$x_2 +$	4.20 $x_3 > 1$
<i>S. granarius</i> (gamma-HCH resistant)	k_1	0.45	$x_1 +$	1.55	$x_2 +$	2.52 $x_3 > 1$
	k_2	0.34	$x_1 +$	1.17	$x_2 +$	1.97 $x_3 > 1$
<i>S. oryzae</i>	k_1	1.16	$x_1 +$	2.61	$x_2 +$	4.03 $x_3 > 1$
	k_2	0.92	$x_1 +$	2.21	$x_2 +$	3.11 $x_3 > 1$
<i>R. dominica</i>	k_1	0.10	$x_1 +$	0.11	$x_2 +$	0.26 $x_3 > 1$
	k_2	0.07	$x_1 +$	0.08	$x_2 +$	0.18 $x_3 > 1$

Table 5.4 a

Minimum cost mixtures calculated from 48 hour bioassay data (dust formulations) to control seven species of stored product insects including a malathion-resistant strain of *T. castaneum* and gamma-HCH resistant *S. granarius* giving greater than 95% kill.

cost ratio		dose of pesticide to be used (mg a.i/kg wheat)		cost C
malathion	etrimfos	X ₁ malathion	X ₂ etrimfos	
1.0	1.0	0	3.846	3.846
1.0	2.5	0	3.846	9.615
1.0	2.6	9.006	0.382	10.000
1.0	10.0	9.006	0.382	12.892
malathion	pirimiphos methyl	malathion	pirimiphos methyl	
1.0	1.0	0	9.091	9.091
1.0	1.1	9.586	0.377	10.000
1.0	2.0	9.586	0.377	10.339
1.0	10.0	9.586	0.377	13.353

Table 5.4 b

Minimum cost mixtures calculated from 48 hour bioassay data (dust formulations) to control seven species of stored product insects including a malathion-resistant strain of *T. castaneum* and gamma-HCH resistant *S. granarius* giving greater than 99% kill.

cost ratio		dose of pesticide to be used (mg a.i/kg wheat)		cost C
malathion	etrimfos	X ₁ malathion	X ₂ etrimfos	
1.0	1.0	0	5.556	5.566
1.0	2.5	0	5.556	13.889
1.0	2.6	13.046	0.482	14.299
1.0	10.0	13.046	0.482	17.868
malathion	pirimiphos methyl	malathion	pirimiphos methyl	
1.0	1.0	0	12.500	12.500
1.0	1.1	0	12.500	13.750
1.0	1.2	13.750	0.469	14.312
1.0	10.0	13.750	0.469	18.440

etrimfos mixtures.

In both cases, three solutions exist, depending on the ratio of the cost of the malathion dust to pirimiphos methyl or etrimfos dust. In the case of malathion : pirimiphos methyl dust mixtures, if the cost ratio of malathion : pirimiphos methyl is more than 1 : 240 then malathion dust applied at 100 mg a.i./kg wheat is cheaper than the use of a mixture, but in the more probable event of the ratio lying between 1 : 1.101 and 1 : 240 then the minimum cost solution defined by point A (Fig 5.4) is to use a mixture containing 9.586 mg malathion a.i. and 0.377 mg pirimiphos methyl a.i./kg wheat. The third solution (9.091 mg pirimiphos methyl/kg wheat) at a cost ratio malathion : pirimiphos methyl of less than 1 : 1.101 is also less interesting than the use of a mixture, given the current costs of dust formulations of these two products. The use of the mixture or the single compounds at these rates will give 95% control or greater of all species tested.

The corresponding results for malathion : etrimfos mixtures are the following three solutions; first if the cost ratio is 1:2.59 , then etrimfos alone (at 3.846 mg a.i./kg wheat) is cheaper ; the second if the cost ratio lying between 1:2.60 and 1:238, then the minimum cost solution defined by point A (Fig. 5.5) is to use a mixture containing 9.006 mg (a.i.) malathion and 0.382 mg (a.i.) etrimfos/kg wheat. Thirdly, if the cost ratio is more than 1 : 238 then malathion applied alone (at 100 mg

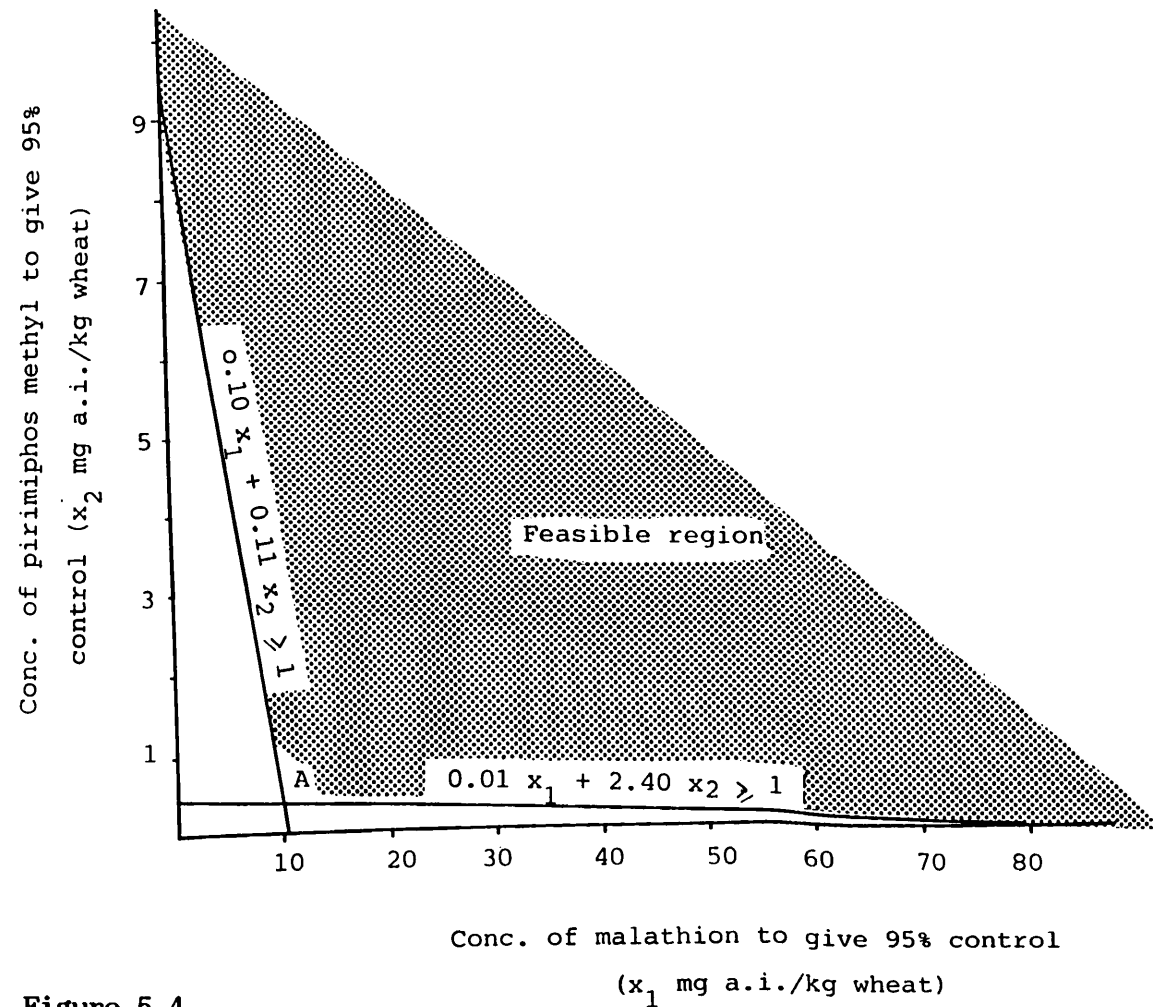


Figure 5.4

Feasible region corresponding to concentrations of malathion (x_1) and pirimiphos methyl (x_2) which jointly give control of both *R. dominica* and malathion-resistant strain of *T. castaneum* at or above the 95% mortality level.

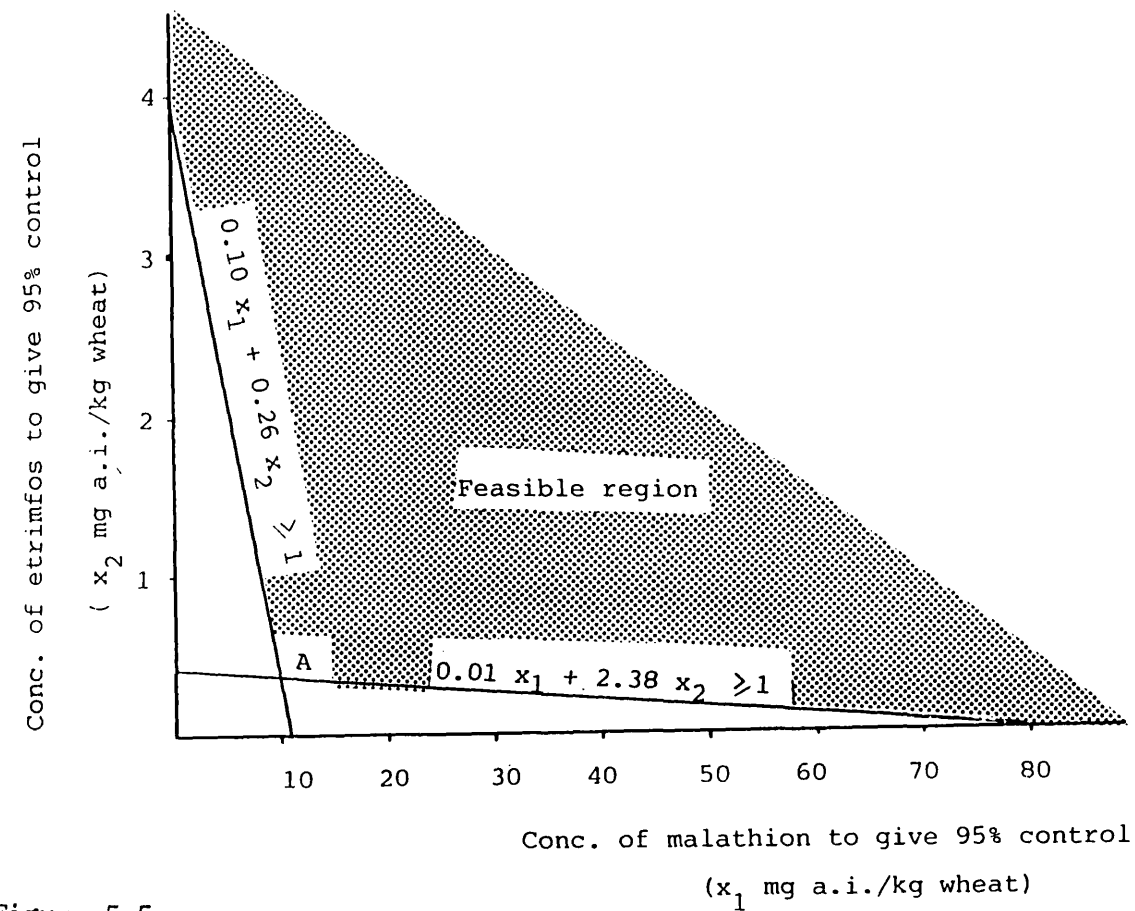


Figure 5.5

Feasible region corresponding to concentrations of malathion (x_1) and etrimfos (x_2) which jointly give control of both *R. dominica* and malathion-resistant strain of *T. castaneum* at or above the 95% mortality level.

a.i./kg wheat) will be cheaper than the use of a mixture.

5.6 Analysis of minimum cost mixtures of malathion with deltamethrin to control mixed populations of *S. granarius* (R) with *T. castaneum* (S) and of gamma-HCH with deltamethrin for control of mixed populations of *S. granarius* (R) with *T. castaneum* (S) (dust admixture, 7 day bioassays).

Constraint equations for the two linear programming problems are shown in Fig. 5.6 and 5.7. together with their corresponding graphs.

In the case of the malathion/deltamethrin mixtures, for cost ratios of malathion/deltamethrin less than 0.6 : 1, malathion alone will give the cheapest control, for cost ratios between 0.5 : 1 and 2.0 : 1 malathion/deltamethrin mixtures give cheapest control, and for cost ratios greater than 1.9 : 1 deltamethrin gives the cheapest control. Fig. 5.6 also gives total costs, in terms of the cost of a unit application of malathion, for minimum cost mixtures at various cost ratios between 0.5 : 1 and 10 : 1.

Similarly, in the case of gamma-HCH/deltamethrin mixtures, deltamethrin dust gives the cheapest control for cost ratios of gamma-HCH/deltamethrin less than 1 : 2.2, mixtures only being necessary (for > 95% control of both species) for cost ratios greater than 1 : 2.1 and less than 1 : 26.

Figure 5.6

Minimum cost mixtures calculated from 7 day bioassay data (dust formulation).

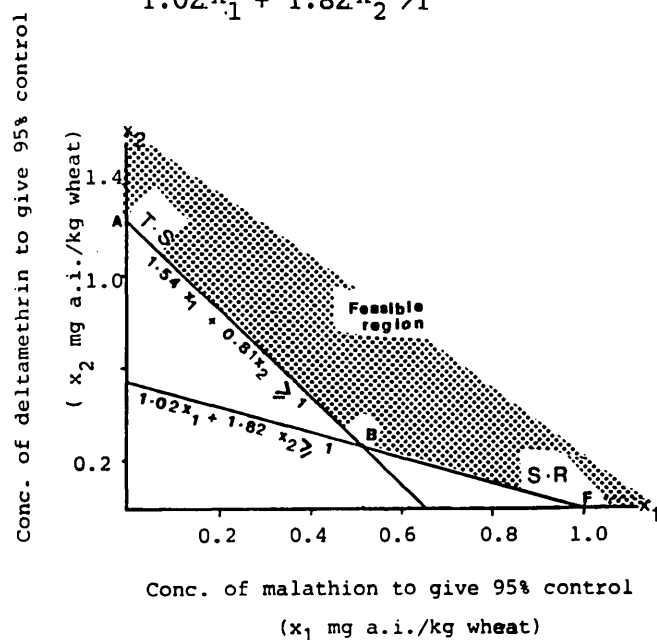
minimise cost function

$$C = c_1x_1 + c_2x_2$$

subject to

$$1.54x_1 + 0.81x_2 \geq 1$$

$$1.02x_1 + 1.82x_2 \geq 1$$



cost ratio malathion	deltamethrin	x_1 malathion	x_2 deltamethrin	cost C	optimal solution
0.5	1.0	0.980	0.0	0.490	F
0.6	1.0	0.511	0.263	0.570	B
1.9	1.0	0.511	0.263	1.234	B
2.0	1.0	0.0	1.235	0.958	A
10.0	1.0	0.0	1.235	1.235	A

S.R = *Sitophilus granarius* (gamma-HCH resistant)

T.S = *Tribolium castaneum* (susceptible strain)

* Feasible region corresponding to concentrations of malathion (x_1) and deltamethrin (x_2) which jointly give control of both *Tribolium castaneum* (susceptible strain) and gamma-HCH resistant strain of *S. granarius* at or above the 95% mortality level.

Figure 5.7

Minimum cost mixtures calculated from 7 day bioassay data (dust formulation).

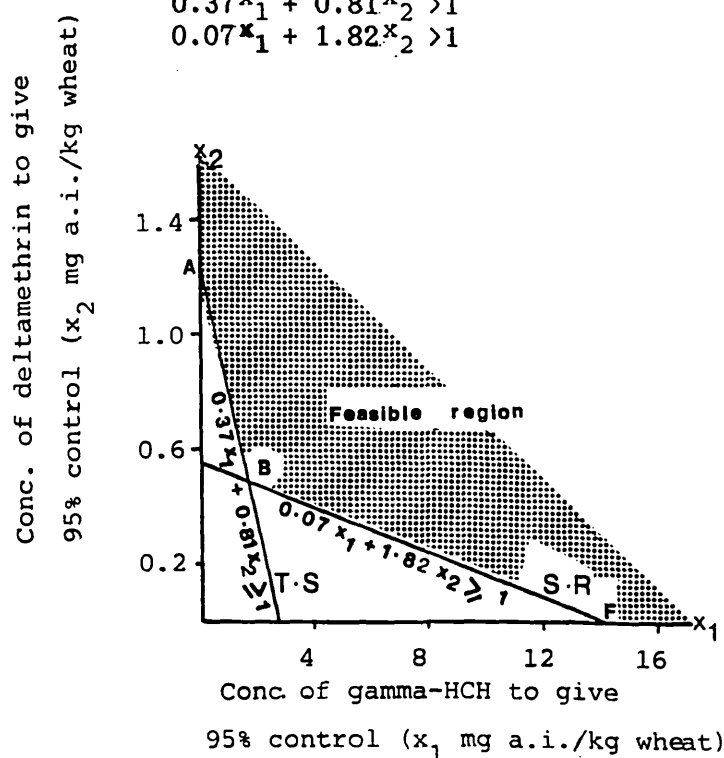
minimise cost function

$$C = c_1x_1 + c_2x_2$$

subject to

$$0.37x_1 + 0.81x_2 > 1$$

$$0.07x_1 + 1.82x_2 > 1$$



cost ratio		x_1	x_2	cost	optimal
gamma-HCH	deltamethrin	gamma-HCH	deltamethrin	C	solution
1.0	1.0	0	1.235	1.235	A
1.0	2.0	0	1.235	2.469	A
1.0	2.1	0	1.235	2.593	A
1.0	2.2	1.638	0.486	2.708	B
1.0	25.0	1.638	0.486	13.799	B
1.0	26	14.286	0	14.286	F

S.R = *Sitophilus granarius* (gamma-HCH resistant)

T.S = *Tribolium castaneum* (susceptible strain)

* Feasible region corresponding to concentrations of gamma-HCH (x_1) and deltamethrin (x_2) which jointly give control of both *T. castaneum* (susceptible strain) and gamma-HCH resistant strain of *S. granarius* at or above the 95% mortality level.

5.7 Analysis of minimum cost mixtures of malathion with deltamethrin and of pirimiphos methyl with deltamethrin to control mixed populations of *R. dominica*, *T. castaneum*, *S. granarius*, and *O. surinamensis*. (residual film bioassay, 48 hr)

The constraint coefficient for the linear programming problem are shown in Table 5.5. Solutions are illustrated in Table 5.6a (using K_{95} values) and Table 5.6b (using K_{99} values) for various deltamethrin : pirimiphos methyl cost ratios. The graph of the linear program requiring control *T. castaneum* and *S. granarius* at or above 95% response level using the optimal mixture of deltamethrin and pirimiphos-methyl is similarly illustrated in Figure 5.8.

5.8 Extensions of the Linear Programming Approach to Optimal Applications of Insecticide Mixtures to Control Stored Grain Pests

Extensions to cover problem of applications which will give greater than permitted maximum residue limits

Additional constraints can be included in the linear programming problems outlined above to eliminate feasible solutions which are

Table 5.5

Values of reciprocal of LC95 ($k_{0.5}$) and LC99 ($k_{0.9}$) calculated for four insect species when bioassayed against deltamethrin, malathion, and pirimiphos methyl (48 hour residual film bioassay)

species		constraint equation coefficient for					
		deltamethrin		malathion		pirimiphos-me	
<i>O. surinamensis</i>	k_1	3.15	+	13.91	+	6.83	> 1
	k_2	0.89	+	9.65	+	4.27	> 1
<i>T. castaneum</i> (susceptible)	k_1	1.64	+	4.32	+	10.01	> 1
	K_2	0.94	+	3.41	+	7.58	> 1
<i>S. granarius</i> (susceptible)	k_1	5.47	+	7.52	+	6.39	> 1
	k_2	2.40	+	6.09	+	4.59	> 1
<i>R. dominica</i>	k_1	0.29	+	0.60	+	0.47	> 1
	k_2	0.12	+	0.42	+	0.33	> 1

Table 5.6 a

Solution of a linear programming problem to control 4 susceptible species of stored product insects giving 95% kill from 48 hour residual film bioassay

cost ratio		dose of pesticide (s) to be used (mg/ml)		Cost (C)
deltamethrin	malathion	X ₁ deltamethrin	X ₂ malathion	
1.0	1.0	0	1.667	1.667
1.0	2.0	0	3.333	3.333
1.0	2.1	3.448	3.448	3.448
1.0	10.0	3.448	3.448	3.448
deltamethrin	pirimiphos methyl	deltamethrin	pirimiphos methyl	
1.0	1.0	0	2.128	2.128
1.0	1.6	0	2.128	3.404
1.0	1.7	3.448	0	3.448
1.0	10.0	3.448	0	3.448

Table 5.6b

Solution of a linear programming problem to control 4 susceptible species of stored product insects giving 99% kill from 48 hour residual film bioassay

cost ratio		dose of pesticide (s) to be used (mg/ml)		Cost (C)
deltamethrin	malathion	X ₁ deltamethrin	X ₂ malathion	
1.0	1.0	0	2.381	2.381
1.0	3.5	0	2.381	8.333
1.0	3.6	8.333	0	8.333
1.0	10.0	8.333	0	8.333
deltamethrin	pirimiphos methyl	deltamethrin	pirimiphos methyl	
1.0	1.0	0	3.030	3.030
1.0	2.7	0	3.030	8.182
1.0	2.8	8.333	0	8.333
1.0	10.0	8.333	0	8.333

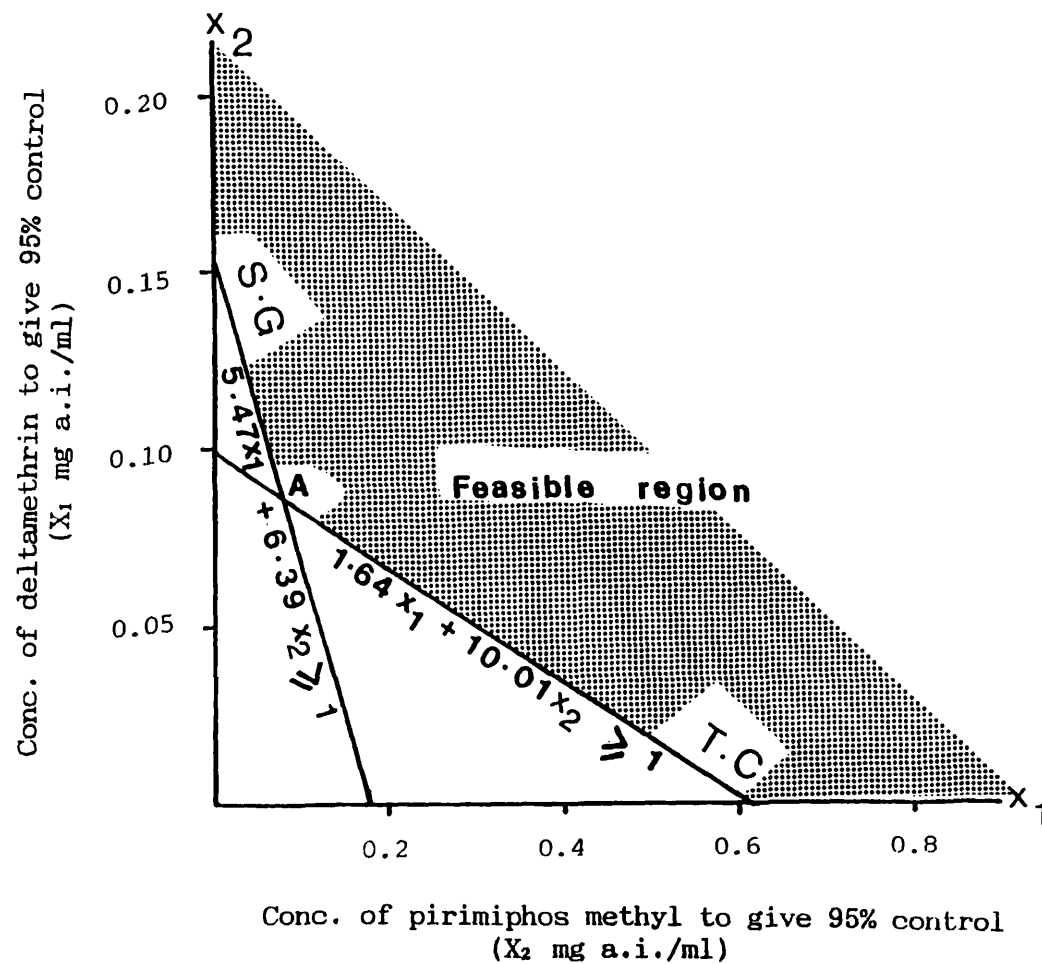


Figure 5.8

Feasible region corresponding to concentrations of deltamethrin (X_1) and pirimiphos methyl (X_2) which jointly give control of both *S. granarius* and malathion-resistant strain of *T. castaneum* at or above the 95% mortality level.

unacceptable on the basis of criteria other than those of required control levels. The patterns of degradation described in Chapter 3 for malathion, pirimiphos methyl and etrimfos can be used to predict the concentration of insecticide in the wheat at any time after application at the temperature, humidity, and initial application rate investigated. For insecticide degradation under conditions of relatively low grain moisture content, it is preferable to use an equation based on a model which incorporates diffusion of insecticide to areas of the wheat grain where it is largely protected from metabolic decay (Section 3.2.5).

For any given storage time t , the maximum residue limit can be substituted for C to calculate the corresponding initial concentration C_0 for any of the insecticides in a mixed formulation on the assumption that similar expressions could be identified for other starting concentrations. This then gives rise to constraint equations of the form

$$x_i < C_{0i}$$

for each insecticide, which may be included in the linear programming problem. The feasible region will be reduced to a finite area, and new optimal solutions may be found, depending on the cost ratios of the components. The situation is illustrated in Figure 5.9.

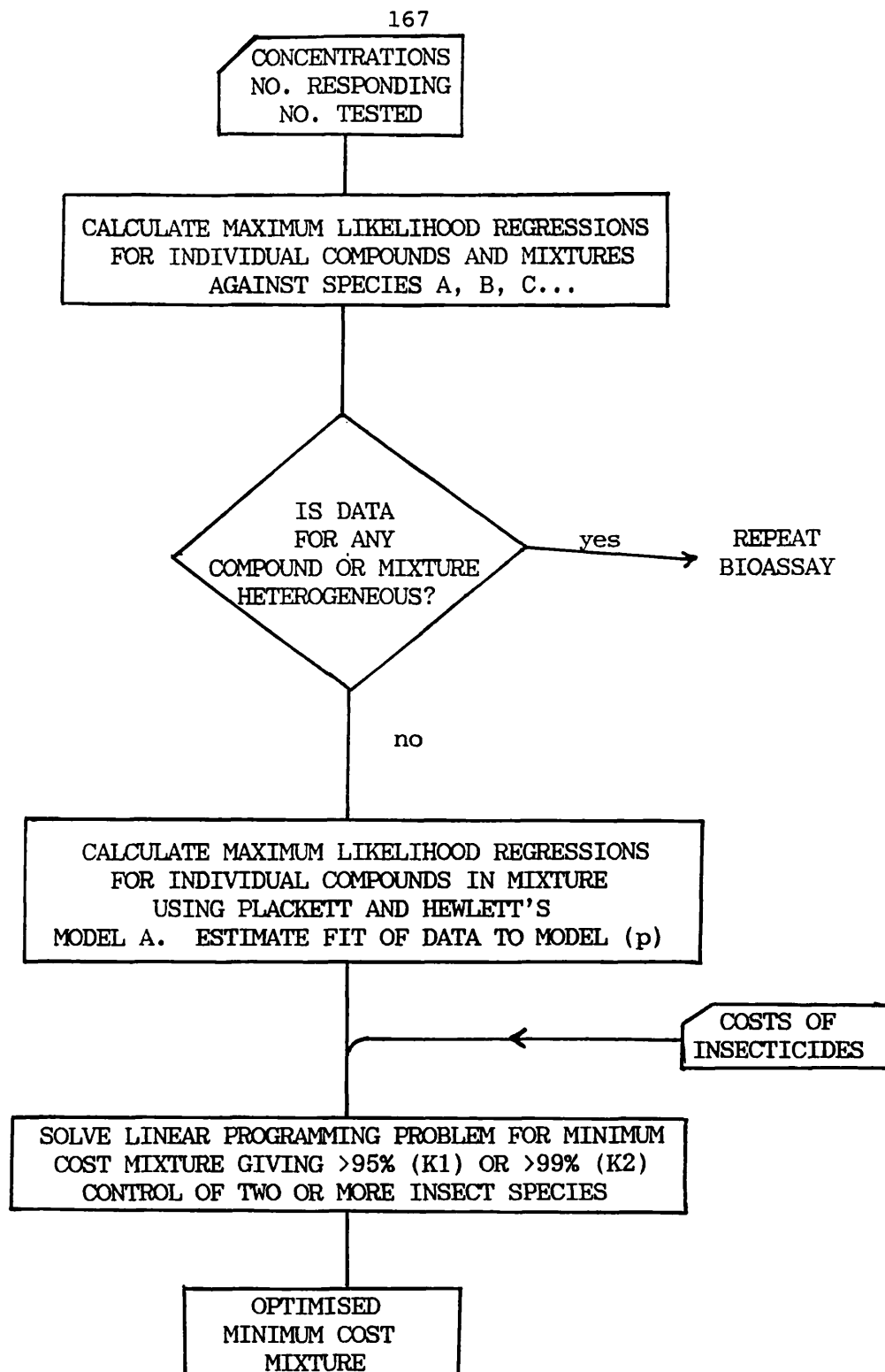


Figure 5.9

Flow chart for calculation of application rate and cost of minimum cost mixture of two or more insecticides to control two or more insect species at or above a chosen level.

5.9 Discussion

Linear programming is widely used in economic models, and involves optimising a utility function, such as profit, subject to a series of linear constraints. In most practical problems the number of constraints is large, frequently over 100, the number of variables may be of a similar order of magnitude, and the location of the optimal solution can only be achieved using a fast digital computer.

The problem of identifying optimum mixtures of insecticides to control a complex of insect pests in stored grain is on a much smaller scale. Mixtures of three or more insecticides have rarely been used in the field and the number of constraints likely to be incorporated into any realistic problem is likely to be in single figures. The questions that arise in the application of the technique to insect pest control are: can the method be used for rapid screening of insecticides against a complex of pests under laboratory conditions? and, are the results likely to be useful in estimating field application rates of mixtures?

In general, screening procedures to establish the usefulness of novel insecticides seek to establish the relation between application rate and time before control breakdown for each of a number of test species under a variety of storage conditions. Careful laboratory assays producing $\log(\text{dose})/\text{n.e.d.}(\text{response})$ regressions which are sufficiently homogeneous

to be of use in testing for additivity of action of mixtures are possibly of secondary importance in the development of mixtures for application. Nevertheless, data of this type has been made available from a company interested in developing a binary mixture for control of strains of *S. oryzae*, *T. castaneum* and *R. dominica* in wheat. The data consisted of bioassay data for 4 individual compounds, the 6 possible 1:1 w/w binary mixtures of them, and cost ratios for the compounds. Using the method described in this section of the thesis, it was possible to calculate the overall optimum mixture composition and an application rate which would, under the bioassay conditions, give > 95% response of the three species (le Patourel, unpublished results). Calculation of an application rate and mixture composition which would decay to this optimum mixture over the period for which >95% control of the three species was required would have required information on the rate of decay of biological activity of the test insecticides under a range of storage conditions. However, had this been available there seems no reason to suppose that any mixture other than the one defined by this process would be more usefully tested in the field.

Rhyzopertha dominica is a well-known example of a species which is more tolerant of organophosphorus insecticides than many other species, yet which has a comparable or lower tolerance of certain insecticides from other groups than the majority of stored beetle pests. Other examples of such natural variations in tolerance found with adult *T. confusum* to many

organophosphorus insecticides than *T. castaneum*. When a strain of a particular species develops resistance to one or more insecticides, as in the cases of malathion-resistance in the Kano strain of *T. castaneum* and gamma-HCH-resistance in the Lamia strain of *S. granarius* then the use of mixtures to provide protection of stored grain from a number of pests including this species would seem to offer advantages of cost over a change to a more expensive compound effective against the pest complex. The FAO survey identified malathion resistance in many strains of *T. castaneum* where other species retained their susceptibility (Champ and Dyte, 1976).

CHAPTER 6

Some Effects of Malathion, Etrimfos and Pirimiphos methyl on *S. granarius* and *R. dominica* Adults Surviving Treatment

6.1 Introduction

The acute toxic effects of stored grain insecticides are easy to measure and are the most commonly used indicators of biological action. However, insecticides may also influence the biological fitness of survivors. In the case of fumigants, survivors of one treatment may be exposed to subsequent treatments during the storage period, and the demonstration by Bond and Upitis (1973) that the time required for survivors of phosphine application to recover full tolerance to the compound varied considerably with insect species suggests that subsequent application rates could be varied depending on the type of infestation.

Residual insecticides are generally applied to grain with the intention of providing protection throughout the storage period, although over this time the concentration of toxicant decreases as a result of chemical decay and biological inactivation. As a result, an increasing proportion of any insect population will survive treatment, and the question arises as to the fate and biological fitness of the survivors, particularly with

respect to their capacity to mate and produce a subsequent generation. The issue is of practical importance, since the progeny of survivors of treatments will emerge into grain which may contain insecticide residues at too low a level to produce significant mortality, and at a time when the grain is due to be inspected for sale or trans-shipment. Decay of insecticides during storage is a useful attribute with respect to consumption of residues by the public, but may make the grain susceptible to infestation during the storage period. An inhibitory effect on progeny production by survivors of a treatment is therefore a useful characteristic of insecticides used for stored grain protection.

This section describes attempts to establish whether or not treatments with pirimiphos methyl, etrimfos and malathion dust formulations had any such effects on populations of *S. granarius* and *R. dominica* which survived treatment with these insecticides.

6.2 Toxicity of etrimfos, malathion, pirimiphos methyl and their binary mixtures to adult populations of *S. granarius* and *R. dominica* (dust-treated wheat, 7 day bioassays).

An initial experiment established the dose/toxicity relationships for etrimfos, malathion, and pirimiphos methyl so that application rates to measure effects of these insecticides on reproduction by survivors could

be related to those producing known levels of mortality in the populations. A 7 day bioassay period was chosen since an exposure time of this length generally gives an end-point mortality for organophosphorus insecticides (Champ and Campbell-Brown 1970a), although no experiments were carried out to check this for the insecticides and insect species used.

Regression equations and associated statistics for the bioassays are given in Tables 6.1 and 6.2 . As in the previous bioassays using shorter exposure periods (Sections 4.6, 4.7 and 4.8.1) etrimfos was most toxic and malathion was least toxic to *S. granarius*. The toxicity of all three compounds was an order of magnitude lower to *R. dominica*, with etrimfos again the most toxic compound and pirimiphos methyl and malathion having comparable toxicities.

Binary mixtures of the insecticides were also tested as a continuation of the studies described in Chapter 4, and the results tested against the hypothesis of additive action using the "MIX" program. The only case which was not consistent with the hypothesis was that for wheat treated with the mixture of etrimfos and malathion dusts and bioassayed against *R. dominica* ($p < 0.05$).

Table 6.1

Response of *S. granarius* to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) applied alone or as 1:1 (w/w) mixtures and analysis of bioassay data for fit to model for additive action (7 day bioassays at 25°C)

insecticide	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate)	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate including mixture data)	LC50 (mg a.i./kg) (95 % fiducial limits)	LC 95 (mg a.i./kg)
etrimfos	$y = 8.4 + 9.9 x$ $p = 0.25$		0.144 0.136-0.152	0.211 0.193-0.230
malathion	$y = 3.5 + 11.0 x$ $p = 0.41$		0.479 0.460-0.499	0.677 0.731-0.726
pirimiphos methyl	$y = 5.4 + 8.4 x$ $p = 0.45$		0.229 0.214-0.244	0.359 0.322-0.399
etrimfos + malathion (1:1)	$y = 4.7 + 8.7 x$ $p = 0.54$	$y = 7.4 + 8.9 x$ $y = 3.3 + 10.5 x$ $p = 0.07$	calculated (1) 0.270 calculated (2) 0.274 experimental 0.288 0.272-0.306	0.321 0.339 0.446 0.407-0.488
pirimiphos met + malathion (1:1)	$y = 4.0 + 8.6 x$ $p = 0.24$	$y = 5.2 + 8.1 x$ $y = 3.5 + 10.8 x$ $p = 0.38$	calculated (1) 0.351 calculated (2) 0.347 experimental 0.339 0.319-0.360	0.468 0.469 0.528 0.475-0.587
pirimiphos met + etrimfos (1:1)	$y = 5.7 + 7.6 x$ $p = 0.57$	$y = 5.0 + 7.9 x$ $y = 7.4 + 8.8 x$ $p = 0.23$	calculated (1) 0.177 calculated (2) 0.178 experimental 0.177 0.167-0.188	0.265 0.277 0.292 0.268-0.318

(1) Calculated values based on regression lines for two compounds bioassayed separately.

(2) Calculated values based on regression lines calculated from compounds bioassayed separately and as 1:1 mixture (w/w dust formulation).

p probability corresponding to chi-square.

Table 6.2

Response of *E. dominica* to malathion (4%), pirimiphos methyl (2%) and etrimfos (2%) applied alone or as 1:1 (w/w) mixtures and analysis of bioassay data for fit to model for additive action (7 day bioassays at 25°C)

insecticide	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate)	regression of N.E.D. response (y) on log dose (x) (Maximum likelihood estimate including mixture data)	LC50 (µg a.i./kg) (95 % fiducial limits)	LC 95 (µg a.i./kg)
etrimfos	$y = -0.4 + 5.0 x$ $p = 0.67$		1.191 1.096-1.293	2.532 2.195-3.038
malathion	$y = -1.9 + 4.0 x$ $p = 0.49$		2.969 2.753-3.205	7.740 6.270-9.600
pirimiphos methyl	$y = -2.4 + 5.4 x$ $p = 0.53$		2.788 2.627-2.959	5.617 4.983-6.330
etrimfos + malathion (1:1)	$y = -1.5 + 6.3 x$ $p = 0.92$	$y = -0.3 + 5.5 x$ $y = -2.0 + 4.5 x$ $p = 0.04$	calculated (1) 1.980 calculated (2) 1.888 experimental 1.739 1.614-1.871	3.806 3.355 3.181 2.830-3.594
pirimiphos me+ malathion (1:1)	$y = -2.9 + 6.5 x$ $p = 0.83$	$y = -2.5 + 5.7 x$ $y = -2.1 + 4.7 x$ $p = 0.08$	calculated (1) 2.910 calculated (2) 2.802 experimental 2.742 2.580-2.913	6.495 5.793 4.914 4.433-5.441
pirimiphos me+ etrimfos (1:1)	$y = -1.7 + 6.0 x$ $p = 0.66$	$y = -2.5 + 5.5 x$ $y = -0.6 + 5.3 x$ $p = 0.09$	calculated (1) 1.668 calculated (2) 1.775 experimental 1.940 1.822-2.065	3.482 3.599 3.665 3.276-4.118

(1) Calculated values based on regression lines for two compounds bioassayed separately.

(2) Calculated values based on regression lines calculated from compounds bioassayed separately and as 1:1 mixture (w/w dust formulation).

P probability corresponding to chi-square.

6.3 Progeny production by *R. dominica* and *S. granarius* by survivors of etrimfos, pirimiphos methyl, or malathion treatments (and binary mixtures) using various dose levels.

Three dose levels were used for both species, corresponding to the LC5, LC10 and LC20 as calculated from the regression equations for 7 day bioassays (Tables 6.1 and 6.2). The 50 adult insects added to each bioassay jar were not sexed and were assumed to consist of an equal number of males and females. Populations of the two species investigated are known to have a sex ratio of 1 : 1 (Richards , 1947; Shires, 1979), and if the culture jars were considered as "large" populations (generally more than 3000 individuals) then the probability of the sex ratio in the sample lying between 0.9 and 1.1 is 0.33 (Binomial test). The adults were removed from the jars at 7 days and the jars then incubated for a further 9 weeks. At the end of this time the wheat grains were examined individually and any which were seen to contain unemerged adults were broken open. In the case of *R. dominica* it was necessary to pierce the grains with a mounted needle to identify those containing adults. The mean number of adult progeny, both dead and alive, for the two replicates are recorded in Appendices 6.1 and 6.2 for *S. granarius* and *R. dominica* respectively, and are presented as Figures 6.1 and 6.2.

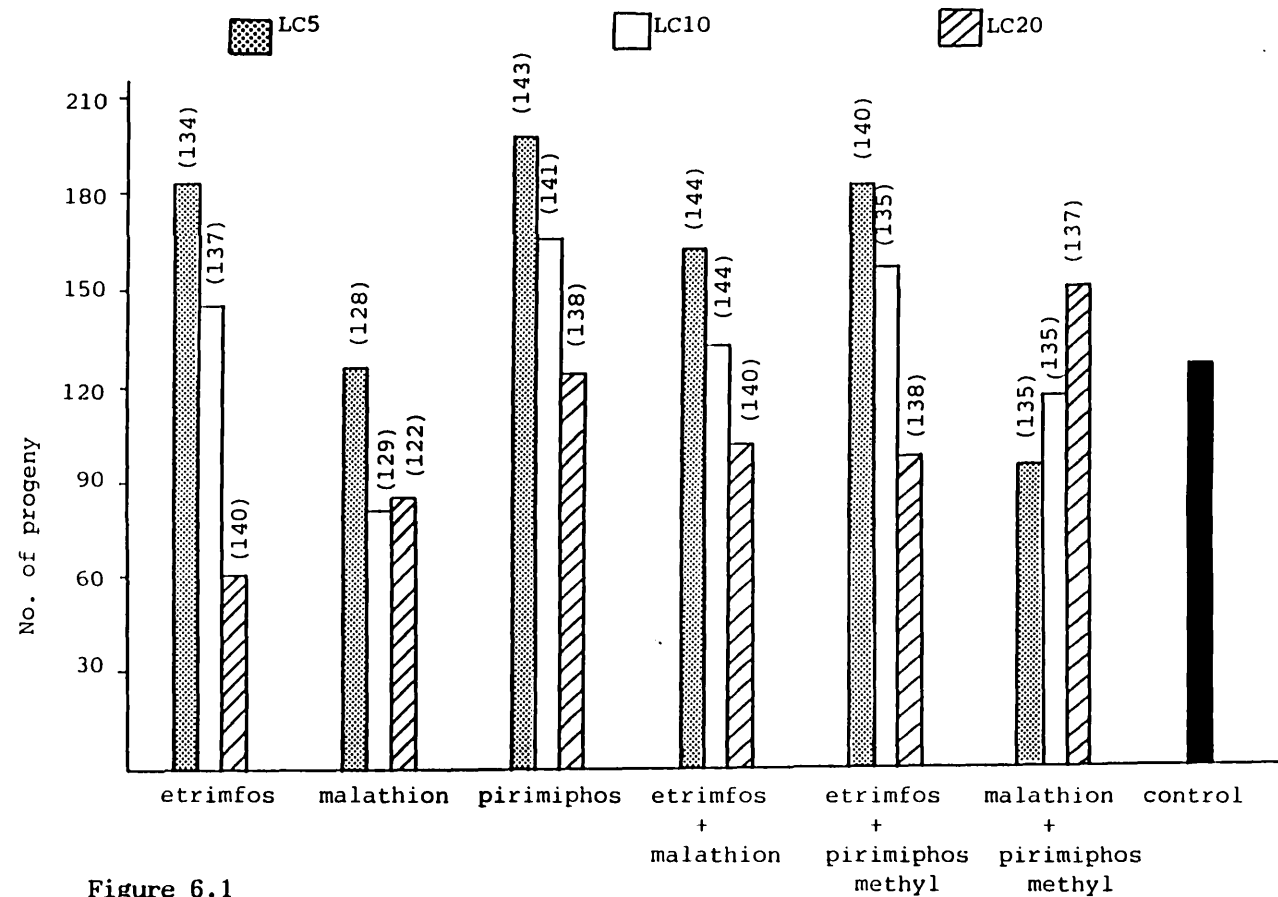


Figure 6.1

The effect of three different concentrations of etrimfos (2%), malathion (4%), pirimiphos methyl (2%) and their binary mixtures admixed with whole wheat on progeny production (F_1) by *S. granarius*.

Figures in brackets at the top of each histogram correspond to expected numbers calculated on the basis of the number of surviving females in the sample assuming 1:1 sex ratio and same fecundity as in control.

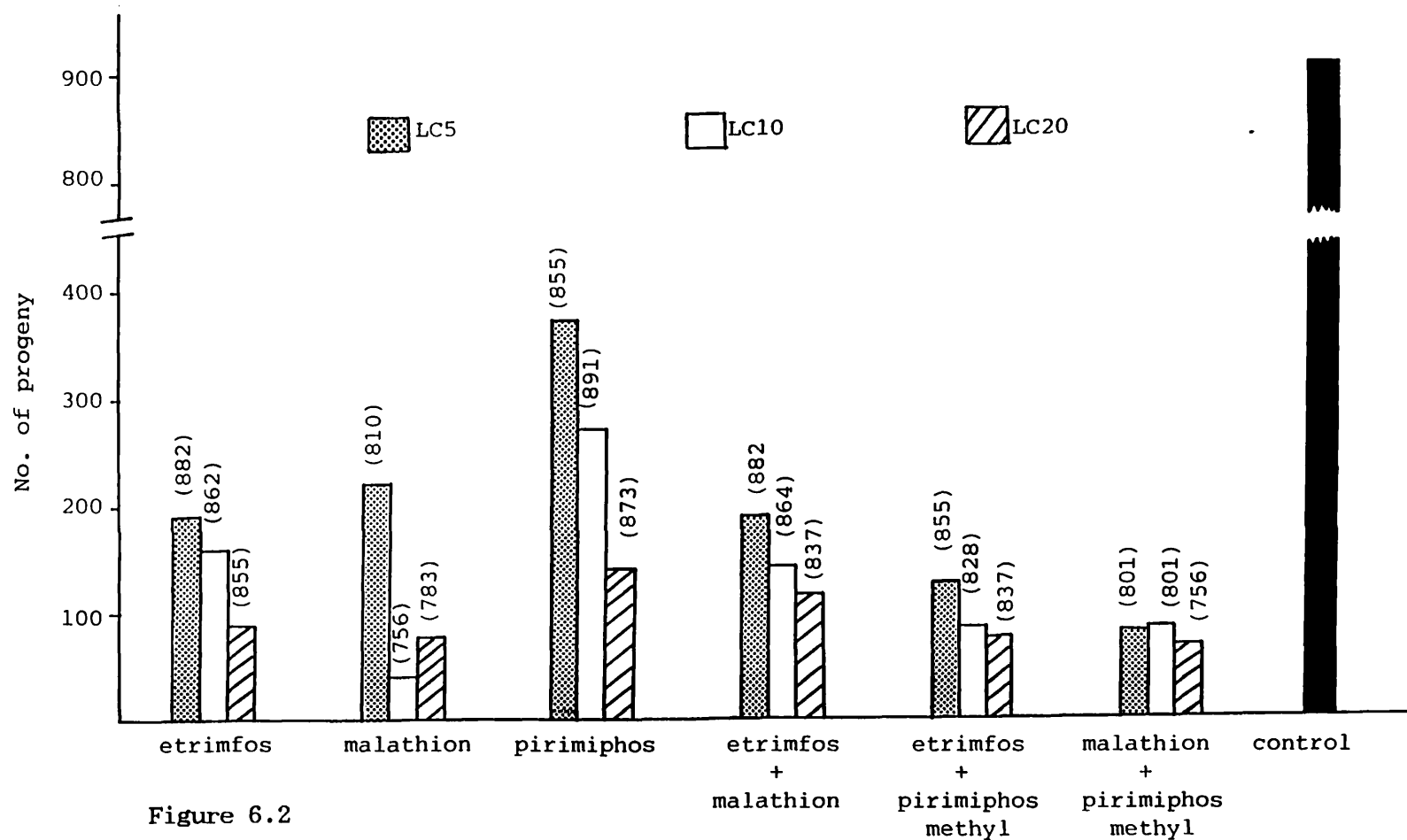


Figure 6.2

The effect of three different concentrations of etrimfos (2%), malathion (4%), pirimiphos methyl (2%) and their binary mixtures admixed with whole wheat on progeny production (F_1) by *R. dominica*.

Figures in brackets at the top of each histogram correspond to expected numbers calculated on the basis of the number of surviving females in the sample assuming 1:1 sex ratio and same fecundity as in control.

The results illustrate the anticipated trend towards lower progeny production as the applied dose of dust formulation of the insecticides and their mixtures was increased. The control experiment indicated a mean of 6 progeny per female for *S. granarius* and 36 per female for *R. dominica* (eggs laid which matured to emerging adults) over the period of the experiment (assuming a 1 : 1 ratio of females to males in the population samples taken). On the conservative assumption that no female which died during the exposure period laid any eggs, the best estimate of the number of progeny expected to emerge from wheat treated at the LC5, LC10 and LC20 doses could be calculated for *S. granarius* and *R. dominica* using the observed number of survivors of each insecticide treatment and again assuming a 1 : 1 sex ratio. These expected numbers are also shown in Figures 6.1 and 6.2.

In the case of *S. granarius* the reduction in progeny production at the three dose levels is broadly in line with that predicted on the basis of mortality of the parent females during the experiment. Only the malathion : pirimiphos methyl mixture showed a discrepancy from the expected trend, but the number of replicates carried out did not permit a test for the significance of the result. The experiment established that any effects of these insecticidal dusts on progeny production by *S. granarius* adults surviving treatment are likely to be small, and unlikely to be of practical significance.

In the case of *R. dominica* there was a large difference between the mean production of progeny in the control experiment and that in all the insecticide treatments. Again, replication was insufficient to test the significance of this difference, but it was of an order of magnitude which made such a test unnecessary. In each case, fewer progeny were produced at the higher dose levels, but no more so than could be explained in terms of adult mortality during the 7 day oviposition period. More replication would be needed to establish the dose/response relationship for this inhibition of progeny production, but it is clear that a large response is produced at a dose which kills only 5% of the adults for all three insecticides, and that there is no great difference between the ability of the three insecticides to produce the response.

6.4 Effect of low doses of malathion, pirimiphos methyl, and etrimfos on the weight of wheat consumed over a 10 week period and the weight of insects surviving treatment

This experiment was carried out with only two replicates for each treatment. Batches of 50 insect were weighed, and then incubated with the calculated LC5, LC10 and LC20 doses of the three insecticides in 100 g wheat for a 7 day period. At the end of the incubation the surviving adults were weighed to determine any change in the mean weight of insects. Interpretation of this measurement could not distinguish between effects

due to feeding inhibition/promotion as a result of sub-lethal action of the insecticide treatments, and any selective action of the insecticides on insects with small or large body weights, but the intention was to investigate any large differences with more detailed experiments. In addition, the mean weight of the progeny which emerged from the wheat was determined after incubation of the wheat for 9 weeks, as was the weight of wheat remaining. Mean progeny weights were determined by weighing at least 10 replicate samples of 10 adult insects emerging from individual jars.

The results of the experiment are shown in Tables 6.3 and 6.4 for *S. granarius* and Tables 6.5 and 6.6 for *R. dominica*.

In the case of *S. granarius* the mean weight of the insects was slightly lower at the end of the incubation period for every treatment investigated, but the level of replication was insufficient to establish the significance of this result for individual treatments. The data for all treatments was pooled, and the null hypothesis that the mean weight of insects after 7 days at all treatment levels was the same as the mean weight at the beginning of the experiment was tested by a t-test. The hypothesis was rejected ($p < 0.001$).

The mean weight of the progeny emerging from the control treatment jar was significantly lower ($p < 0.001$) than the mean weight of the parent generation (obtained by pooling the mean initial weights of insects for

Table 6.3

Effect of low doses of dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) on *S. granarius* progeny

dose (mg a.i./kg)	expected % mortality*	% adult mortality at 7 days (2 replicates)	% mortality of progeny (2 replicates)
control	0.0	3 (2,4)	10.6 (8.3,12.9)
etrimfos			
0.101	7.3	11 (6,16)	14.1 (13.7,14.5)
0.109	13.0	9 (2,16)	9.0 (9.7,8.3)
0.118	21.5	7 (4,10)	10.4 (18,8.2)
malathion			
0.343	5.4	15 (14,16)	7.0 (7.9,6.1)
0.366	9.7	14 (20,8)	7.0 (6.0,7.9)
0.417	24.9	19 (20,18)	16.9 (13.2,20.5)
pirimiphos methyl			
0.148	5.8	5 (8,2)	9.5 (10.7,8.3)
0.163	11.2	6 (10,12)	8.4 (10.1,6.6)
0.183	21.4	8 (8,8)	8.1 (6.9,9.2)

* From data of Table 6.1

Table 6.3 (Cont.)

Effect of low doses of dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) on *S. granarius* progeny

dose (mg a.i./kg)	expected % mortality*	% adult mortality at 7 days (2 replicates)	% mortality of progeny (2 replicates)
etrimfos + malathion			
0.187	3.3	4 (0,8)	7.1 (6.2,7.9)
0.206	7.1	4 (0,8)	6.7 (3.4,10.0)
0.232	15.4	7 (6,8)	22.7 (22.4,22.9)
etrimfos + pirimiphos methyl			
0.109	5.3	7 (4,10)	15.1 (17.6,12.6)
0.122	10.7	10 (12,8)	5.8 (4.2,7.4)
0.139	20.8	8 (6,10)	8.0 (7.9,8.1)
malathion + pirimiphos methyl			
0.216	4.3	10 (14,6)	5.5 (3.8,7.2)
0.242	9.7	10 (12,8)	5.7 (5.8,5.6)
0.274	20.2	9 (12,6)	14.9 (10.4,19.2)

* From data of Table 6.1

Table 6.4

Comparison of body weight of *S. granarius* weevils surviving treatments with dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) with those of untreated populations

dose (mg a.i./kg)	mean initial weight of insects (mg) (2 replicates)	mean weight of insects surviving treatment (mg) (2 replicates)	mean weight of emerged insects (mg) ± s.e.
control	2.85 (2.86, 2.84)	2.99 (3.04, 2.94)	2.51 [0.04]
etrimfos			
0.101	2.73 (2.74, 2.71)	2.49 (2.61, 2.31)	2.49 [0.20]
0.109	2.88 (2.82, 2.94)	2.73 (2.80, 2.65)	2.35 [0.13]
0.118	2.82 (2.80, 2.83)	2.70 (2.74, 2.62)	2.29 [0.12]
malathion			
0.343	2.86 (2.84, 2.87)	2.51 (2.47, 2.54)	2.53 [0.21]
0.366	2.55 (2.72, 2.37)	2.24 (2.23, 2.24)	2.13 [0.51]
0.417	2.83 (2.73, 2.93)	2.31 (2.17, 2.45)	2.42 [0.20]
pirimiphos methyl			
0.148	2.86 (2.88, 2.84)	2.38 (2.03, 2.73)	2.46 [0.17]
0.163	2.72 (2.86, 2.57)	2.54 (2.86, 2.22)	2.36 [0.17]
0.183	2.85 (2.83, 2.86)	2.59 (2.54, 2.63)	2.26 [0.07]

Table 6.4 (Cont.)

Comparison of body weight of *S. granarius* weevils surviving treatments with dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) with those of untreated populations

dose (mg a.i./kg)	mean initial weight of insects (mg) (2 replicates)	mean weight of insects surviving treatment (mg) (2 replicates)	mean weight of emerged insects (mg) ± s.e.
etrimfos + malathion			
0.187	2.74 (2.74, 2.74)	2.65 (2.78, 2.52)	2.42 [0.18]
0.206	2.82 (2.97, 2.67)	2.78 (3.10, 2.46)	2.28 [0.20]
0.232	2.73 (2.67, 2.77)	2.18 (2.39, 1.97)	2.68 [0.18]
etrimfos + pirimiphos methyl			
0.109	2.82 (2.77, 2.86)	2.65 (2.84, 2.46)	2.76 [0.19]
0.122	2.79 (2.76, 2.82)	2.58 (2.51, 2.64)	2.28 [0.20]
0.139	2.82 (2.90, 2.73)	2.54 (2.68, 2.39)	2.68 [0.18]
malathion + pirimiphos methyl			
0.216	2.65 (2.65, 2.65)	2.42 (2.39, 2.44)	2.38 [0.16]
0.242	2.76 (2.73, 2.79)	2.47 (2.42, 2.52)	2.31 [0.22]
0.272	2.95 (3.11, 2.79)	2.75 (2.86, 2.64)	2.46 [0.16]

Table 6.5

Effect of low doses of dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) on *R. dominica* progeny

dose (mg a.i./kg)	expected % mortality*	% adult mortality at 7 days (2 replicates)	% mortality of progeny (2 replicates)
control	0	0 (0,0)	2.0 (1.1,2.9)
etrimfos			
0.567	5.2	2 (2,2)	0 (0,0)
0.664	9.9	4 (4,4)	1.2 (0.0,2.3)
0.803	19.0	5 (8,2)	0.5 (1.0,0)
malathion			
1.109	4.3	10 (6,14)	4.1 (7.4,0.7)
1.380	9.0	16 (18,14)	0 (0,0)
1.765	18.1	13 (10,16)	0 (0,0)
pirimiphos methyl			
1.385	5.1	5 (4,6)	0.6 (0.3,0.9)
1.626	10.4	1 (0.0,2)	1.3 (1.0,1.6)
1.972	20.9	3 (2,4)	0.7 (0.0,1.3)

* From data of Table 6.2

Table 6.5 (Cont.)

Effect of low doses of dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) on *R. dominica* progeny

dose (mg a.i./kg)	expected % mortality*	% adult mortality at 7 days (2 replicates)	% mortality of progeny (2 replicates)
etrimfos + malathion			
0.972	5.7	2 (2,2)	1.6 (1.2,2.0)
1.096	10.6	4 (6,2)	0.9 (1.7,0.0)
1.316	22.7	7 (6,8)	0.5 (0.9,0.0)
etrimfos + pirimiphos methyl			
1.036	5.4	5 (4,6)	1.2 (0.0,2.4)
1.187	10.6	8 (8,8)	0 (0,0)
1.423	21.7	7 (4,10)	2.3 (3.5,1.1)
malathion + pirimiphos methyl			
1.541	4.7	11 (12,10)	0.5 (0.9,0.0)
1.749	9.3	11 (14,8)	1.3 (2.6,0.0)
2.046	19.0	16 (10,22)	1.1 (2.1,0.0)

* From data of Table 6.2

Table 6.6

Comparison of body weight of *R. dominica* weevils surviving treatments with dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) with those of untreated populations

dose (mg a.i./kg)	mean initial weight of insects (mg) (2 replicates)	mean weight of insects surviving treatment (mg) (2 replicates)	mean weight of emerged insects (mg) ± s.e.
control	1.57 (1.58,1.56)	1.55 (1.56,1.54)	1.56 [0.19]
etrimfos			
0.567	1.59 (1.58,1.60)	1.59 (1.59,1.59)	1.46 [0.06]
0.664	1.62 (1.60,1.64)	1.60 (1.61,1.58)	1.48 [0.05]
0.803	1.60 (1.59,1.61)	1.65 (1.63,1.66)	1.46 [0.06]
malathion			
1.109	1.56 (1.54,1.58)	1.57 (1.52,1.61)	1.46 [0.08]
1.380	1.58 (1.56,1.60)	1.57 (1.57,1.57)	1.53 [0.02]
1.765	1.56 (1.52,1.60)	1.60 (1.58,1.61)	1.39 [0.07]
pirimiphos methyl			
1.385	1.62 (1.57,1.66)	1.58 (1.56,1.60)	1.51 [0.08]
1.626	1.58 (1.61,1.54)	1.54 (1.52,1.55)	1.57 [0.08]
1.972	1.61 (1.61,1.60)	1.61 (1.61,1.60)	1.57 [0.07]

Table 6.6 (Cont.)

Comparison of body weight of *R. dominica* weevils surviving treatments with dust formulations of etrimfos (2%), malathion (4%) and pirimiphos methyl (2%) with those of untreated populations

Dose (mg a.i./kg)	mean initial weight of insects (mg) (2 replicates)	mean weight of insects surviving treatment (mg) (2 replicates)	mean weight of emerged insects (mg) ± s.e.
etrimfos + malathion			
0.972	1.58 (1.56,1.60)	1.59 (1.57,1.60)	1.62 [0.07]
1.096	1.54 (1.54,1.54)	1.54 (1.56,1.52)	1.49 [0.04]
1.316	1.55 (1.54,1.56)	1.65 (1.62,1.67)	1.46 [0.07]
etrimfos + pirimiphos methyl			
1.036	1.53 (1.54,1.52)	1.59 (1.59,1.58)	1.62 [0.06]
1.187	1.50 (1.50,1.49)	1.52 (1.55,1.48)	1.54 [0.00]
1.423	1.54 (1.59,1.49)	1.57 (1.64,1.49)	1.56 [0.06]
malathion + pirimiphos methyl			
1.541	1.60 (1.54,1.65)	1.55 (1.49,1.60)	1.56 [0.08]
1.749	1.56 (1.55,1.57)	1.57 (1.52,1.619)	1.51 [0.06]
2.046	1.55 (1.56,1.54)	1.52 (1.51,1.52)	1.50 [0.06]

all treatments). The interpretation of this result is not clear; it is unlikely that competition for favourable egg-laying sites between adult females or for resources between developing larvae was greater in the experimental jars than in the main culture jar, given the ratio of wheat to insects in each. However, there was no significant difference between the mean weight of progeny emerging from the control jar and that of the mean weight from any of the treatment jars ($p > 0.05$ in all cases). Similarly, the weight of wheat consumed by the progeny during development (Figure 6.3) did not indicate any major difference between control and treatment when the expected weight of wheat consumed (calculated on the basis of the wheat consumption per developing larva in the control jars) was compared with the observed weight loss.

In the case of *R. dominica* no such difference between the mean weight of insects surviving the treatments and the mean weight of the insects at the start of the incubation period was evident (means not significantly different, $p > 0.5$), and neither was there a significant difference between the mean weight of the progeny of insects surviving treatment and that of the mean weight of the parent generation. Also, the amount of wheat consumed by the developing larvae was comparable with that which would be expected on a per larva basis from the control experiment (Figure 6.4).

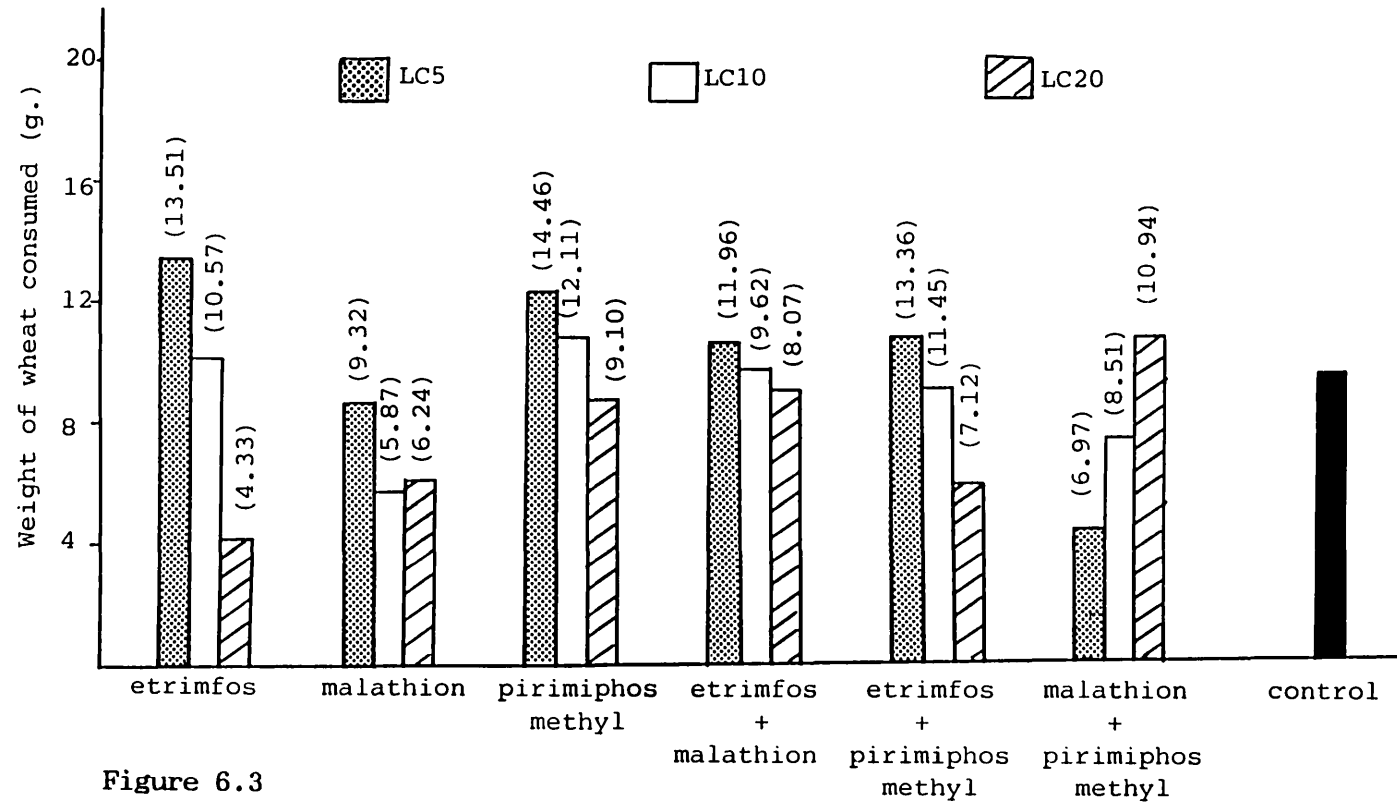


Figure 6.3

Weight of wheat consumed by *S. granarius* (0.0734 g/ progeny)

Figures in brackets at the top of each histogram correspond to expected wheat consumed by the developing larvae on a per larva basis calculated from the control value.

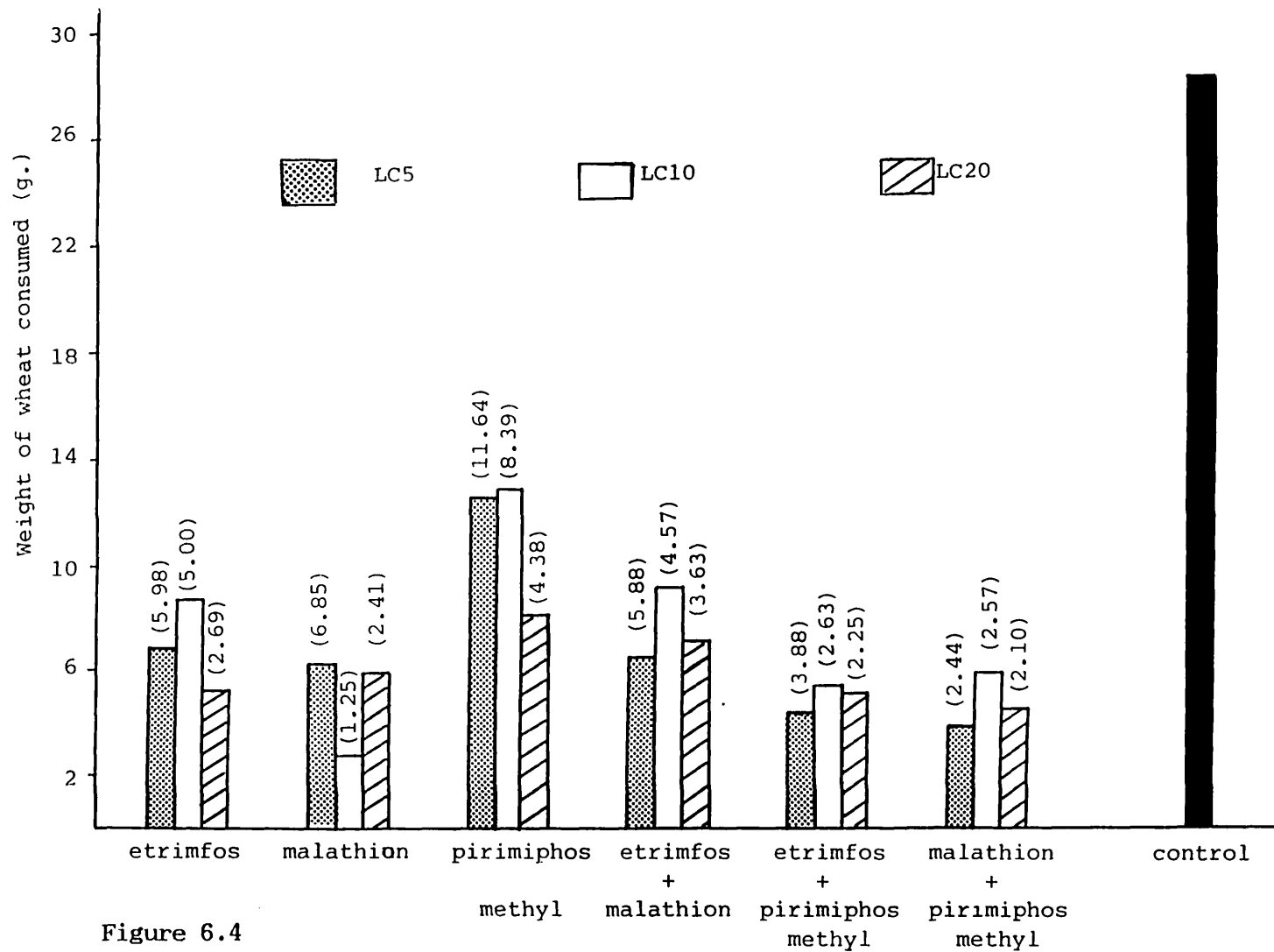


Figure 6.4

Weight of wheat consumed by *R. dominica* (0.0313 g/ progeny)

Figures in brackets at the top of each histogram correspond to expected wheat consumed by the developing larvae on a per larva basis calculated from the control value.

6.5 Discussion

In principle, insecticides can act on adult females by inhibiting mating, the number of eggs laid and the viability of those eggs. However, if adults are introduced into insecticide-treated grain, allowed to mate and lay eggs, and then the progeny allowed to develop, the effect on progeny production may be due both to the effect on the ability of the female to deposit normal numbers of viable eggs and on direct effects of the insecticide on egg hatch and survival of the larvae. Experimentally, one may wish to separate these effects, although in the field inhibition of progeny production would be due to a combination of them.

There is accumulating evidence to suggest that when insecticides are found to inhibit progeny production it is because surviving females lay fewer eggs than normal. Zettler and Lecato (1974) studied the effect of malathion on the black carpet beetle *Attagenus megatoma* by topically dosing females and pairing them individually prior to putting them on the culture medium. They demonstrated that survivors of treatment produced significantly fewer progeny, and that reduced fecundity was the principal cause of this effect. Loschiavo (1955) demonstrated that the effect of DDT on surviving female *T. confusum* which led to reduction in progeny production was specifically due to reduction in the number of eggs laid per day, and since the insects were allowed to lay their eggs in untreated medium this effect was not due to any contact effect of the insecticide on

the ovipositors. On the other hand, Hodges and Meik (1986) showed that 0.5% permethrin dust in maize had a strong inhibitory effect on progeny production by *T. castaneum* and that this was associated with a preference for oviposition on untreated maize. They suggested a direct contact effect of the compound on the ovipositor, a suggestion which was also advanced by Tenhet (1947) to explain the reduction in fecundity of tobacco beetles surviving doses of pyrethrins.

There is little data available on the effect of insecticides applied to the surface of wheat grain on the development and survival of eggs and larvae which, like *S. granarius* and *R. dominica*, develop inside the grain. Rowlands (1967) has shown that malathion applied to wheat grain in organic solvents accumulates in the endosperm soon after application, and would thus be available to the developing larvae. No comparable data appears to be available for dust formulations, although it would be anticipated on the basis of the physical separation between the relatively large proportion of the formulation which is trapped on the hairs and held proud of the surface of the wheat, that the extent of penetration would be much less than for wheat treated with emulsion formulations. On this basis, effects of dust treatments on progeny production which were greater than could be explained on the basis of the mortality of the a proportion of the females during the experiment might reasonably be ascribed to effects on mating and oviposition, rather than to toxic effects on the developing larvae.

The results of the experiments described in this Chapter suggest that sublethal doses of all three organophosphorus insecticides investigated inhibit progeny production by *R. dominica* to a considerable extent, but have no comparable effect on *S. granarius*. The lack of effect of the treatments on body weight of progeny and weight of wheat consumed suggests that eggs of both species which hatched subsequently completed a normal larval development and were not affected by residual insecticidal dust on the grain surface. It therefore appears likely that reduced progeny production by *R. dominica* can be attributed either to a reduction in numbers of eggs laid, possibly mediated by an effect on oviposition, or a reduction in egg hatch.

The two species differ in their egg laying behaviour: *S. granarius* females excavate a hole in the grain into which the egg is deposited, and then seal the hole with a mucilaginous plug as the ovipositor is withdrawn; *R. dominica* lays its eggs either singly or in batches in loose frass produced by the insect, and on the grain surface. Intuitively, one would expect *S. granarius* to be the species more vulnerable to any effects of insecticides which produced behavioural changes or muscular discoordination in the ovipositor. Kuenen (1958) claimed that progeny production by *S. granarius* was 20% greater than controls when adults were allowed to mate and lay eggs in grain treated with DDT at levels producing low mortality although this result was not replicated. Harein (1960)

exposed *S. oryzae* to wheat treated with fenchlorphos at a rate producing 7% mortality in the adult population, and obtained a 94% reduction in progeny production, and both Chadwick (1962) and Boles (1974) showed progeny reductions when this species was exposed to sub-LC50 doses in wheat. On the other hand, Hodges and Meik (1986) found permethrin dust to have no effect on progeny production by either *S. oryzae* or *S. zeamais* when adult populations were exposed to treated maize. The evidence for sub-lethal effects on progeny production by *Sitophilus* spp. due to insecticides is thus variable.

CONCLUSIONS

1. The pattern of breakdown of malathion and pirimiphos methyl dusts on wheat was similar to that reported in studies on emulsion formulations of these insecticides applied to wheat, and showed a similar dependence on wheat moisture content and temperature. Etrimfos, reported to have a prolonged persistence on wheat under cool storage conditions, was shown to have a comparable persistence to that of pirimiphos methyl under the conditions of intermediate temperature/high wheat moisture content and high temperature/low wheat moisture content investigated in this work.
2. A two-compartment first-order kinetic model of insecticide disappearance was found to give a better fit both to the breakdown patterns investigated in this work and in the published literature to these and other insecticides on stored grain than a simple first order kinetic model. It is suggested that the two-compartment model would be more suitable for predicting the amount of insecticide left as a residue in the wheat at any time after application.
3. Mixtures of dust formulations of etrimfos, pirimiphos methyl, malathion, deltamethrin and gamma-HCH were shown to act additively in most cases against a number of insect pests of stored grain. A methodology for testing the hypothesis of additive action based on that of Plackett and Hewlett (1963) was used.

4. A technique for determining the minimum cost application rate of an mixture of insecticides optimised to control two or more species of insects at or above some chosen response level was developed for cases in which insecticide formulations are shown to act additively, and was illustrated using the bioassay data generated when testing for the additive action of pairs of insecticide dusts. The technique provides a useful starting point for designing mixtures for field testing.

5. The effects of malathion, pirimiphos methyl, and etrimfos on progeny production by *R.dominica* and *S.granarius* were investigated. All three compounds were shown to suppress progeny production by *R.dominica* but to have little or no effect on *S.granarius*.

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Appendix 2.1

The mean body weights of 2-3 weeks adult of the different species used in the bioassay

species	mean weight mg/insect	$\pm$ standard error (mg)
<i>T.castaneum</i> (susceptible)	1.843 c *	0.017
<i>T.castaneum</i> (malathion-resistant)	2.109 b	0.093
<i>T.confusum</i>	2.177 a	0.037
<i>S.granarius</i> (susceptible)	2.748 a	0.070
<i>S.granarius</i> (gamma-HCH resistant)	2.555 b	0.149
<i>S.oryzae</i>	2.211 c	0.136
<i>O.surinamensis</i>	0.462 b	0.014
<i>O.surinamensis</i> (Greek strain)	0.432 b	0.069
<i>O.mercator</i>	0.530 a	0.030
<i>R.dominica</i>	1.519	0.039

* Mean weights within each genus not significantly different if followed by the same letter (F multiple range test, $P > 0.05$) each species sepaarately ($P > 0.05$).

Appendix 2.2

Peak heights of standard concentrations of pirimiphos methyl in methanol, acetone, and methanol : acetone (1 : 1 v/v) under standard chromatographic conditions

concentration (mg/l)	peak height (mm)		
	acetone	methanol	1:1 mixture
0.5	52	47	44
	49	47	46
1.0	92	95	87
	99	90	96
2.0	163	140	155
	161	148	157

Appendix 3.1

The effect of storage at - 5°C over a 20 week period on the stability of insecticides in methanolic extracts of wheat stored at two different conditions prior to extraction

pesticide	sample storage condition before extraction	weeks after storage at - 5°C								
		1	4	6	9	12	14	16	18	20
		concentration of insecticide in extract (mg/l)								
malathion	25°C/HMC*	13.95 (0.74)**	13.90 (0.355)	13.25 (0.99)	13.20 (1.485)	13.44 (0.09)	12.85 (0.21)	12.25 (0.35)	12.75 (0.705)	12.825 (0.460)
	30°C/LMC	13.35 (0.46)	13.05 (0.885)	12.15 (0.53)	13.025 (1.235)	12.315 (0.795)	12.575 (0.81)	12.00 (1.415)	12.525 (0.885)	12.750 (1.06)
etrimfos	25°C/HMC	7.4 (0.7)	7.4 (0.14)	7.2 (0.07)	7.75 (0.21)	7.6 (0.07)	7.35 (0.15)	7.55 (0.105)	7.8 (0.320)	7.65 (0.00)
	30°C/LMC	7.8 (0.00)	7.85 (0.07)	7.8 (0.285)	8.0 (0.07)	7.95 (0.80)	8.2 (0.60)	7.85 (0.49)	8.4 (0.355)	7.95 (0.425)
pirimiphos-methyl	25°C/HMC	8.75 (1.06)	8.75 (0.355)	7.75 (0.355)	7.6 (0.60)	7.35 (0.46)	7.5 (0.705)	8.0 (0.705)	7.5 (0.625)	7.90 (1.06)
	30°C/LMC	9.20 (0.46)	9.375 (0.885)	8.1 (0.60)	7.5 (0.705)	7.45 (0.105)	8.0 (0.92)	7.875 (0.885)	7.5 (0.705)	8.25 (0.845)

* HMC= high moisture content(14.3%); LMC= low moisture content(10.1%).

** ± Standard error

Appendix 3.2

Mean values of pesticide residues remaining on wheat grain stored under two sets of conditions over a 20 week period

pesticide applied dose	storage conditions	weeks after treatment									
		0	1	4	6	9	12	14	16	18	20
		concentration on wheat (mg a.i./kg)									
malathion (16 mg a.i./kg)	25°C/HMC*	13.95 (0.74)**	12.40 (0.175)	6.70 (0.425)	4.20 (0.265)	2.95 (0.07)	2.25 (0.355)	1.875 (0.175)	1.325 (0.105)	1.325 (0.06)	1.00 (0.00)
	30°C/LMC	13.35 (0.46)	14.10 (1.875)	9.50 (0.00)	7.5 (0.705)	6.8 (0.885)	5.875 (0.175)	6.30 (0.375)	4.75 (0.355)	4.625 (0.175)	4.275 (0.175)
etrimfos (10 mg a.i./kg)	25°C/HMC	7.4 (0.7)	6.30 (0.78)	5.40 (0.35)	3.50 (0.285)	3.45 (0.07)	3.25 (0.00)	2.650 (0.035)	2.35 (0.015)	2.45 (0.14)	2.35 (0.025)
	30°C/LMC	7.8 (0.00)	7.55 (0.14)	6.55 (0.21)	5.45 (0.185)	5.45 (0.14)	5.35 (0.14)	4.5 (0.14)	4.15 (0.085)	3.9 (0.065)	4.15 (0.07)
pirimiphos-methyl (10 mg a.i./kg)	25°C/HMC	8.75 (1.06)	8.15 (0.00)	5.625 (0.885)	4.50 (0.495)	4.40 (0.355)	3.45 (0.20)	3.825 (0.26)	3.25 (0.14)	2.60 (0.11)	2.90 (0.155)
	30°C/LMC	9.20 (0.46)	9.25 (0.105)	6.7 (0.125)	6.00 (0.15)	4.50 (0.09)	4.55 (0.14)	4.325 (0.11)	4.21 (0.105)	3.6 (0.13)	4.075 (0.105)

* HMC= high moisture content(14.9%); LMC= low moisture content(10.1%).

** ± Standard error

Appendix 3.3

Mortality of 50 *R. dominica* adults exposed to etrimfos (2%) , malathion (4%), or pirimiphos methyl (2%) admixed with with 14.9% m.c wheat grain at 25°C (48 hour bioassay at different storage intervals, 2 replicates at each time)

storage time (week)	insecticide		
	etrimfos (10 mg a.i/kg)	pirimiphos-methyl (10 mg a.i/kg)	malathion (16 mg a.i/kg)
0	50,50	49,47	50,50
1	50,50	31,36	41,36
2	-	16,17	24,22
3	28,29	13,15	14,16
4	11,13	11,7	10,2
5	5,5	2,5	2,6
6	2,1	0,0	2,2

Appendix 3.4

Mortality of 50 *R. dominica* adult exposed to etrimfos (2%), malathion (4%), or pirimiphos methyl (2%) admixed with with 10.1% m.c wheat grain at 30°C (48 hour bioassay at different storage intervals, 2 replicates at each time)

storage time (week)	pesticide			
	etrimfos (10 mg a.i./kg)	pirimiphos-methyl (10 mg a.i./kg)	malathion (16 mg a.i./kg)	
			1st	2nd
			experiment	experiment
0	50,50	50,50	50,50	50,50
1	50,50	47,48	50,50	-
2	-	43,40	50,50	50,50
3	46,42	33,29	50,50	-
4	44,44	30,30	-	50,50
5	30,41	22,26	50,50	-
6	11,6	6,5	50,50	-
7	3,0	-	-	-
8	-	-	-	48,48
10	-	-	50,48	42,40
12	-	-	49,49	-
14	-	-	-	38,38
16	-	-	-	-
18	-	-	47,48	37,35
20	-	-	41,42	40,32

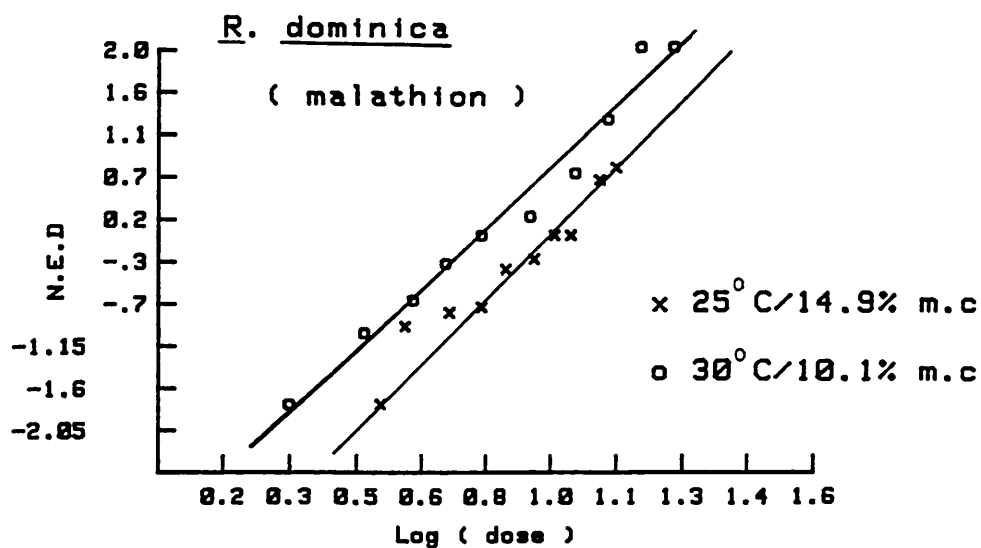
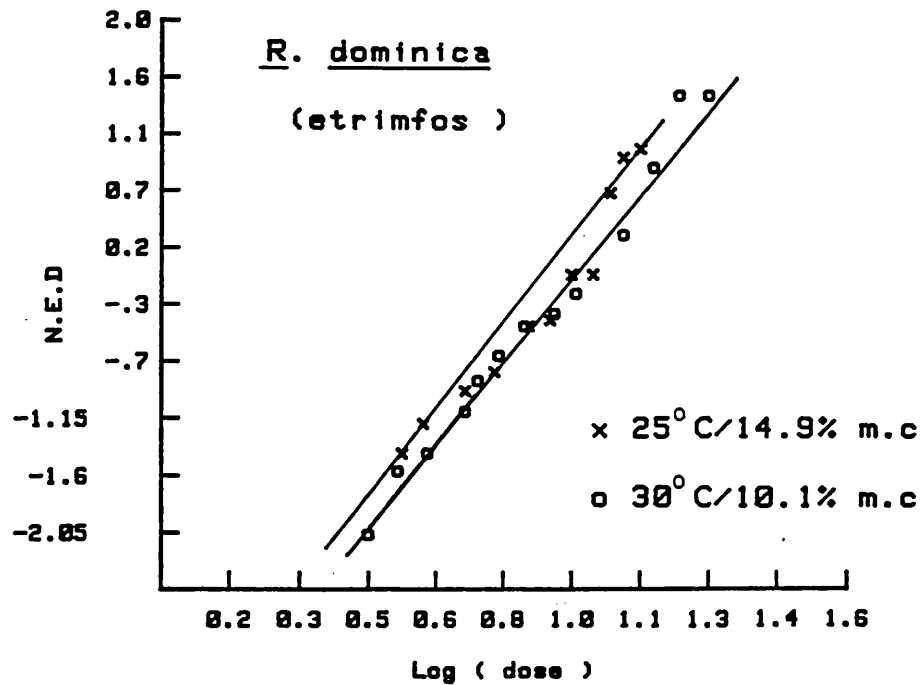
Appendix 3.5a

Response of *R. dominica* to malathion (4%), etrimfos (2%), and pirimiphos-methyl (2%) dust formulations admixed with whole wheat at 25°C/14.9% m.c and 30°C/10.1% m.c. (48 hour bioassay)

insecticide	temperature/ wheat moisture content	N.E.D.response(y)/ log dose (x) regression	LC50 mg(formulation)/ 100g wheat	p
etrimfos	25°C/14.9%	$y = -4.2 + 4.6 x$	8.5	0.11
	30°C/10.1%	$y = -4.0 + 4.2 x$	9.04	0.63
malathion	25°C/14.9%	$y = -3.4 + 3.6 x$	8.8	0.58
	30°C/10.1%	$y = -3.0 + 3.8 x$	6.07	0.77
pirimiphos methyl	25°C/14.9%	$y = -6.7 + 5.0 x$	21.70	0.34
	30°C/10.1%	$y = -3.6 + 2.9 x$	16.98	0.66

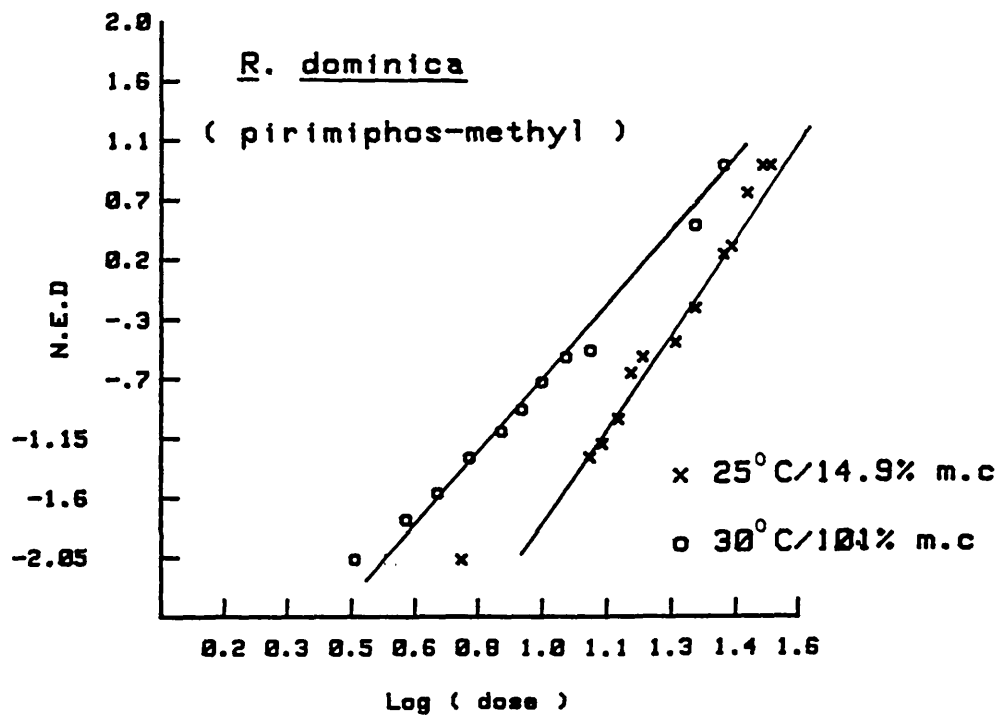
Appendix 3.5b

Log (dose)/n.e.d. (response) regressions for *R. dominica* bioassayed against etrimfos (2% a.i.) and malathion (4% a.i.) dust formulations (48 hr)



Appendix 3.5c

Log (dose)/n.e.d. (response) regression for *R. dominica* bioassayed against pirimiphos methyl (2% a.i.) dust formulation (48 hr)



Appendix 3.6

Mortality of 50 *R.dominica* adults exposed for 48 hour on wheat treated with etrimfos (2%), malathion (4%), pirimiphos-methyl (2%), and their dust formulation mixtures (1:1 w/w) stored at 25°C and 75% r.h . (2 replicates at each time)

pesticide (mg a.i/kg)	days after treatment								
	0	5	10	15	20	25	30	35	40
etrimfos (6.3)	50,50	44,46	35,36	18,20	2,1	2,1	0,0	-	-
malathion (17.1)	50,50	47,50	41,45	32,31	12,17	6,9	2,4	1,1	0,0
pirimiphos- methyl (13.6)	50,50	50,48	43,47	37,40	31,35	15,24	9,15	3,1	0,0
etrimfos + malathion (3.1 + 6.2)	50,50	47,43	34,31	11,13	5,3	3,2	1,0	-	-
pirimiphos- me.+ malathion (4.7 + 9.4)	50,50	50,49	43,41	34,35	20,27	8,11	2,0	-	-
etrimfos + pirimiphos-me. (3.7 + 7.4)	50,50	48,49	45,45	23,17	10,4	4,2	3,0	0,0	-

Appendix 3.7

Mortality of 50 *S. granarius* adults exposed to etrimfos (2%), malathion (4%), or pirimiphos methyl (2%) admixed with with 14.9% m.c wheat grain at 25°C (48 hour bioassay at different storage intervals, 2 replicates at each time)

storage time (week)	insecticide		
	etrimfos (10 mg a.i/kg)	pirimiphos-methyl (10 mg a.i/kg)	malathion (16 mg a.i/kg)
0	50,50	50,50	50,50
4	50,50	47,48	21,19
5	50,50	46,47	10,8
6	44,44	45,47	5,4
7	-	-	4,1
8	39,38	36,33	2,1
9	29,29	24,26	-
10	17,18	16,16	-

Appendix 3.8

Mortality of 50 *S. granarius* adults exposed to etrimfos (2%), malathion (4%), or pirimiphos methyl (2%) admixed with with 10.1% m.c wheat grain at 30°C (48 hour bioassay at different storage intervals, 2 replicates at each time)

storage time (week)	insecticide		
	etrimfos (10 mg a.i/kg)	pirimiphos-methyl (10 mg a.i/kg)	malathion (16 mg a.i/kg)
0	50,50	50,50	50,50
2	50,50	50,50	50,50
4	50,50	50,50	50,50
6	-	-	-
8	50,50	50,49	49,48
10	48,42	48,41	46,44
12	-	-	-
14	38,37	46,44	38,39
16	-	-	-
18	38,42	42,41	27,28
20	42,35	45,41	28,25

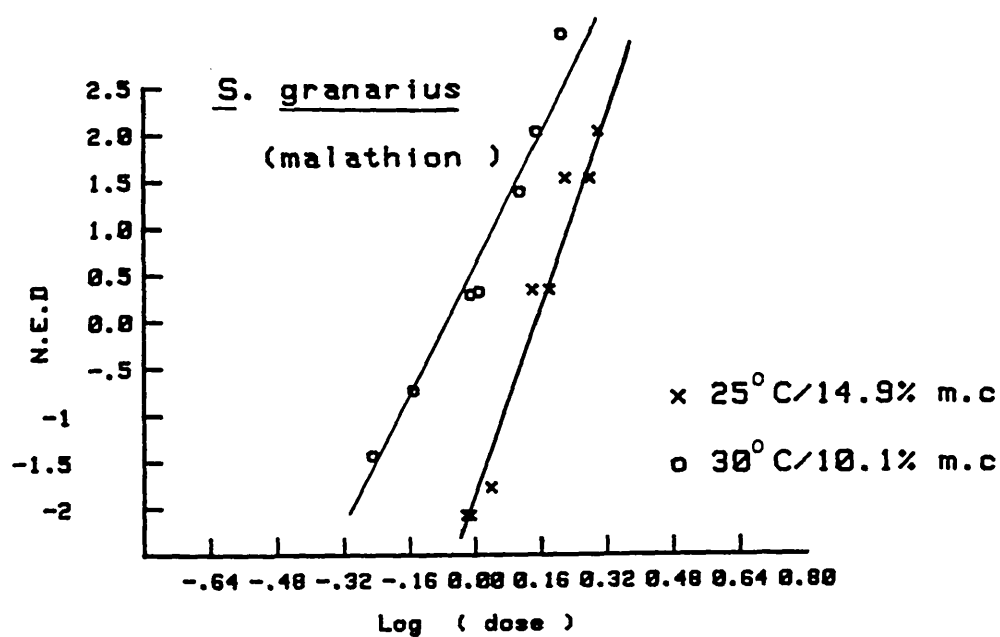
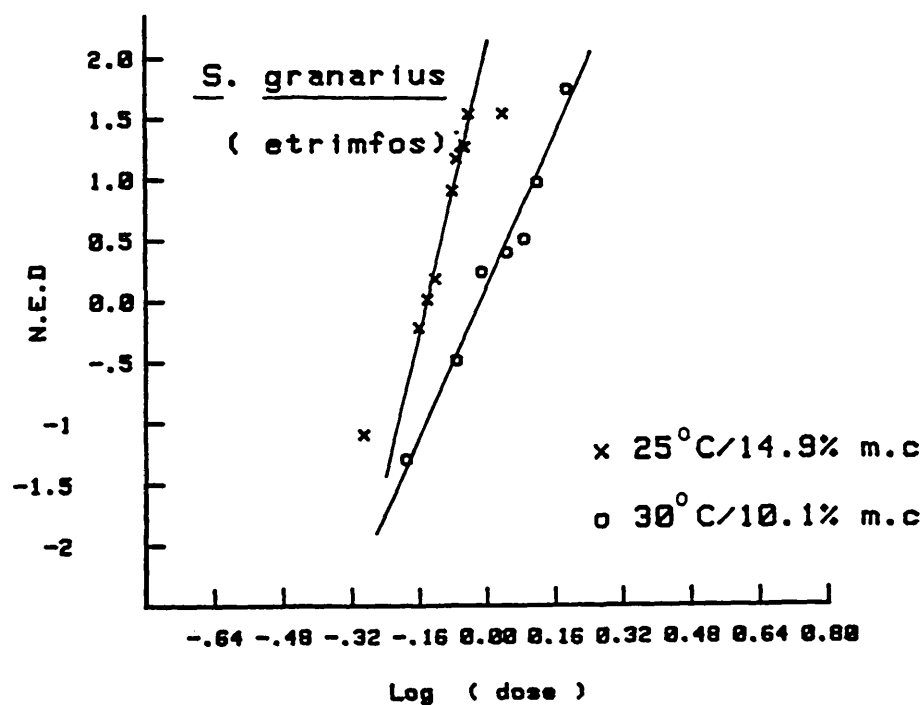
Appendix 3.9a

Response of *S. granarius* to malathion (4%), etrimfos (2%), and pirimiphos-methyl (2%) dust formulations admixed with whole wheat at 25°C/14.9% m.c and 30°C/10.1% m.c. (48 hour bioassay)

insecticide	temperature/ wheat moisture content	N.E.D.response(y)/ log dose (x) regression	LC50 mg(formulation)/ 100g wheat	p
etrimfos	25°C/14.9%	y= 1.6 + 9.7 x	0.69	0.29
	30°C/10.1%	y= 0.1 + 6.7 x	0.96	0.39
malathion	25°C/14.9%	y= -1.9 + 13.7 x	1.37	0.11
	30°C/10.1%	y= 0.5 + 8.3 x	0.88	0.80
pirimiphos methyl	25°C/14.9%	y= -1.4 + 14.4 x	1.24	0.36
	30°C/10.1%	y= -1.3 + 6.9 x	1.52	0.71

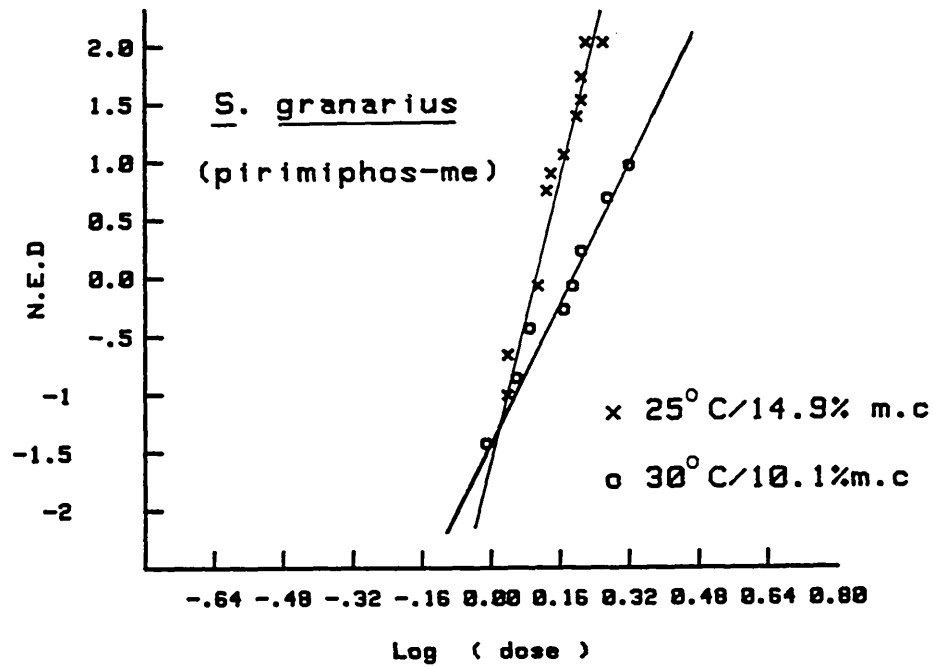
Appendix 3.9b

Log (dose)/n.e.d. (response) regressions for *S. granarius* bioassayed against etrimfos (2% a.i.) and malathion (4% a.i.) dust formulations (48 hr)



Appendix 3.9c

Log (dose)/n.e.d. (response) regression for *S. granarius* bioassayed against pirimiphos methyl (2% a.i.) dust formulation (48 hr)



Appendix 4.1

"MIX" - A program to fit $n.e.d.(response)/\log(dose)$ parameters to dose/response data for two toxicants applied singly and together using the maximum likelihood technique of Plackett and Hewlett (1963).

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Procedures

1. enterdata lines 340 -600

The data required for the program are the doses of two toxicants (1 and 2) applied to the species under test, together with the proportion of samples of a population of the species responding to these doses applied individually (1 bioassay for each toxicant) and together (1 bioassay for mixtures with a constant ratio of toxicant 1 to toxicant 2). A mean value for control mortality in the test species under the experimental conditions is also required.

2. calculatedosages lines 710 - 930

The proportion of each sample population responding to each dose is determined, with a correction for control mortality applied using Abbott's formula. Negative proportions (where the response to the toxicant is less than the mean control response) are set equal to zero. The log (base 10) of each applied dose is calculated for the bioassays in which the toxicants are applied singly. The log of the dose of each component applied as a mixture is also calculated.

3. leastsquares lines 940 - 1160

Provisional linear regressions of $\log(dose)$ against normal equivalent deviate (response) for the two toxicants bioassayed independently are calculated for all proportions responding which are both less than 1 and greater than zero, using the method of least squares.

4. The counter ITERATIONS is set equal to 0. The subsequent seven procedures constitute the maximum likelihood method for fitting the best set of values to parameters a_1 , b_1 , a_2 and b_2 of the two regressions of $n.e.d.(response)$ on $\log(dose)$, under the assumption that the components of the mixture act additively when toxicants 1 and 2 are bioassayed together. Initial estimates of these parameters are iteratively improved until the difference between successive values of all four parameters is less than 0.01 (lines 200 - 260).

5. expectedneds lines 1170 - 1230

Appendix 4.1 (Cont.)

Expected values for normal equivalent deviate (responses) are calculated for doses of toxicants applied singly using these provisional linear regressions. Corresponding expected values for n.e.d.'s of responses to the dose of each component of the mixture are similarly calculated

6. bisection lines 1240 - 1360

A bisection routine is used to solve the equation

$$10^{(x_1 - x)/b_1} + 10^{(x_2 - x)/b_2} = 1 \quad (i)$$

for x , where

x_1 is the expected n.e.d. response to the dose of toxicant 1 in the mixture,
 x_2 is the expected n.e.d. response to the dose of toxicant 2 in the mixture,
 x is the expected n.e.d. response to these doses applied together,
 b_1 and b_2 are the provisional slopes of the $\log(\text{dose})/\text{n.e.d.}(\text{response})$ regressions for toxicants 1 and 2.

The equation is that of Plackett and Hewlett (1963) for two toxicants acting similarly and with complete correlation of tolerances (additive action).

7. weights lines 1360 - 1500

Weights are assigned to each value of an expected proportion of the population responding to a dose (individual toxicants and mixture) using the standard method described by Finney (1971).

8. derivatives lines 1510 - 1580

The partial derivatives of equation (i) with respect both parameters of the two $\log(\text{dose})/\text{n.e.d.}(\text{response})$ regressions are calculated. The equations for these four partial derivatives are given in Plackett and Hewlett (1963) (equations 18 and 19).

9. sums lines 1600 - 1920; calculations lines 1940 - 2000
increments lines 2010 - 2070

Corrections to the initial and subsequent estimates of the a_1 and b_1 are calculated using equations (20) and (21) of Hewlett and Plackett (1963):

Appendix 4.1 (Cont.)

Similar equations are used to calculate the corrections to b_1 and b_2 .

Here:

W_i = weight coefficient attached to the response to a dose x_i , multiplied by the number of insects in the bioassay

$z_i = \log x_i$

P^+ = observed response (as a proportion)

P^* = expected response (as a proportion)

Z = ordinate of the normal curve at z_i

Subscripts 1 and 2 indicate the respective data for toxicants 1 and 2 applied separately.

The corrections are calculated and added to the current values of a_1 , b_1 , a_2 , and b_2 . If all four increments are less than 0.01 the iteration is terminated, and the current estimates of a_1 , b_1 , a_2 , and b_2 are output from the maximum likelihood determination.

10. calculatechisquared1 and calculatechisquared2 lines 2080 -2240

The proportions of the population expected to respond to each dose tested according to the maximum likelihood regressions and the assumption of additive action for the components of the mixture are calculated, and compared with the experimental values. A chi-square value for goodness-of-fit of the data to the model with $n-4$ degrees of freedom is calculated, where n is the number of doses tested. The procedure probability is called to calculate the proportion of cases in which the observed discrepancies between expected and observed proportions would be expected to occur by chance, assuming a chi-squared distribution. Values of $p > 0.05$ are taken to indicate an adequate fit of the data to the model for additive action of the components of the mixture.

11. printout and printtable lines 2250 - 2510 and 3410 - 3530

The following calculated values are printed out:

chi-square for goodness-of-fit of data to model, with degrees of freedom and corresponding probability value;
 number of iterations required for regression parameters to converge to within pre-set error of 0.01
 maximum likelihood regression equations for toxicants 1 and 2
 a table of input data, expected and experimental n.e.d.(responses), and contribution of each data point to total chi-square value.

Appendix 4.1 (Cont.)

12. recombine lines 3170 -3400

Extreme values of n.e.d.(responses) can make an unrepresentative contribution to the total chi-squared value for goodness-of-fit (Finney, 1971). This procedure uses Finney's method for recombining data with expected n.e.d.'s greater or less than a pre-set value to eliminate this spurious contribution to the total chi-square. The appropriate value depends on the number of animals used in the bioassays, and can be found in the Table given in Finney's book. It is input at lines 3190 and 3200. The procedure is available as an option if the total chi-square calculated in procedures (10) is less than 0.05

Functions

1. ORD line 2530

Calculates the ordinate value y of a normal curve given a value of the abscissa x .

2. INV lines 2540 - 2630

Calculates the area under a normal curve from $-\infty$ to x for a given value of the abscissa x .

3. NED lines 2650 - 2790

Calculates the abscissa x for a given value of the area under the curve from $-\infty$ to x

REFERENCES

Finney D.J. (1971), Probit Analysis, Cambridge University Press, Cambridge 3rd Edition.

Plackett R.L. and Hewlett P.S. (1963), A unified theory for quantal responses to mixtures of drugs: the fitting to data of certain models for two non-interactive drugs with complete positive correlation of tolerances, Biometrics 19, 517-531

Appendix 4.1 (Cont.)

```

10 REM:*****
20 REM MIXTURES PROGRAM: FITS HEWLETT AND PLACKETT MODEL A TO MIXTURE DATA
30 REM  REVISED 14.4.87
40 REM:*****
50 MODE 7:CLS
60 PROCtitle
65 MODE 131
70 PROCcenterdata
80 PROCdimensionarrays
90 PROCcalculatedosages
100 PROCleastssquares
110 REM *****
120 ITERATION = 0
130 PROCexpectedneds
140 PROCbisection
150 PROCweights
160 PROCderivatives
170 PROCsums
180 PROCcalculations
190 PROCincrements
200 IF ABS(DELA1)<0.01 AND ABS(DELB1)<0.01 THEN GOTO 230
210 A1=A1+DELA1:A2=A2+DELA2:B1=B1+DELB1:B2=B2+DELB2
220 ITERATION=ITERATION+1:GOTO 130
230 IF ABS(DELA2)<0.01 AND ABS(DELB2)<0.01 THEN GOTO 270
240 A1=A1+DELA1:A2=A2+DELA2:B1=B1+DELB1:B2=B2+DELB2
250 ITERATION=ITERATION+1:GOTO 130
260 REM *****
270 PROCcalculatetechnisquared1
280 PROCcalculatetechnisquared2
290 PRINT CHISQUARE;L+M+N-4:INPUT "PROBABILITY Y/N";ANS$
300 IF ANS$="N" THEN PROB=0: IF ANS$="N" THEN 320
310 PROCprobability(L+M+N-4,CHISQUARE)
320 PROCprintout
330 END
340 REM *****
350 DEFPROCcenterdata
360 CLS:PRINT:PRINT:PRINT
370 INPUT "Input no of doses of insecticide 1 ";L
380 INPUT "Input no of doses of insecticide 2 ";M
390 INPUT "Input no of doses of mixture";N
400 CLS:PRINT:PRINT
410 DIM I(3): I(1)=L:I(2)=M:I(3)=N:MAX=0
420 MAX=L:IF MAX<M THEN MAX=M
425 IF MAX<N THEN MAX=N
430 DIM Z1(MAX),N1(MAX),B1(MAX),Z2(MAX),N2(MAX),B2(MAX),Z31(MAX)
440 DIM Z32(MAX),Z33(MAX),N3(MAX),B3(MAX)
450 DIM LX1(MAX),LX2(MAX),LX31(MAX),LX32(MAX),ZA3(MAX)

```

Appendix 4.1 (Cont.)

```

460 PRINT "Input dose, total tested,":PRINT "total responding for insecticide1"
470 FOR I= 1 TO L: INPUT Z1(I),N1(I),R1(I): NEXT
480 PRINT "Input dose, total tested,":PRINT "total responding for INSECTICIDE2"
490 FOR I= 1 TO M: INPUT Z2(I),N2(I),R2(I): NEXT
500 PRINT "Input dose of insecticide1,"
510 PRINT "dose of insecticide2, total tested,"
520 PRINT "total responding for MIXTURE"
530 FOR I=1 TO N:INPUT Z3(I),Z32(I),N3(I),R3(I):NEXT
540 PRINT
550 INPUT "Input control mortality as a proportion"; C
560 PRINT:INPUT "Name of species";S$
570 PRINT:INPUT "Name of insecticide1";S1$
580 PRINT:INPUT "Name of insecticide2";S2$
590 PRINT:INPUT "Bioassay time";S3$
600 ENDPROC
610 REM *****
620 DEFPROCdimensionarrays
630 DIM NED1(MAX),NED2(MAX),NED31(MAX),NED32(MAX),NED3(MAX),Y1(MAX),Y2(MAX),Y3(MAX)
640 DIM BNED1(MAX),BNED2(MAX),BNED31(MAX),BNED32(MAX),BNED3(MAX),PB1(MAX),PB2(MAX),PB3(MAX)
650 DIM W1(MAX),W2(MAX),W3(MAX),P1(MAX),P2(MAX),P3(MAX),LX3(MAX),CHI1(MAX),CHI2(MAX), CHI3(MAX)
660 DIM CHINUM1(MAX),CHINUM2(MAX),CHINUM3(MAX),CHIDEN1(MAX),CHIDEN2(MAX)
670 DIM CHIDEN3(MAX),FLAG1(MAX),FLAG2(MAX),FLAG3(MAX),PB1(MAX),PB2(MAX),PB3(MAX)
680 DIM DA1(MAX),DA2(MAX),DB1(MAX),DB2(MAX)
690 ENDPROC
700 REM *****
710 DEFPROCcalculatedosages
720 FOR A = 1 TO L: PR1(A)=(R1(A)/N1(A)-C)/(1-C):NEXT
730 FOR A = 1 TO M: PR2(A)=(R2(A)/N2(A)-C)/(1-C):NEXT
740 FOR A = 1 TO N: PR3(A)=(R3(A)/N3(A)-C)/(1-C):NEXT
750 I=1
760 FLAG=0: IF PR1(I) < 0 THEN FLAG=1
770 IF FLAG=1 THEN PR1(I)=0
780 IF I=L THEN GOTO 800
790 I=I+1:GOTO 760
800 I=1
810 FLAG=0: IF PR2(I) < 0 THEN FLAG=1
820 IF FLAG=1 THEN PR2(I)=0
830 IF I=M THEN GOTO 850
840 I=I+1:GOTO 810
850 I=1
860 FLAG=0: IF PR3(I) < 0 THEN FLAG=1
870 IF FLAG=1 THEN PR3(I)=0
880 IF I=N THEN GOTO 900
890 I=I+1:GOTO 860
900 FOR I=1 TO L: LX1(I)=LOG(Z1(I)):NEXT

```

Appendix 4.1 (Cont.)

```

910 FOR I=1 TO M: LX2(I)=LOG(Z2(I)):NEXT
920 FOR I=1 TO N: LX3(I)=LOG(Z3(I)):LX32(I)=LOG(Z32(I)):NEXT
930 ENDPROC
940 REM *****
950 DEFPROCleastsquares
960 SX1=0:SY1=0:SY1=0:MX1=0:LX1=0:LX21=0
970 C1=1:C2=0
980 IF PR1(C1) <= 0 OR PR1(C1) >= 1 GOTO 1030
990 NED1(C1) = FWNED(PR1(C1))
1000 SY1=SY1+LX1(C1)*NED1(C1): SX21=SY21+LX1(C1)*LX1(C1)
1010 SX1 =SX1+LX1(C1):SY1=SY1+NED1(C1)
1020 C2=C2+1
1030 C1=C1+1: IF C1<M+1 GOTO 980
1040 MX1=SY1/C2:MY1=SY1/C2:LX1=C2*MX1:MY1=LX21=C2*MX1*MX1
1050 B1=(SY1-LX1)/(SX21-LX21):A1=MY1-B1*MX1
1060 SX2=0:SY2=0:SY2=0:MX2=0:LX2=0:LX22=0
1070 C1=1:C2=0
1080 IF PR2(C1) <= 0 OR PR2(C1) >= 1 GOTO 1130
1090 NED2(C1) = FWNED(PR2(C1))
1100 SY2=SY2+LX2(C1)*NED2(C1): SX22=SY22+LX2(C1)*LX2(C1)
1110 SX2 =SX2+LX2(C1):SY2=SY2+NED2(C1)
1120 C2=C2+1
1130 C1=C1+1: IF C1<M+1 GOTO 1080
1140 MX2=SY2/C2:MY2=SY2/C2:LX2=C2*MX2:MY2=LX22=C2*MX2*MX2
1150 B2=(SY2-LX2)/(SX22-LX22):A2=MY2-B2*MX2
1160 ENDPROC
1170 REM *****
1180 DEFPROCexpectedneds
1190 FOR I=1 TO L: ENED1(I)=A1+B1*LX1(I):NEXT
1200 FOR I=1 TO M: ENED2(I)=A2+B2*LX2(I):NEXT
1210 FOR I=1 TO N: ENED3(I)=A1+B1*LX3(I): ENED32(I)=A2+B2*LX32(I): NED3(I)=FWNED(PR3(I)):NEXT
1220 ENDPROC
1230 REM *****
1240 DEFPROCbisection
1250 Q = 1
1260 LOWER = -4: UPPER = 4
1270 ENED3(Q)=(LOWER+UPPER)/2
1280 IF ABS(LOWER-UPPER) < 0.001 GOTO 1330
1290 S1=1 - 10^((ENED31(Q)-ENED3(Q))/B1)-10^((ENED32(Q)-ENED3(Q))/B2)
1300 S2=1 - 10^((ENED31(Q)-LOWER)/B1) - 10^((ENED32(Q)-LOWER)/B2)
1310 IF S1*S2>0 THEN LOWER = ENED3(Q) ELSE UPPER = ENED3(Q)
1320 GOTO 1270
1330 IF Q=N THEN GOTO 1380
1340 Q=Q+1: GOTO 1260
1350 ENDPROC

```

Appendix 4.1 (Cont.)

```

1360 REM *****
1370 DEFPROCweights
1380 FOR I=1 TO L: PE1(I)=FNINV(ENED1(I)): NEXT
1390 FOR I=1 TO M: PE2(I)=FNINV(ENED2(I)): NEXT
1400 FOR I=1 TO N: PE3(I)=FNINV(ENED3(I)): NEXT
1410 FOR I=1 TO L: Y1(I)=FNORD(ENED1(I))
1420 W1(I)=Y1(I)*Y1(I)*W1(I)/(1-PE1(I))/(PE1(I)+C/(1-C)): NEXT
1430 FOR I=1 TO M: Y2(I)=FNORD(ENED2(I))
1440 W2(I)=Y2(I)*Y2(I)*W2(I)/(1-PE2(I))/(PE2(I)+C/(1-C)): NEXT
1450 FOR I=1 TO N: Y3(I)=FNORD(ENED3(I))
1460 W3(I)=Y3(I)*Y3(I)*W3(I)/(1-PE3(I))/(PE3(I)+C/(1-C)): NEXT
1470 FOR I=1 TO L: P1(I)=(PE1(I)-PE1(I))/FNORD(ENED1(I)):NEXT
1480 FOR I=1 TO M: P2(I)=(PE2(I)-PE2(I))/FNORD(ENED2(I)):NEXT
1490 FOR I=1 TO N: P3(I)=(PE3(I)-PE3(I))/FNORD(ENED3(I)):NEXT
1500 ENDPROC
1510 REM *****
1520 DEFPROCderivatives
1530 FOR I=1 TO N
1540 DA1(I)=B2/(B2-B1+B1*(10^((ENED3(I)-ENED31(I))/B1)))
1550 DA2(I)=B1/(B1-B2+B2*(10^((ENED3(I)-ENED32(I))/B2))): NEXT
1560 FOR I=1 TO N: DB1(I)=DA1(I)*(ENED3(I)-A1)/B1
1570 DB2(I)=DA2(I)*(ENED3(I)-A2)/B2: NEXT
1580 ENDPROC
1590 REM *****
1600 DEFPROCsums
1610 SW1=0:SWL1=0:SWP1=0:SWLS1=0:SWLP1=0:SW2=0:SWL2=0:SWP2=0:SWLS2=0:SWLP2=0
1620 I=1
1630 SW1=W1(I)+SW1: SWL1=W1(I)*LX1(I)+SWL1: SWLS1=W1(I)*LX1(I)*LX1(I)+SWLS1
1640 SWP1=W1(I)*P1(I)+SWP1:SWLP1=W1(I)*P1(I)*LX1(I)+SWLP1: I=I+1
1650 IF I=MAX+1 GOTO 1670
1660 GOTO 1630
1670 I=1
1680 SW2=W2(I)+SW2: SWL2=W2(I)*LX2(I)+SWL2: SWLS2=W2(I)*LX2(I)*LX2(I)+SWLS2
1690 SWP2=W2(I)*P2(I)+SWP2:SWLP2=W2(I)*P2(I)*LX2(I)+SWLP2: I=I+1
1700 IF I= MAX+1 GOTO 1720
1710 GOTO 1680
1720 SWDAS31=0:SWDBS31=0:SWDAB31=0:SWDAP31=0:SWDBP31=0
1730 I=1
1740 SWDAS31=SWDAS31+W3(I)*DA1(I)*DA1(I)
1750 SWDBS31=SWDBS31+W3(I)*DB1(I)*DB1(I)
1760 SWDAB31=SWDAB31+W3(I)*DA1(I)*DB1(I)
1770 SWDAP31=SWDAP31+W3(I)*DA1(I)*P3(I)
1780 SWDBP31=SWDBP31+W3(I)*DB1(I)*P3(I)
1790 I=I+1
1800 IF I=MAX+1 GOTO 1820

```

```

>

```

Appendix 4.1 (Cont.)

```

1810 GOTO 1740
1820 SWDAS32=0:SWDBS32=0:SWDAB32=0:SWDAP32=0:SWDBP32=0
1830 I=1
1840 SWDAS32=SWDAS32+W3(I)*DA2(I)*DA2(I)
1850 SWDBS32=SWDBS32+W3(I)*DB2(I)*DB2(I)
1860 SWDAB32=SWDAB32+W3(I)*DA2(I)*DB2(I)
1870 SWDAP32=SWDAP32+W3(I)*DA2(I)*P3(I)
1880 SWDBP32=SWDBP32+W3(I)*DB2(I)*P3(I)
1890 I=I+1
1900 IF I=MAX+1 GOTO 1950
1910 GOTO 1840
1920 ENDPROC
1930 REM *****
1940 DEFPROCcalculations
1950 F1=SW1+SWDAS31:F2=SWL1+SWDAB31:F3=SWP1+SWDAP31:F4=F2
1960 F5=SWLS1+SWDBS31:F6=SWLP1+SWDBP31
1970 G1=SW2+SWDAS32: G2=SWL2+SWDAB32: G3=SWP2+SWDAP32: G4=G2
1980 G5=SWLS2+SWDBS32: G6=SWLP2+SWDBP32
1990 ENDPROC
2000 REM *****
2010 DEFPROCincrements
2020 DELA1=(F5*F3-F2*F6)/(F1*F5-F2*F4)
2030 DELB1=(F1*F6-F4*F3)/(F1*F5-F2*F4)
2040 DELA2=(G5*G3-G2*G6)/(G1*G5-G2*G4)
2050 DELB2=(G1*G6-G4*G3)/(G1*G5-G2*G4)
2060 ENDPROC
2070 REM *****
2080 DEFPROCcalculatechisquared1
2090 FOR I=1 TO L:CHINUM1(I)=N1(I)*(PB1(I)-PB1(I))
2100 CHIDEN1(I)=N1(I)*PB1(I)*(1-PB1(I)):NEXT
2110 FOR I=1 TO M:CHINUM2(I)=N2(I)*(PB2(I)-PB2(I))
2120 CHIDEN2(I)=N2(I)*PB2(I)*(1-PB2(I)):NEXT
2130 FOR I=1 TO N:CHINUM3(I)=N3(I)*(PB3(I)-PB3(I))
2140 CHIDEN3(I)=N3(I)*PB3(I)*(1-PB3(I)):NEXT
2150 REM*****
2160 DEFPROCcalculatechisquared2
2170 FOR I=1 TO MAX:CHI1(I)=0:CHI2(I)=0:CHI3(I)=0:NEXT
2180 CHI1=0:CHI2=0:CHI3=0:CHISQUARE=0
2190 FOR I=1 TO L:CHI1(I)=CHINUM1(I)^2/CHIDEN1(I):CHI1=CHI1+CHI1(I):NEXT
2200 FOR I=1 TO M:CHI2(I)=CHINUM2(I)^2/CHIDEN2(I):CHI2=CHI2+CHI2(I):NEXT
2210 FOR I=1 TO N:CHI3(I)=CHINUM3(I)^2/CHIDEN3(I):CHI3=CHI3+CHI3(I):NEXT
2220 CHISQUARE=CHI1+CHI2+CHI3
2230 ENDPROC
2240 REM *****
2250 DEFPROCprintout

```

Appendix 4.1 (Cont.)

```

2260 VDU2:PRINT:GX=10002030A
2270 PRINT:PRINT S$
2280 PRINT S1$;" and ";S2$
2290 PRINT S3$
2300 PRINT:PRINT "Total chi" = ";CHISQUARE
2310 PRINT "D.F. = ";L+M+N-4
2320 PRINT "P = ";PROB
2330 PRINT:PRINT "Number of iterations = "; ITERATION
2340 PRINT:PRINT "Regression equation for ";S1$;" is y = ";A1;" + ";B1;"x"
2350 PRINT:PRINT "Regression equation for ";S2$;" is y = ";A2;" + ";B2;"x"
2360 PROCprinttable
2370 INPUT "Implement recombination option Y/N";ANS$
2380 IF ANS$ = "N" THEN 2500
2390 IF PROB<0.05 THEN PRINT "Data is heterogeneous: recombining:"
2400 IF PROB<0.05 THEN PROCrecombine ELSE 2500
2410 PROCcalculatechisquared2
2420 C=0
2430 FOR I=1 TO L:IF FLAG1(I)=1 THEN C=C+1:NEXT
2440 FOR I=1 TO M:IF FLAG2(I)=1 THEN C=C+1:NEXT
2450 FOR I=1 TO N:IF FLAG3(I)=1 THEN C=C+1:NEXT
2460 PROCprobability(N-C-1,CHISQUARE)
2470 PRINT "P = ";PROB;" CHISQUARE = ";CHISQUARE
2480 IF PROB<0.05 THEN PRINT "Still heterogeneous after recombining".
2490 PROCprinttable
2500 VDU3:GX=10
2510 ENDPROC
2520 REM *****
2530 DEFPNORD(X)=0.39894228*EXP(-X*X/2)
2540 DEFPNINV(X)
2550 LOCAL Z,Z1,Z2,Z3,X1,A1,A2,A3,A4,A5,A6
2560 A1=0.2316419:A2=0.31938153:A3=-0.356563782
2570 A4=1.781477937:A5=-1.821255978:A6=1.330274429
2580 IF X>0 THEN Z=1 ELSE Z=0
2590 Z1=0.3989423*EXP(-X*X/2)
2600 Z2=1/((1+A1*(ABS(X)))
2610 Z3=Z1*(((A6*Z2+A5)*Z2+A4)*Z2+A3)*Z2+A2)*Z2
2620 IF Z=1 THEN X1=1-Z3 ELSE X1=Z3
2630 =X1
2640 REM *****
2650 DEFPNWD(X)
2660 LOCAL Z,Z1,Z2,X1,X2,A1,A2,A3,A4,A5,A6
2670 IF X<=0 THEN GOTO 2770
2680 IF X=1 THEN GOTO 2780
2690 A1=2.515517:A2=0.802853:A3=0.010328
2700 A4=1.432788:A5=0.189269:A6=0.001308

```

Appendix 4.1 (Cont.)

```

2700 A4=1.432788:A5=0.189269:A6=0.001308
2710 IF X<=0.5 THEN Z=1 ELSE Z=0
2720 IF Z=0 THEN X1=1-X ELSE X1=X
2730 Z1=LN(1/X1/X1):Z2=SQR(Z1)
2740 X2=Z2-(A1+A2*Z2+A3*Z1)/(1+A4*Z2+A5*Z1+A6*Z1*Z2)
2750 IF Z=1 THEN X2=-X2
2760 GOTO 2790
2770 X2=-25:GOTO 2790
2780 X2=25:GOTO 2790
2790 =X2
2800 REM *****
2810 DEFPROCdbl(W$,U,W)
2820 PRINTTAB(U,W);CHR$(141);W$
2830 PRINT TAB(U,W+1);CHR$(141);W$
2840 ENDPROC
2850 DEFPROCtitle
2860 PROCdbl("MIXTURES",8,11)
2870 PROCdbl("G.LE PATOUREL",20,18)
2880 PROCdbl("1985",28,20)
2890 PORT=1703000:EXIT
2900 ENDPROC
2910 REM *****
2920 DEFPROCprobability(DF,CHISQUARE)
2930 LOCAL R,K,W,V,J,L,M,V
2940 V=DF
2950 IF V<42 GOTO 2980
2960 PRINT "Too many degrees of freedom for probability algorithm"
2970 PROB = 0:GOTO 3150
2980 IF V=0 THEN 3150
2990 R=1
3000 FOR I=V TO 2 STEP -2
3010 R=R*I
3020 NEXT
3030 K=CHISQUARE^(INT((V+1)/2))*EXP(-CHISQUARE/2)/R
3040 IF INT(V/2)=V/2 THEN 3070
3050 J=SQR(2/CHISQUARE/PI)
3060 GOTO 3080
3070 J=1
3080 L=1:M=1
3090 V=V+2
3100 M=M*CHISQUARE/V
3110 IF M<0.0000001 THEN 3140
3120 L=L*M
3130 GOTO 3090
3140 PROB=1-J*K*L
3150 ENDPROC
3160 REM *****
3170 DEFPROCrecombine
3180 FOR I = 1 TO L:FLAG1(I)=0
3190 IF ENED1(I)> 1 THEN FLAG1(I)=1
3200 IF ENED1(I)<-1 THEN FLAG1(I)=1

```

Appendix 4.1 (Cont.)

```

3210 NEXT
3220 FOR I=1 TO L-1
3230 IF FLAG1(I)=1 AND FLAG1(I+1)=1 THEN CHINUM1(I+1)=CHINUM1(I)+CHINUM1(I+1)
3240 IF FLAG1(I)=1 AND FLAG1(I+1)=1 THEN CHINUM1(I)=0:NEXT
3250 FOR I = 1 TO M:FLAG2(I)=0
3260 IF BNED2(I)> 1 THEN FLAG2(I)=1
3270 IF BNED2(I)<-1 THEN FLAG2(I)=1
3280 NEXT
3290 FOR I=1 TO M-1
3300 IF FLAG2(I)=1 AND FLAG2(I+1)=1 THEN CHINUM2(I+1)=CHINUM2(I)+CHINUM2(I+1)
3310 IF FLAG2(I)=1 AND FLAG2(I+1)=1 THEN CHINUM2(I)=0:NEXT
3320 FOR I = 1 TO N:FLAG3(I)=0
3330 IF BNED3(I)> 1 THEN FLAG3(I)=1
3340 IF BNED3(I)<-1 THEN FLAG3(I)=1
3350 NEXT
3360 FOR I=1 TO N-1
3370 IF FLAG3(I)=1 AND FLAG3(I+1)=1 THEN CHINUM3(I+1)=CHINUM3(I)+CHINUM3(I+1)
3380 IF FLAG3(I)=1 AND FLAG3(I+1)=1 THEN CHINUM3(I)=0:NEXT
3390 ENDPROC
3400 REM*****
3410 DEFPROCprinttable
3420 VDU 2,1,27,1,143,1
3430 PRINT TAB(20);S1$;TAB(80);S2$
3440 PRINT TAB(0);"dose1";TAB(10);"logdose1";TAB(20);"n.e.d.(1)";TAB(30);"exp n.e.d.";TAB(40);"diff";TAB(50);"chi squ"
AB(75);"logdose2";TAB(85);"n.e.d.";TAB(95);"exp n.e.d.";TAB(105);"diff";TAB(115);"chi squ"
3450 FOR I=1 TO MAX
3460 PRINT TAB(0);Z1(I);TAB(10);LX1(I);TAB(20);NED1(I);TAB(30);BNED1(I);TAB(40);NED1(I)-BNED1(I);TAB(50);CHI1(I)
I2(I);TAB(85);NED2(I);TAB(95);BNED2(I);TAB(105);NED2(I)-BNED2(I);TAB(115);CHI2(I):NEXT
3470 PRINT:PRINT TAB(50);"1:1 mixture"
3480 PRINT TAB(0);"logdose1";TAB(10);"logdose2";TAB(20);"n.e.d.";TAB(30)"exp n.e.d.";TAB(40);"diff";TAB(50);"chi squared"
3490 FOR I = 1 TO N
3500 PRINT TAB(0);LX31(I);TAB(10);LX32(I);TAB(20);NED3(I);TAB(30);BNED3(I);TAB(40);NED3(I)-BNED3(I);TAB(50);CHI3(I):NEXT
3510 VDU 3
3520 ENDPROC
3530 REM*****
)

```

Appendix 6.1

Mean number of *S. granarius* progeny produced by survivors of treatments with etrimfos, pirimiphos methyl, and malathion dust formulations and their mixtures admixed with wheat at calculated LC5, LC10, and LC20

insecticide concentration (mg a.i/ kg)	mean number of progeny (2 replicates)	insecticide concentration (mg a.i/ kg)	mean number of progeny (2 replicates)
control	128 (154,101)		
etrimfos		etrimfos + malathion (1:1 w/w)	
0.101	184 (190,171)	0.187	163 (198,128)
0.109	144 (132,155)	0.206	131 (171,90)
0.118	59 (69,49)	0.232	110 (91,128)
malathion		etrimfos + pirimiphos methyl (1:1 w/w)	
0.343	127 (116,138)	0.109	182 (168,195)
0.366	80 (78,82)	0.122	156 (137,174)
0.417	85 (112,58)	0.139	97 (70,124)
pirimiphos methyl		malathion + pirimiphos methyl (1:1 w/w)	
0.148	197 (183,211)	0.216	95 (100,90)
0.163	165 (170,160)	0.242	116 (131,101)
0.183	124 (149,99)	0.274	149 (172,126)

Appendix 6.2

Mean number of *R. dominica* progeny produced by survivors of treatments with etrimfos, pirimiphos methyl, and malathion dust formulations and their mixtures admixed with wheat at calculated LC5, LC10, and LC20

insecticide concentration (mg a.i/ kg)	mean number of progeny (2 replicates)	insecticide concentration (mg a.i/ kg)	mean number of progeny (2 replicates)
control	908 (1136,679)		
etrimfos		etrimfos + malathion	
0.567	191 (206,175)	0.972	188 (172,203)
0.664	160 (147,172)	1.096	146 (112,180)
0.803	86 (100,72)	1.316	116 (115,117)
malathion		etrimfos + pirimiphos methyl	
1.109	219 (296,142)	1.036	124 (125,123)
1.380	40 (38,41)	1.187	84 (87,80)
1.765	77 (89,64)	1.423	72 (55,89)
pirimiphos methyl		malathion + pirimiphos methyl	
1.385	372 (302,441)	1.541	78 (115,41)
1.626	268 (286,250)	1.749	82 (87,76)
1.972	140 (126,153)	2.046	67 (41,92)