3.
Rodent Problems and their Control

Chairman: Mr D. C. DRUMMOND
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THE OCCURRENCE AND SIGNIFICANCE OF RODENTICIDE-RESISTANCE IN THE U.K.

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ABSTRACT

Since the first reports of warfarin-resistance in rodents in the early 1960's many animals have been received at our laboratory for resistance testing. This paper summarises both the positive and negative results of testing a range of anticoagulant rodenticides against *Rattus norvegicus* and *Mus domesticus* during the period 1977-1986. These results are collated with respect to the date and county where each group of rodents was captured. The significance of these results is discussed in relation to possible further incidence of resistance to anticoagulant rodenticides.

INTRODUCTION

Anticoagulant rodenticides, notably warfarin, effectively replaced single dose fast acting poisons as the major means of rodent control in the 1950's. This new class of rodenticides dramatically changed rodent control, because unlike their highly toxic predecessors anticoagulants act over a period of days rather than minutes or hours. Rats and mice did not develop bait-shyness and there was not a need, therefore, for costly prebaiting. In addition vitamin K was an effective antidote and could be used to restore normal blood-clotting in poisoned animals or people.

Occurrence of anticoagulant-rodenticide resistance 1958-1976

The first report of a warfarin-resistant population of *Rattus norvegicus* came from Scotland in 1958 (Boyle 1960). This was followed by a similar report from an area on the Wales-England border near Shrewsbury (Drummond and Bentley 1967), and reports of problems in controlling populations of *Mus domesticus* (Dodsworth 1961). Anticoagulant-resistance in rodents was not confined to the United Kingdom but occurred frequently in those countries which had a high use of anticoagulant rodenticides (reviewed: Greaves 1985, MacNicoll 1986).

Widespread use of warfarin and other anticoagulant rodenticides effectively applied an evolutionary pressure to rodent populations. Investigations carried out on rats trapped in Wales and Scotland showed that resistance was inherited and that animals from the two separate areas had different alleles of the same warfarin-resistance gene (Greaves and Ayres 1969, 1982). Similar studies on warfarin-resistance in the house mouse also demonstrated that there was a single major warfarin-resistance gene (Wallace and MacSwiney 1976). Other studies have suggested, however, an induceable tolerance in this species which is under polygenic control (Rowe and Redfern 1965, Roll 1966).

Continued evolutionary pressure from the use of warfarin and other anticoagulant rodenticides resulted in the spread of resistance from the initial centre, and some populations of resistant rodents covered areas

of several thousand square kilometres (Greaves 1970). In the 1960's the spread of resistance was limited by using non-anticoagulant rodenticides when a new warfarin-resistant population was identified. This strategy was successfully employed to apparently eliminate resistance problems in seven out of eleven known cases in the U.K. (Drummond 1971). This approach was not, however, effective in controlling the populations of warfarin-resistant *R.norvegicus* that became established between Glasgow and Edinburgh in Scotland, on the Wales-England border and in Hampshire.

ANTICOAGULANT RESISTANCE 1977-1986

The second generation anticoagulants, difenacoum, bromadiolone and brodifacoum, became available for use as rodent control agents during this period and were considered to be effective against warfarin-resistant rodents (reviewed: Lund 1985). The introduction of these new, more toxic, compounds was thought likely to eliminate the problems caused by warfarin-resistance. This was unfortunately not the case and reports of difenacoum and bromadiolone resistance were made in several countries (Lund 1985). In the UK the most significant problem that became apparent during this period was that some warfarin-resistant rats in Hampshire were also resistant to difenacoum (Redfern and Gill 1978). A study carried out in 1980 (Greaves *et al.* 1982a) showed that of 202 rats trapped on 42 farms in north-east Hampshire, 85% were resistant to warfarin and 14% were also resistant to difenacoum. Examination of the effectiveness of bromadiolone and brodifacoum on farms in Hampshire in 1981 (Greaves *et al.* 1982b) showed that treatments with these compounds were protracted, and indicated that at least some of the rats had a level of resistance to these alternative anticoagulants.

The situation on the Wales-England border was different in that the second-generation compounds appear to be effective against warfarin-resistant rats. Staff from MAFF Tolworth Laboratory have been monitoring warfarin-resistance in this area, and the results presented in Table 1 summarise the results of testing for warfarin-resistance carried out by the method of Martin *et al.* (1979) on rats trapped during the winter months of 1985-6. These results demonstrate that warfarin-resistant rats still form a significant part of the total population in this area, even though it is unlikely that warfarin is still used for practical control purposes.

TABLE 1

Warfarin-resistance[1] in *R.norvegicus* on the Wales-England border in 1985/86

County	Resistant/Tested
Clwyd	6/40
Powys	37/166
Shropshire	116/257

[1]Warfarin-resistance testing was carried out as described by Martin *et al.* (1979)

The second-generation anticoagulants are reported to have the same mechanism of action as warfarin (Breckenbridge *et al.* 1978, Whitlon *et*

al. 1978, Leck and Park 1981, Hildebrandt and Suttie 1982) and their increased toxicity is assigned to the highly lipophilic nature of these compounds when compared to warfarin (Hadler and Shadbolt 1975, Dubock and Kaukeinen 1978). Since the mode of action of the anticoagulant rodenticides is the same then cross-resistance, such as has occurred in Hampshire, should not be unexpected. Reports of resistance to second generation anticoagulants include cross-resistance of warfarin-resistant *M.domesticus* to difenacoum and bromadiolone (Rowe *et al.* 1981, Lund 1981) and a level of increased tolerance to brodifacoum (Lund 1981). Bromadiolone-resistance was reported as a major problem for rodent control in Jutland, Denmark (D.P.I.L. 1982).

Despite these examples of resistance to second-generation anticoagulant rodenticides there has not been an organised system of resistance monitoring in the UK. The results presented in Tables 2-5 are a summary of results obtained from resistance tests carried out at Tolworth on rodents trapped in England (excluding Shropshire) between 1977 and 1986. It should be noted that this does not represent an organised comprehensive survey, as the results are only drawn from experimental study areas and from those areas with reported rodent control problems. It is possible, therefore, that other anticoagulant-resistant rodent populations have developed that we are not aware of.

TABLE 2

Warfarin-resistance[1] in *R.norvegicus* in England[2] between 1977 and 1986

County	Year	Resistant/Tested
Avon	1978	1/21
Bedfordshire	1981	1/1
Berkshire	1979	12/14
	1980	18/27
	1981	8/9
	1983	11/11
Buckinghamshire	1977	26/26
Hampshire	1979	77/108
	1980	76/89
	1983	7/18
Kent	1977	2/2
Lancashire	1978	15/33
South Humberside	1978	6/15
Surrey	1979	0/10
	1981	2/41
	1982	2/15
	1983	0/3
Wiltshire	1981	6/6
Worcestershire	1981	11/12

[1]Warfarin-resistance testing was carried out as described by Martin *et al.* (1979).

[2]Excluding Shropshire

The information summarised in Table 2 was obtained over a 10 year period using the method described by Martin *et al.* (1979) for measuring

blood clotting after co-administration of warfarin and vitamin K_1-2,3-epoxide to *R.norvegicus*. The results show that widespread use of anticoagulant rodenticides has resulted in a detectable level of warfarin-resistance in wild rat populations. Many of these animals may, however, be susceptible to the second-generation compounds. Difenacoum-resistance in wild trapped *R.norvegicus* (Table 3) was determined by a no-choice feeding test with 50 mg of difenacoum/kg diet (0.005%) as described by Greaves *et al.* (1982a).

The results that are summarised in Table 3 indicate that there is a detectable level of difenacoum-resistance in populations of *R.norvegicus* in three counties of southern England. This probably represents a dispersion from the original centre of anticoagulant-resistance in Hampshire.

TABLE 3

Difenacoum-resistance[1] in *R.norvegicus* in England between 1977 and 1986

County	Year	Resistant/Tested
Bedfordshire	1981	0/1
Berkshire	1979	2/9
	1980	5/24
	1981	5/9
Buckinghamshire	1978	0/11
Hampshire	1978	2/10
	1979	23/90
	1980	43/129
	1981	1/2
Surrey	1981	0/2
Wiltshire	1985	7/16

[1]Difenacoum resistance testing was carried out as described by Greaves *et al.* (1982a).

A smaller number of tests have been carried out for brodifacoum-resistance in *R.norvegicus* which are summarised in Table 4. These tests involved a no choice feeding of 5 or 6 mg of brodifacoum/kg diet (0.0005 or 0.0006%) for 6 or 7 days and recording deaths over a further 21 days. Some tests in 1983 included 3.5 mg of vitamin K_1/kg of diet. The animals were monitored for a further 21 days and deaths recorded. Only one rat of those tested survived a six day feeding test of 0.0005% brodifacoum with vitamin K_1 supplement. This same male animal survived a further feeding test without vitamin K_1, but was killed by a third, 7 day, feeding on 0.0005% brodifacoum. It may be significant that over 30% of the rats trapped for this study in Hampshire and Berkshire in 1982 and 1983 died during the acclimatisation period before test showing symptoms that could be interpreted as either anticoagulant poisoning or vitamin K deficiency. An increased dietary vitamin K requirement is a characteristic of some anticoagulant-resistant *R.norvegicus*, and it could be suggested that the animals dying before test were the resistant members of the population.

TABLE 4

Brodifacoum-resistance in *R.norvegicus* in England between 1977 and 1986

County	Year	Resistant/Tested
Berkshire	1982[1]	0/10
	1983[2]	1/14
	1983[1]	1/1
	1984[3]	0/1
Hampshire	1981[4]	0/4
	1982[1]	0/13
	1983[2]	0/4

[1] 5 mg brodifacoum/kg diet, 6 days no choice feeding
[2] 5 mg brodifacoum and 3.5 mg vitamin K_1/kg diet, 6 days no choice feeding
[3] 5 mg brodifacoum/kg diet, 7 days no choice feeding
[4] 6 mg brodifacoum/kg diet, 4 days no choice feeding

The data presented in Table 5 summarises the results obtained from testing wild *M.domesticus* trapped on a farm where bromadiolone-resistance was suspected. The observation that animals survived 21 days of no choice feeding, and a further 21 days after test, on diets containing highly toxic second-generation anticoagulants indicates a considerable degree of resistance to these rodenticides.

TABLE 5

Anticoagulant-resistance in *M.domesticus* in England between 1977 and 1986

Anticoagulant	County	Year	Resistant/Tested
Bromadiolone[1]	Gloucestershire	1984	11/30
Brodifacoum[2]	Gloucestershire	1984	1/11

[1] 50 mg bromadiolone/kg diet, 21 days no choice
[2] 20 mg brodifacoum/kg diet, 21 days no choice

SIGNIFICANCE OF ANTICOAGULANT RODENTICIDE RESISTANCE IN THE U.K.

Warfarin, and the other first generation anticoagulants, largely replaced the older acute poisons primarily because of increased efficiency of rodent control, but also because of greater safety margins. When warfarin-resistance developed, or was selected for, in wild rodent populations there was a marked fall in the efficiency of rodent control operations in many areas. This led to investigations by both industry and Government laboratories, and to the development of second-generation anticoagulants. These new rodenticides were considered to be effective against warfarin-resistant rats and development of rodenticides with alternative mechanisms of action was postponed. At the same time monitoring of rodenticide-resistance became a low priority requirement.

The cross-resistance problem in Hampshire was identified, however, within a few years of the introduction of difenacoum onto the market. It is now apparent that warfarin is more effective at controlling warfarin-susceptible populations, which cover the majority of the country, than the second generation compounds are for controlling infestations in a large, but ill-defined, area of southern England. Reduced efficiency of rodent control results in increased costs to both the pest control industry and the consumer as well as increased losses due to damage and spoilage, together with an increased risk of disease transmission. The renewed search for alternative rodenticides, and other control techniques, depletes the resources of the pesticide industry, and Government organisations that provide advice and information on rodent control.

Publicity about warfarin-resistant rodents led to a rapid uptake of the more toxic, and potentially more hazardous second-generation compounds when this may not have been necessary. This has effectively removed the option to switch to the more toxic compounds if and when resistance develops. The shortage of alternative rodent control methods, which do not use anticoagulants, implies that there will be continued evolutionary pressure on rodent populations and it is likely that there will be an increase in reports of resistance to the second-generation compounds. This would imply that efficiency of rodent control in those areas would be considerably reduced, and result in an increase in rodent infestations with all of their undesirable associated problems. Our laboratory is re-evaluating the need to test samples of animals trapped from the field in situations where control operations have not been effective. This is particularly important in relation to maintaining our ability to advise the agricultural industry on rodent control. By recommendation of changes in rodenticide usage to individuals and their neighbours, it may be possible to minimise or postpone serious problems of resistance to second-generation anticoagulants.

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FEEDING BEHAVIOUR, SOCIAL INTERACTIONS AND POISON BAIT CONSUMPTION BY A FAMILY GROUP OF WILD RATS LIVING IN SEMI-NATURAL CONDITIONS

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ABSTRACT

The behaviour of a family of wild rats (Rattus norvegicus) living in a large covered arena was video recorded and their feeding continually monitored using electronic balances connected to a remote microprocessor. After a period of neophobia, regular feeding patterns emerged. The mean amounts eaten by individual rats varied between 16.3 and 84.6g/day. The number of meals varied from 5 to 11/day, almost all of which occurred at night. As colony size increased, more meals were taken during daylight. Individuals exhibited different degrees of neophobia towards new foods or bait containers. Difenacoum[1] bait in a familiar container was accepted in preference to standard diet in a novel feeder. Within five days of the poison being introduced all feeding activity ceased.

INTRODUCTION

The domesticated rat, confined alone in a small cage, is not a good model for the behaviour of wild rats in their natural environments. Behaviour is influenced by a host of factors including age, sex, position in the social hierarchy, the availability of food, water or shelter, as well as temperature, lighting etc. Confined colonies of wild rats provide the best opportunity to observe the effects of manipulation of such factors. Unfortunately naturally confined colonies, such as island populations, are rarely available. Rats can be artificially confined in large outdoor colonies (eg. Calhoun 1963, Boice & Adams 1980) but there, little control of environmental conditions is possible and it may be impracticable to remove introduced toxicants. In small, 'single-room', indoor colonies the population density rapidly becomes excessive (Calhoun 1962). A small colony housed in a large covered arena seems to offer the best compromise between the need for experimental control and relevance to the natural situation.

A major methodological problem when studying confined colonies has been the identification of individuals and the quantitification of their food consumption. However the relatively recent availability of electronic balances capable of sending data to a remotely sited microprocessor now enables detailed, accurate recording of each visit to a food source (Sterner 1982). We have used this method to monitor the feeding activity of members of a small colony of wild rats living in a large covered arena, in addition to observing their behaviour either directly or from infra-red video recordings. Our aims are a) to gather basic information on individual differences in feeding behaviour, and b) to see how feeding patterns change in relation to new baits and bait containers.

[1]Mention of commercial products in the text does not imply endorsement.

MATERIALS AND METHODS

Subjects

The colonizers were three wild Common rats (Rattus norvegicus) trapped on a local farm and kept singly in laboratory cages throughout a quarantine period. The rats were a male (482.6g) and two females (331.2g and 252.9g).

Enclosure

The rats lived in a stack of hay bales (1.5 x 3 x 1m) containing five nest boxes, in one of two adjacent pens (each 5 x 10 x 1.5m with concrete floors and 'Aluzinc'-coated steel walls) located in a large, bird-proof, agricultural building (see Plate 1). Four large rooflights provided daylight illumination. At night eight 60W red bulbs illuminated the interior and four 500 W infra-red spotlights lit the feeding and drinking areas. A raised observation hut was situated at one end and video cameras, attached to roof members, were directed at the feeding area and the "blind" side of the stack. Water was continuously available from a poultry font near the feeding sites. Food pots (120mm diam x 100 mm deep) containing powdered diet 41B (Labsure), were situated 1m on either side of the font, each mounted on the weigh-head of a 'Galaxy 4000' electronic balance. The balances were housed in a nearby building which also contained a 'System 96' micro-processor that logged all weight changes.

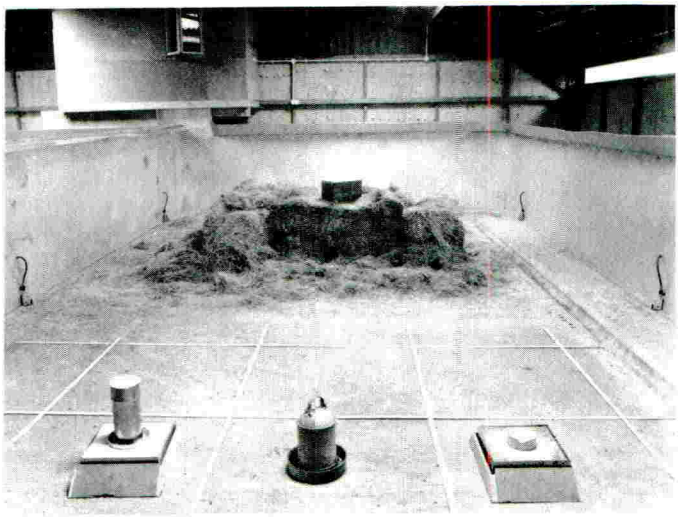

Plate 1. The rat arena: in the foreground are the feeders, novel on the left, familiar on the right, each mounted on an electronic balance, between them is the water font, behind this the stack with the nest boxes and in the background the observation hut.

Procedures

The balances registered each change of weight on the pan after automatically applying a 'stability criterion'. These changes were logged, together with the time, by the micro-processor. Thus a rat stepping on the balance to feed would be weighed and thereby identified. When it left the

balance the amount of food eaten could be calculated by subtracting the new weight of the pan from the previous weight. The food pots were refilled and the balances re-set each morning.

For one week after the rats were first introduced, their behaviour was observed directly each evening and activity in the feeding area video-taped for the remainder of the night. Subsequently various experimental manipulations were made, as described together with the relevant findings in the following section.

RESULTS

Habituation to the pen

At first the rats were reluctant to venture far from the stack but by the end of the first week they had explored the entire pen. The male was the boldest in this exploration, although he did not feed until the sixth night whilst the females began feeding on the fourth night.

Growth and reproduction

The male grew steadily to approximately 540g on day 46 and maintained this weight thereafter. The smaller female was found dead in the stack on day 24 and at death weighed only 269g. The larger female grew until she weighed 450g on day 80; this weight was subsequently maintained. Her first two litters each consisted of a single pup: a male (J) and a female (j) respectively. Both reached 200g in 61 days. When the colony was terminated after 21 weeks, J was 125 days old and weighed 360g, whilst j was 97 days old and weighed 263g. The third litter consisted of four males and four females; these weighed 341g, 331g, 228g, 217g and 281g, 272g, 253g and 234g at autopsy when they were 73 days old. The last litter contained four females and these weighed 140g, 131g, 117g and 113g at autopsy when 44 days old.

Daily activity

Until the death of the smaller female, feeding occurred at any time from mid-afternoon to mid-morning but after her death feeding was confined to the hours of darkness whilst the colony was small. However, as colony size increased, more time was spent feeding and often this occurred during daylight (Fig. 1).

Feeding patterns of individuals

In order to derive an objective definition of 'a meal' we plotted the log survivorship functions of the inter-feeding-bout-intervals of the adult male and female and the juveniles J and j when aged 66 and 39 days respectively. When behaviour patterns are organized in bouts the slope of this function is steep to begin with before changing sharply to a gradual decline. The point of change of slope is the most realistic estimate of the end of a bout (Slater 1974). As there were no significant differences between individuals in the overall shape of their survivorship functions, all the data were pooled. The resulting curve (Fig. 2) clearly indicates that for our rats a meal may be defined as any series of consecutive bouts of feeding separated by intervals of less than 13 mins.

Table 1 summarises the results of an analysis of the feeding patterns of these four rats during four consecutive 24 hour periods. There are many significant differences. The female ate the most and her meals were longer and larger than the others. Her feeding rate during a meal was however not significantly greater than the adult male who had fewer and shorter meals. The older juvenile had as many meals per night as the adult female but these were much shorter than hers and also his feeding

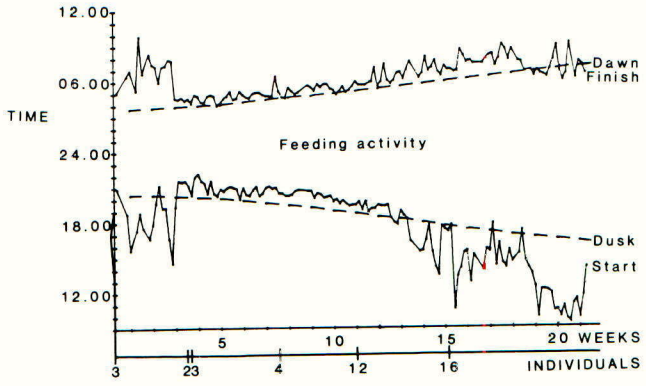

Fig. 1. The start and finish of the main period of feeding activity by the colony and the effects of day length and colony size.

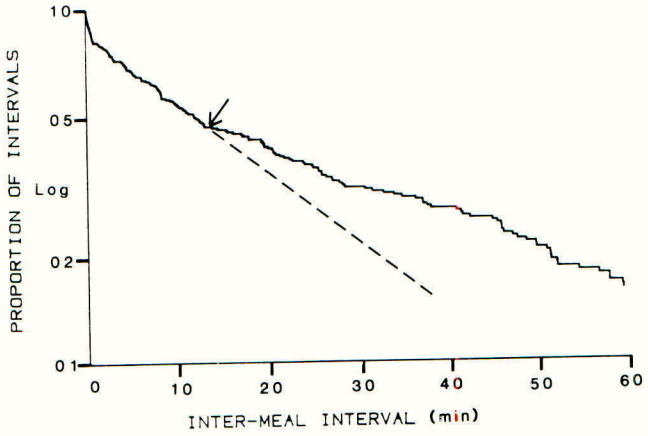

Fig. 2. Log survivorship curve of intermeal intervals for pooled data of four rats over four days, the graph plots the proportion of intermeal intervals which exceed time (t). The arrow indicates the point of discontinuity.

rate during a meal was significantly less. The younger juvenile ate the least, having fewer meals than the larger juvenile but approximately the same meal length and feeding rate.

The male frequently fed at the same feeder as the younger juvenile and only very rarely with the others. The female frequently shared a feeder with the younger juvenile but not the older. The juveniles often fed together, and the younger juvenile seldom fed alone.

TABLE 1

Means of measures of feeding by four individual rats monitored during four consecutive 24 hour periods.

	♂	♀	J(♂)	j(♀)	SED	p<
Total food eaten (g/day)	28.0	84.6	32.1	16.3	6.5	0.001
Total time spent feeding (s/day)	4688	12062	7674	5820	536	0.001
Number of meals (meals/day)	5.25	9.50	11.50	7.00	0.99	0.001
Duration of meals (s/meals)	914	1313	670	897	163	0.05
Size of meal (g)	5.30	9.22	2.80	2.64	1.18	0.001
Rate of feeding (g/min)	0.355	0.420	0.249	0.169	0.05	0.01

Bars connect individuals that are not significantly different from each other at $p<0.05$.

The introduction of a new food

On day 14, one of the feeders was emptied and refilled with whole wheat grain. This food had not previously been presented in the pens but had been an alternative to diet 41B during the early part of the rats' quarantine period. The male fed on the wheat immediately, ate equal amounts of wheat and 41B on the second night, ate mostly 41B on the third night and thensubsequently ignored the wheat. The larger female ate wheat only on the third night after its introduction. The smaller female opted for a sampling approach eating 0.1g of wheat on the first night, and then various amounts on subsequent nights until switching back entirely to 41B on the seventh night.

The introduction of a novel food

On day 21 the wheat feeder was emptied and refilled with a mixture of pinhead oatmeal, sugar and corn oil (90:5:5) which we considered to be

both novel and particularly palatable. The adult rats ignored the novel food for four nights. The male then fed mainly on the oatmeal mixture and maintained his preference for that feeding point even after the food pot had been refilled with diet 41B on day 29. The remaining female also came to prefer the novel diet but her preference for this feeding point stopped immediately the oatmeal was withdrawn.

The introduction of a novel feeding container

By day 126 the colony had grown to 16 rats, and a new larger container similar to the drinking font was placed on one of the balances. At first all rats avoided the novel feeder although it contained the usual diet 41B. They drank from the side of the water font furthest from it and continually glanced toward it when feeding from the familiar feeder. On the first day only 86 visits were made to the familiar feeder, compared with 464 visits made the day before the novel container was introduced. The total food consumption was also reduced; 236g compared with 310.2g during the control day. On the second day there were 1636 visits to the familiar feeder and consumption rose to 338.6g. Thereafter the number of visits to, and the food consumption from, the familiar feeder stabilised at about the levels previously recorded from both balances prior to the introduction of the novel feeder.

On the fifth day the food in the familiar feeder temporarily became exhausted yet the rats still avoided the novel feeder. It was not until the eleventh day, when the food in the familiar feeder again became exhausted, that any food was eaten from the novel container. The older juvenile was the first to feed from the novel feeder, followed by the younger juvenile and the litter of infants. Their visits were very tentative; 48% resulted in no measurable food consumption and no meal was greater than 2g. The adults continued to visit the familiar feeder until at last on the twelfth night they also fed from the novel feeder. On that night there were 904 visits to the novel container but only 62g were eaten; there were also 230 fruitless visits to the empty familiar feeder.

On the thirteenth day the familiar feeder was replenished and food consumption at the novel one fell to 3.3g, derived from only seven visits; 383.5g were eaten from the familiar feeder derived from 1386 visits. During the following two days visits to the novel feeder increased to 349 (18.4g consumed) and 198 (12.0g consumed) respectively. There was still, however, far more feeding activity around the familiar container, 3585 visits (405.6g consumed) and 1578 visits (375.5g consumed).

On the sixteenth day the feeders were switched between the balances. Feeding preference immediately shifted to the balance now containing the familiar feeder. The rats continued to feed exclusively from the familiar feeder in the new position until the food was exhausted on the eighteenth day when some feeding from the novel feeder occurred. The rats fed again exclusively from the familiar container after it has been replenished on the nineteenth day.

The introduction of a rodenticide bait

As the colony was rapidly growing too large for the arena we decided to introduce, on day 147, a rodenticide bait and to monitor the responses of the rats. The novel container was filled with diet 41B but the familiar feeder was emptied and refilled with a bait of medium oatmeal mixed with 50 mg/kg difenacoum using a commercial Neosorexa 'mastermix'. When the rats were presented with this choice, of a familiar food in the now 'not-so-novel'

container and and a novel poison bait in a familiar container, we were surprised to find that they preferred the latter.

A neophobic response was observed on the first day when there was a reduction in food consumption from the familiar feeder and a marked increase in the number of short visits to it (see Table 2), particularly in visits on which no food was consumed. There was also a slight compensatory increase in both visits to the novel container and the amount of food taken from it. The neophobic response had waned by the second day when both the number of visits and the amount of food eaten from the familiar feeder were of the same order as during the day prior to the introduction of the poison: the number of visits to, and the amount of food consumed from the novel container decreased. The third day revealed the same pattern as the second, but on the fourth day there was a dramatic fall both in visits and food consumed. Only 7 visits were made throughout the fifth day and all were to the novel container from which 9.8 g were taken. No further activity was observed from the colony. The individual(s) that had learnt to associate the effects of the poison with the novel bait in the familiar feeder had learnt this association too late.

TABLE 2

The choice between a poison bait on B1 and a novel container on B2 (figures for group, n = 16)

Day	Visits to balance		Food eaten from balance (g/d)	
	B1	B2	B1	B2
0 (control)	1282	203	703.5	18.9
1	1899	840	329.1	102.0
2	1234	109	409.4	10.6
3	836	16	350.3	0
4	23	0	29.1	0
5	0	7	0	9.8
6*	0	0	0	0

*Dead rats were found in the pen

DISCUSSION

Although one of our female colonizers died quite soon after the colony was started, almost certainly as a result of aggression from the other female, we were encouraged by the steady growth in numbers after this unpromising start. The surviving female was pregnant almost continuously and produced litters on days 25, 53, 77 and 106 with an abortion on day 30. The weight gains of the colonizers and of the pups J and j support the view (Calhoun 1963) that rats in large pens are usually healthier and larger than those in laboratory cages. Our estimates of the amount of food eaten, and the way it was consumed, were broadly in accordance with earlier studies using wild rats (Shepherd 1986) although there appeared to be more variation between individuals. The use of electronic balances linked to a microprocessor proved a reliable technique.

Many of our results from this initial study could have important implications for rat pest control. There were wide variations in the total amount of food eaten which may be correlated with the size, sex and reproductive status of the individual. The adult female who was suckling a litter of eight pups which had been born 15 days previously and was pregnant with a further litter ate quite exceptional amounts. The size of meals and rate of feeding also differed significantly between individuals. Even in our relatively small colony, social interactions appeared to force some rats to feed at times or in places which were not those they would choose given free choice. In particular the sudden reduction in the duration of feeding after the death of the smaller female suggests that she had been avoiding the dominant female who deterred even the males from using the same food source. The wide differences in the total amount of food eaten indicate that, even when the bait base is a familiar food, the amount of poison consumed by individuals would vary considerably and not necessarily in proportion to body weight. Differences in the rate of feeding or the number and size of meals imply that with rapidly acting acute poisons there are likely to be differences between individuals in the susceptability to poison shyness resultant from the ingestion of sub-lethal doses.

The considerable differences in the degree of neophobia exhibited by individual rats may be exhibited as differences in trappability or susceptability to poison baiting. These differences cannot always be simply ascribed to sex, age, (Misanin et al. 1985) or dominance (Robertson 1982). When first introduced to the pen the adult male was the boldest in exploring the floor area but the last to feed on our balances. The juveniles usually followed their parents to food as we expected (Galef and Clark 1972), but when they were faced with the choice between using a novel container and starving, it was the older juveniles who first exploited the new food source; they were followed by their siblings and then finally by their parents. Therefore, we have two possible answers to the question: "Which rats do not eat the poison when a treatment fails?" The first is that it is the subordinates, who have been excluded from the "delicious" rat bait by the dominant animals. In this case a little perseverance should do the trick since once the dominants have been eliminated, the subordinates will eat the bait in their turn. The second answer is that the survivors are the dominant animals who are most food neophobic and have not been tempted away from their familiar diet. In this case simply persevering with the treatment is unlikely to succeed. Analysis of the weights and age of the individuals killed should indicate which alternative is correct in any given situation.

Our data suggest that neophobia towards a palatable new food, even one containing poison, is likely to be transient compared to the neophobia towards a new object such as our food container. Others have already drawn attention to container neophobia (Mitchell et al. 1973), but we were interested to observe that it waned very slowly, was apparently extinguished when the need for food increased, and reappeared when a less novel alternative was again available. It may be that when poison baiting rats, a non-salient, preferably familiar, bait container is more important than familiarity with the bait base and that the conventional prebait period of one week is inadequate. Although we must clearly gather data from several more colonies before we can reach any firm conclusions, our studies so far have confirmed that remote electronic monitoring reveals details of individual behaviour which have hitherto been impossible to record. We are confident that these facilities will enable us to considerably extend our understanding of a wide range of problems from bait acceptance to trap responses.

ACKNOWLEDGEMENTS

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THE CONTRIBUTION OF FIELD STUDIES TO STORED PRODUCT RODENT CONTROL

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ABSTRACT

Field studies are selectively reviewed to provide basic information on the movements of wild rats and the population dynamics and social structure of rat infestations. This information is used in an attempt to answer such questions as what is the likelihood of colonisation of food stores from outlying infestations, how fast is the growth of established infestations and what is the optimum baiting strategy. The potential for long-distance colonisation is shown to be surprisingly high whereas the expected growth of infestations is found to be considerably less than some sources have suggested. Baiting strategies are discussed in the light of social and behavioural factors and a modified hypothesis for the success of pulsed baiting is proposed.

INTRODUCTION

According to Agriculture Canada (1979), rats and mice are "beyond doubt, the most widespread and destructive pests in the world". The same source contends that "a pair of rats, in three years, could have a total of 350 000 000 offspring [descendants] under favourable conditions" and "a single rat will eat, spoil or damage $20 - $25 worth of goods per year". It is unclear how the authors derived these figures, but they do illustrate the popular perception of rat population explosions. However, since the world is not carpeted with rats, it does not require a biologist to point out that conditions cannot always be favourable; although it must be said that, except possibly for lack of water, it is hard to imagine a more favourable habitat for rodents than food stores.

With so much at stake, one might think that great effort would have been expended in studying rats in the wild; however, as we will show, this is not the case, and at present, more questions must be begged than can be answered. The purpose of this paper is therefore to outline the actual and potential contributions of field studies to the realistic assessment and control of rat infestations in farm buildings and other food stores. Biologists would expect the behaviour and ecology of rats to be complex and variable; our argument is that better understanding of that complexity will facilitate better pest management. The topics that will be addressed are: where do infestations originate, how quickly do they grow and what is the best way of stopping them?

THE START OF THE PROBLEM - MOVEMENT

It is widely held among farmers that new rat infestations do not arise from survivors of the last poisoning campaign but from rats moving in from the farm next door. Whether or not this is true will depend on how far rats move and how often.

The longest recorded single movement was a round trip of 3.3 km made by a male rat in a single night, discovered during a radio-tracking study (Taylor & Quy 1978, Hardy & Taylor 1979). This single excursion exceeds the longest recorded distance travelled over a month, 954 m, as revealed by capture-mark-recapture (Hartley & Bishop 1979). Similarly, studies based on trapping (e.g. Davis et al. 1948, Hartley & Bishop 1979, Stroud 1982) tend to indicate much smaller average ranges than do those based on radio-tracking. Hartley &

Bishop found the mean range size of 11 male rats on farmland in Wales to be 66 m, whereas Taylor (1978), using radio-tracking, found the mean range of 7 males on a farm in the south of England to be exactly ten times as great. In the absence of further details about differences in ecology between the two farms, it seems likely that these disparities reflect differences in methodology rather than in the actual movements of the animals.

It is clear, from the few published radio-tracking studies, that much of the variation in the extent and pattern of movements by rats can be attributed to the abundance and distribution of food. Specifically, rats associated with food sources in buildings generally show very restricted movements, whereas rats living away from food stores make long journeys to such concentrated food sources, or range widely when foraging (Taylor & Quy 1978, Taylor 1978, Hardy Taylor 1979, Fenn & Macdonald unpubl.). Consequently, a colony of rats some 3 km distant from a food store could provide the basis for an infestation, and our radio-tracking studies show there is a significant risk of immigration from rats within 0.5 km (see also Hardy & Taylor 1979).

Knowledge of the pattern of rat movements is also important in understanding the spread of resistance to rodenticides. The monitoring of an outbreak of resistance on the England/Wales border showed a radial spread of about 4.8 km/year (Greaves & Rennison 1973), but in exceptional circumstances the spread could possibly be much more rapid; Homolka (1983), quoting Kozlov (1979), states that "in the unploughed grounds of Kazakhstan the Norway rats populated places 10 km or more distant from villages and within a year the limits of the areas populated by them were shifted 70 km away". These figures may be extreme, but few data are available on the dispersal of rats or the distances moved by them. Similarly, it is unclear how these two elements vary with ecological circumstances or how they interact with other factors, such as the selection pressure from continued baiting, which are involved with the maintenance and spread of resistance (Smith & Greaves 1986).

Perhaps as important as the question of where an infestation develops is that of when it develops. A survey of 1 584 farms in Wales by Huson & Rennison (1981) confirmed previous suspicions of an increase of rat infestations of farm buildings in winter, regardless of whether the buildings were used for food storage or animal harbourage. To explore the basis of this pattern, we have trapped and radio-collared rats on farmland throughout the year. The results reveal a large increase in the movements of rats in the fields after harvest. Many of these itinerant rats become established around permanent food sources, such as woodland pheasant-feeders, for the autumn and winter and only a small proportion of the rats travelling the fields in late summer maintain stable home ranges there over the winter. Similarly, Errington (1935) found that as winter progressed in Wisconsin, field-living rats moved into corn shocks and then to buildings. Taylor (1978) found a marked increase in the movements of rats when supplementary food was removed from bait boxes in winter, one of his radio-tracked rats visited five boxes (instead of the usual one) and covered nearly 1 500 m in two hours.

Our radio-tracking studies and those of others mentioned previously confirm the potential for immigrant rats to seed new infestations. However, the relative contribution of such immigrants in initiating a new infestation, as opposed to that of local survivors of previous control, is unknown. This is a difficult topic to study, not least because of the problems of completely ridding farm buildings of rats, or of monitoring the small number of rats likely to survive or to immigrate. However, what field studies have shown is that, on lowland farms, the potential for reinfestation is probably enhanced by the greater mobility of field rats following harvest. This argues in

favour of applying prophylactic measures in the autumn. These measures might include the repair and renewal of rat-proofing, the removal of edible waste and possibly the replenishment of permanent bait stations. However, Smith & Greaves (1986) point out that permanent baiting should be discounted as soon as it becomes less than 100% effective, to minimise the risk of selecting for rodenticide-resistant rats.

THE SCALE OF THE PROBLEM - POPULATION DYNAMICS

A knowledge of the population dynamics of any stored product pest is vital to planning its management. How quickly do a pair of rats become a plague and at what stage is it economically advantageous to institute control measures? As well as answering these sorts of questions, a knowledge of the characteristics of the pest population also gives some idea of the speed at which resistance to pesticides may develop.

A review of 13 field studies by Brooks (1973) showed that the potential number of young rats produced per female per year ranged from 18.5 to 55.7, with a non-weighted average of 36.81. Data on rat biology in food stores are not available but corn ricks may provide a close approximation. In corn ricks, Leslie et al. (1952) found an average of about nine embryos per pregnant female. Under ideal, but patently absurd conditions, this would lead to an annual productivity of 108 offspring per female per year, and a yearly total of over 450 000 descendants from a single pair. The latter figure was calculated on the assumptions of no mortality, sexual maturity in two months, a sex ratio of 1:1 and no density-dependent regulation of population size. What factors are likely to constrain this enormous potential for increase? The answers are obvious in principle to the population biologist. Nevertheless, we will list them here, largely to illustrate how few of the relevant factors have been adequately studied in the case of rats.

The assumption of no mortality is clearly unrealistic. A rat's potential lifespan might be three years or more, but Davis (1948) found that Maryland farm rats had only a 5% chance of survival to the end of a year. Although food stores might afford protection against many natural predators – 5/11 radio-tracked rats were killed by predators in a study by Taylor (1978) they might also be well stocked with cats (see Elton 1953) and provide above average conditions for disease.

The ideal of sexual maturity after two months is unlikely to apply, even in a favourable environment such as a food store. In corn ricks, Leslie et al. (1945) concluded that females were capable of conception at the age of eleven or twelve weeks.

The sex ratio in an environment such as a food store is unlikely to be 1:1. Kendall (1984), in a review of sex ratios in different field environments found that males tended to be displaced from the more favourable habitats. Leslie et al. (1952) found that the percentage of male rick rats fell from 50.62 in young juveniles (< 44 g) to 41.34 in adults (> 195 g), suggesting that it is sexually mature males that are displaced. Since the social system of wild rats is largely unknown, the operational sex ratio (i.e. the ratio of reproductively active individuals of each sex) is unknown. However, the idea that social pressures affect sex ratio is supported by the observations of Calhoun (1962). He showed that several separate social groups existed within his enclosure (see the following section of this paper) and that one criterion for judging the ascendancy of a group was the sex ratio, the higher the proportion of females, the higher the social rank of the group (group rank was also indicated by other criteria such as number of

wounds and reproductive success). Clearly, the more females in a group, the greater potential productivity. Since food stores are ideal habitats one might expect them to support highly productive rat populations, due both to female condition (leading to larger litters) and a skewed sex ratio (leading to a higher proportion of females in the population).

On the other hand, a density-dependent inhibition of reproduction operates when rat populations become too dense (Calhoun 1949) and so in reality their population processes are probably described by a logistic curve. This is a population growth curve where the rate of increase (r) is progressively reduced as the population reaches carrying capacity (k); a study of the rate of recovery of decimated populations of rats in the wild suggested exactly this type of growth (Emlen et al. 1948).

So having illustrated some of the reasons why a pair of rats will not produce 450 000 descendants, we will now attempt a more realistic estimation. If we use an estimate of r derived from the rick study (.094/rat/week), a pair of rats could produce a maximum of about 214 descendants in a year. This figure does not allow for any restriction imposed by the carrying capacity of the environment. Calhoun (1962) suggested that the capacity of his enclosure (30 x 30 m) was 2 000 rats. If, in the absence of a more appropriate figure, we assume this to be the carrying capacity of our notional food store, the population at the end of the year falls to about 195, a far cry from the 450 000 predicted earlier, but quite enough to warrant control measures.

SOLVING THE PROBLEM - SOCIAL STRUCTURE AND BEHAVIOUR

Caughley (1977) states that "populations are more vulnerable to a manipulation of their habitat than they are to a direct manipulation of their numbers". This emphasises the importance of good hygiene and rat-proofing and indicates that complete eradication of rat infestations can only be achieved by removal from the area of a part of the rat's requirements, a condition that is unlikely to be met in food stores except possibly in the case of water. The result therefore, is that anticoagulant poisoning has been (and is likely to be in the forseeable future) the most practical method of control. We suggest that a knowledge of the social structure and behaviour of the rat population may be crucial in the design of a successful poisoning campaign.

In contrast to mice, rats show a marked avoidance of any new object in their environment, a phenomenon known as neophobia. Additionally, because the symptoms have caused them to cease feeding before taking a lethal dose, rodents which have fed on acute poisons and survived, will be "bait-shy". That is, they will be averse to taking the bait again for up to four months (Barnett 1948). Both these problems can be overcome by the use of 'saturation baiting' with chronic anticoagulants, the best known of which is warfarin. However, this technique is both labour-intensive and expensive, involving the continual replenishment of several (often about a dozen), large (250 g) bait points until no more bait is taken (Dubock 1984). The principle underlying saturation baiting is that it allows rats to overcome their neophobia, so that they feed regularly until death. The use of a chronic anticoagulant also means that the rats will already have ingested a lethal dose by the time that adverse effects develop, thus overcoming bait-shyness. However, as reported by Dubock (1984) and other authors, baiting campaigns in the field often result in less effective or slower control than expected on the basis of laboratory studies.

To explain these results, it was hypothesised that some "first-feeding" rats were preventing others from taking the bait, and, due to the delay in onset of symptoms, were continuing to monopolise the bait even after ingesting a lethal dose themselves. This idea gave rise to the technique of 'pulsed baiting' (Dubock 1979). In this case many more (often > 100) smaller (5 - 15 g) bait points are replenished at weekly rather than daily intervals, giving the "first-feeders" time to die before offering new bait. Pulsed baiting reduces labour and bait costs and obtains maximum advantage from the newer single feed anticoagulant rodenticides such as brodifacoum. Dubock, (1984) cites examples where the technique worked, but it is hard to reconcile the suggestion of bait monopoly by certain individual rats with field observations.

Calhoun (1962) records 10 to 15 rats grouped around a food hopper and over 30 rats have been recorded feeding within a few feet of each other on a rural refuse tip (Fenn. unpubl.). Certainly, both observations were made when there was a surplus of food, and Calhoun reported increased antagonism when food ran short, but the provision of poison bait would be expected to provide a temporary abundance of food and therefore lessen antagonism. Calhoun's (loc. cit., p283) analysis of the activity of marked rats showed that they consistently arrived to feed at a hopper at particular but different times, resulting in a marked segregation.

Taking these and other observations, we propose a slightly different hypothesis. Our proposal also stems from the idea that pulsed baiting succeeds because it overcomes the exclusion of rats from bait points. However, we suggest that exclusion rests not on the behaviour of individuals, but rather on spatial subdivisions within the rat population. If the colony of rats in an area constitutes several subgroups or 'clans', then clan members might defend an area containing a bait point, perhaps with temporal stratification occuring within a clan, but with members of other clans being excluded. In short, the root of the problem would lie in group territoriality rather than individual dominance. Since there appears to be no convention for the nomenclature of rat groupings, in following discussion we shall use the word 'colony' to describe the whole infestation and the word 'clan' to describe a subgroup within a colony; where a word appears between single quotation marks, this indicates the usage by the quoted source.

Is there any evidence for social subunits, such as clans, in rat colonies? Hardy & Taylor (1979) found a high degree of overlap in the ranges of five male and two female rats radio-tracked around farm buildings. Telle (1966) found that the smaller the size of a rat 'flock' the less tendency there was to accept strange rats and Steiniger (1950), working in a small enclosure, found that stable 'ingroups' often developed around a family structure, and that members of these groups would attack any alien rats. Calhoun (1962) reported large male rats dominating fairly extensive areas in his larger experimental enclosure. These areas coincided almost exactly with discrete 'colonies'. However, lower ranking rats always managed to find a spatial or temporal window in which to feed at the communal food source, probably because this was an area where rats were prevented from establishing homesites and which consequently was not controlled by any single 'colony'.

Two studies shed light on control success relative to patterns of bait placement. Fenn et al. (in prep.) found, in a pilot study, that four radio-tracked rats from at least three discrete areas of an Oxfordshire farm fed from the same source, a building housing a grain mill. The rat population on this farm was apparently completely destroyed by the application of several

kg of anticoagulant at a single bait point in the grain mill. However, Buckle et al. (1987), using chemical bait markers, found that a larger number of bait points increased both the amount of bait taken by each rat and the number of rats that took bait. In the light of Buckle's results the success of our single bait point would be surprising if we did not expect social organisation to vary with ecological circumstances. The unusual degree of control in our study may have been achieved because it was mid-winter and there was little other food available to the rats and because no single clan was established at the bait site due to the lack of harbourage in the building.

In conclusion, we hope to have shown that any social, and in particular spatial subdivision within a food store colony will probably affect the access of rats to poison bait sites. Therefore, an understanding of rat society and its ecological determinants will be crucial in deciding upon the optimum baiting strategy for a particular infestation.

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PERFORMANCE AND SAFETY OF THE NEW ANTICOAGULANT RODENTICIDE FLOCOUMAFEN

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ABSTRACT

Flocoumafen is a new, highly potent, single-feed anticoagulant rodenticide from Shell Research Limited, effective against all species of rats and mice tested, including those resistant to earlier anticoagulant products. Marketed under the trade mark Storm®, the ready-to-use bait is a wax-bound block. This bait formulation has given excellent control in a range of operational uses. The blocks are convenient to handle and retain their good palatability in storage. Storage and field use characteristics are improved by the addition of insecticidal and antimicrobial agents. Non-target safety is enhanced by the block's lack of attractiveness to birds, whilst the use of a taste deterrent looks promising as a means to improve human safety.

INTRODUCTION

Many different methods have been devised to control rodent pests but, in practice, effective control largely relies on the use of anticoagulant rodenticides baits. Anticoagulant rodenticides have many advantages over alternative chemical control agents (mainly acute poisons), including their good palatability, the lack of bait shyness, and the availability of an effective and practical antidote.

Commercial anticoagulant bait formulations vary considerably in their potency to the different pest species. The practical implications associated with differences in biological performance are further complicated by the fact that certain strains, particularly of the commensal rodents, are resistant to the earlier, so-called first generation anticoagulants (Greaves 1985).

The threat of resistance becoming widespread prompted the development of the more potent second generation products difenacoum (commercialised in 1974), bromadiolone (1976) and brodifacoum (1978). Although isolated cases of resistance to difenacoum and bromadiolone have been reported (Lund 1984), no resistance of practical importance has yet been encountered with the most efficacious compound, brodifacoum. In addition to their effectiveness against resistant strains, the availability of these highly potent rodenticides stimulated interest in the development of alternative bait formulations and in a more cost effective application technique referred to as minimal or pulsed baiting (Dubock 1982).

Flocoumafen is a new, highly potent anticoagulant rodenticide from Shell Research Limited. It is being marketed under the trade mark Storm. Information on flocoumafen's essential features has already been reported (Bowler, Entwistle and Porter 1984, Johnson and Scott 1986). This paper reviews more recent performance data and advances in formulation development aimed at producing a safer, more convenient and effective commercial bait.

FLOCOUMAFEN

Numerous studies have been carried out in the laboratory to determine the range and degree of flocoumafen's rodenticidal activity as measured by its toxicity, palatability and effect on resistant strains.

Toxicity

The acute oral toxicity of flocoumafen to various rodent pest species is given in Table 1. For most species the acute oral LD50 is less than 1.0mg/kg.

TABLE 1 : The acute oral toxicity of technical flocoumafen to various rodent species

Species (sex) and strain	Acute oral LD50 (mg/kg)	Days to Death Range	Mean
R A T S			
Bandicota indica (M&F)	0.48	-	10
Praeomys natalensis (M&F)	1.30	3-7	5
Rattus argentiventer (M)	0.48	4-18	9
Rattus argentiventer (F)	0.56	4-19	7
R. norvegicus (M&F) F344	0.25	5-7	6
R. norvegicus (M) Wistar	0.46	5-8	6
R. norvegicus (F) Wistar	0.56	5-8	6
R. rattus (M)	1.80	6-11	9
R. rattus (F)	1.00	4-13	8
R. r. diardii (M&F)	0.65	3-20	7
R. tiomanicus (M)	0.28	3-17	5
R. tiomanicus (F)	0.42	3-10	7
Sigmodon hispidus (M)	1.21	6-14	8
M I C E			
Mus domesticus (M) C57BL/10	0.79	4-11	7
M. domesticus (F) C57BL/10	1.50	6-10	7
M. domesticus (M&F) CF1	2.40	2-10	6
Apodemus flavicollis (M&F)	4.20	4-6	5
V O L E S			
Arvicola terrestris (M&F)	0.20	3-18	7
Clethrionomys glareolus (M&F)	0.23	2-6	4
Microtus arvalis (M&F)	0.12	2-8	4

With the exception of R. rattus, males are slightly more sensitive to flocoumafen than the females. Typically rats and mice die within four to eight days after the administration of a lethal dose.

The high potency of flocoumafen means that it is extremely effective even at very low bait concentrations. In general, rats and mice need to eat approximately 10% and 20% respectively of their bodyweight each day. The theoretical amount of 0.005% bait required to deliver an acute oral LD50 dose represents only a small proportion (often 10% or less) of the daily food requirements (Table 2).

TABLE 2 : Theoretical amounts of 0.005% flocoumafen bait required to deliver an acute oral LD50 to several rat species and the house mouse

Species	Body Weight (g)	Bait (g)
R A T S		
Bandicota indica	400	3.8
Rattus argentiventer	150	1.6
R. norvegicus	250	1.3-2.8
R. rattus	200	5.5
R. r. diardii	150	2.0
R. tiomanicus	150	0.8-2.0
Sigmodon hispidus	150	3.6
M O U S E		
Mus domesticus	20	0.5-1.0

Laboratory studies involving single, no-choice bait feeds aimed at determining the bioavailability of flocoumafen following its ingestion have confirmed this single feed potency for R. norvegicus (Johnson and Scott 1986).

The potency of flocoumafen to rats is compared to that of other anticoagulants in Table 3.

TABLE 3 : Comparative potencies of various anticoagulant rodenticides to Rattus norvegicus

	Acute Oral LD50(mg/kg)	Bait Conc (mg/kg)	Theoretical amount of bait (g) required to deliver LD50 dose to a 250g rat
First generation anticoagulants			
Diphacinone	3.0	50	15.0
Chlorophacinone	20.5	50	102.5
Coumatetralyl	16.5	375	11.0
Pindone	50.0	250	50.0
Warfarin	58.0	250	58.0
Second generation anticoagulants			
Brodifacoum	0.26	50	1.3
Bromadiolone	1.12	50	5.6
Difenacoum	1.80	50	9.0
Flocoumafen	0.25-0.56	50	1.3-2.8

The single feed potency of flocoumafen is matched only by brodifacoum.

Palatability

Flocoumafen's good intrinsic palatability has been confirmed in a range of bait formulations using several pest species (Johnson and Scott 1986, Parshad and Chopra 1986, Rowe, Bradfield and Swinney 1985).

The palatability of the current wax block formulation under laboratory and field conditions is discussed later.

Effect on Resistant Strains

A precise measure of an anticoagulant's activity against resistant rodents can be gauged from the resistance factor (RF).

$$\text{Resistance factor (RF)} = \frac{\text{acute oral LD50 (mg/kg) in resistant strain}}{\text{acute oral LD50 (mg/kg) in non-resistant strain}}$$

For each resistant strain, the higher the resistance factor, the less effective the rodenticide. An RF of more than 5 would indicate a potential field problem. Typically, flocoumafen has given RF values close to 1.0 for the warfarin-resistant rat strains tested to date (Table 4).

TABLE 4 : Acute oral toxicity of technical flocoumafen to warfarin-resistant strains of rats and mice

Species (sex)	Acute oral LD50 (mg/kg) Non-resistant	Resistant	Resistance Factor (RF)
R A T			
Rattus norvegicus (M)	0.46	0.46	1.0
Rattus norvegicus (F)	0.56	0.42	0.8
Rattus rattus (M)	1.80	ca. 1.90	ca. 1.1
Rattus rattus (F)	1.00	ca. 1.40	ca. 1.4
Rattus tiomanicus (M)	0.28	0.28	1.0
Rattus tiomanicus (F)	0.42	0.65	1.5
M O U S E			
Mus domesticus (M&F)	1.30	5.30	3.0

The toxicity of flocoumafen to resistant R. norvegicus is compared to that of some other rodenticides in Table 5 (Hadler and Shadbolt 1975, Dubock and Kaukeinen 1978). Here, the RF values quoted have been based on the ED50 for prothrombin time response, rather than on the LD50 itself.

TABLE 5 : Resistance factors of anticoagulants against R. norvegicus

	Prothrombin ED50 (mg/kg) Wistar	Homozygous	Resistance Factor (RF)
First generation anticoagulants			
Coumatetralyl	0.31	4.4	14.2
Chlorophacinone	0.22	>20.0	> 90.9
Diphacinone	0.22	>50.0	>227.3
Warfarin (S-)	0.30	>50.0	>166.7
Second generation anticoagulants			
Brodifacoum	0.08	0.10	1.3
Difenacoum	0.17	0.32	1.9
Flocoumafen	0.10	0.13	1.3

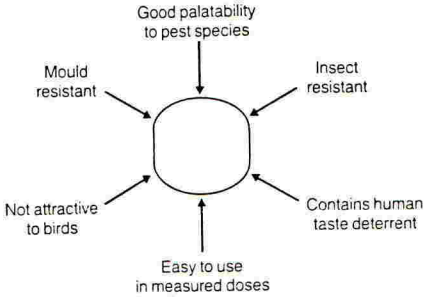
FORMULATION DEVELOPMENT AND FIELD PERFORMANCE

Laboratory studies have confirmed that flocoumafen is a highly efficacious anticoagulant rodenticide with good palatability. The ultimate test of any rodenticide is, of course, how well the product performs under field conditions.

However potent a rodenticide, its acceptance in a field bait in the presence of competing alternative food is of critical importance. The principal bait formulation selected for field trials and subsequent commercialisation is the wax-bound block. This type of formulation was originally developed more than twenty five years ago for use in damp conditions, such as sewers, and for rodent control in tropical tree crops (Marsh and Plesse 1960, Smith 1967, Wood 1969). Recent advances in the formulation of the flocoumafen block bait are intended to broaden its application, particularly against the commensal rodents.

The important features of the flocoumafen block bait are summarised in Figure 1.

Fig. 1 **Features of STORM Block Bait**



Insect and mould resistance

Wax block formulations are inherently more resistant to insects and mould than the more traditional loose grain formulations. For use in damp conditions or in permanent bait points, and to improve storage, this feature has been greatly improved by the addition of an insecticide and an antimicrobial agent. This improved insect and mould resistance has been achieved without adversely affecting palatability.

Palatability and efficacy

One reason for the present rather limited use of wax block baits is that they have, in general, been considered to be the least palatable of the available formulations. The lower potencies of earlier rodenticides such as warfarin require the palatability of the bait to be as high as possible.

Laboratory tests have indeed shown the flocoumafen block bait to be slightly less palatabile than equivalent loose grain formulations. However, the block bait is still sufficiently palatable to compete with alternative food sources in the field and, because of the outstanding

potency of flocoumafen, rodents are still easily able to consume a lethal dose in a single encounter.

An extensive series of performance trials have been carried out to investigate the palatability and efficacy of the flocoumafen block bait in a range of real use situations. One such trial is described below.

Experimental flocoumafen blocks of moderate palatability were tested in the field by the Ministry of Agriculture, Fisheries and Food (MAFF), to control farm infestations of warfarin-resistant R. norvegicus (MAFF 1986). Six farms with moderate to heavy infestations were treated with 0.005% flocoumafen wax blocks, using a modified pulse baiting technique (baiting at 0, 3, 7, 14 and 21 days).

Rat activity was monitored during the pre- and post-treatment census periods by visually scoring footprint activity and by determining the amounts of census bait consumed. Each bait point consisted of three 15g wax blocks (45g) and one bait block was laid in every burrow entrance. The number of bait points ranged from 38 to 110 (mean = 67) and the number of holes baited from 11 to 69 (mean = 32).

On the six farms there were numerous instances where bait points were located in and around animal feed and grain stores, which provided an attractive alternative food. The farms therefore provided a good challenge for the acceptance of the experimental block bait. The results of the trials are summarised below:

Number of farms	6
Mean number of bait points	67
Mean number of active bait points	54 (81%)
Mean total bait take (kg)	6.15
Mean take per active bait point (kg)	0.114
Mean estimated level of control	99%

The mean number of blocks laid per farm throughout the treatment period was 596 (range 318-1020), of which 410 (range 186-745) or 69%, were taken. This is equivalent to a mean bait take of 6.15kg of bait per farm (range 2.79-11.18kg).

Of the bait taken during each treatment, a mean of 70% was taken during the first week and a further 20% during the second week. The block baits were clearly readily taken by the rats and gave complete eradication of three of the infestations and virtually complete of the remainder (mean level of control 99%).

The above results are typical of those achieved in other trials. The flocoumafen block bait is readily accepted by rodents in the field, and rodent infestations are rapidly and thoroughly controlled.

Safety of the wax block formulation.

A perceived safety problem associated with the use of wax block baits in and around buildings is their carriage by the rats. Occasionally during a treatment rats may carry blocks to positions where they feel more secure, either to concealed places above ground or back to their nests. A problem may arise if blocks are dropped by the

rats in exposed positions.

There is little quantitative information published on the incidence of this phenomenon. As part of the above field trials programme therefore, MAFF recorded the number of block baits found in exposed positions on each of the farms as follows:

(i) during the treatment

(ii) found by farmers during routine farm activities for a period of 6-months after the treatment.

On four of the six farms, a total of 13 blocks were found in exposed positions away from their original well protected bait points during the first week of baiting and three blocks on one farm during the second week. This total of 16 blocks represents 0.7% of the total bait take. During the 6 month post-treatment observation period a total of 25 hoarded blocks (1.0% of the total bait take) were discovered in covered positions during the normal course of farming. While the amount of bait likely to be left in exposed positions is very small it could, if not removed by an operator, still represent some potential safety hazard.

No obvious solution has been found to prevent the carriage of block bait by rats without significantly increasing the cost of the treatment. However, it may be possible to reduce carriage to negligible proportions by limiting the number of blocks laid and, where appropriate, emphasising hole baiting. These possibilities are presently being investigated.

Another approach to solving this problem is to make the block bait unattractive to non-target species perceived to be at risk. The flocoumafen block formulation has been developed with this in mind. Two key features are the lack of attractiveness of the block itself to birds, and the incorporation of a human taste deterrent.

Attractiveness of block baits to birds.

Although comparatively few incidents of bird poisoning involving anticoagulant rodenticides are reported each year, domesticated and wild birds are at risk particularly from bait placed outdoors. In a simple field experiment aimed at determining the attractiveness to wild birds of different wheat-based bait formulations, 30g of six different formulations were randomly arranged on a bird table and exposed to wild birds for periods ranging from 1-12 hours. These formulations contained no flocoumafen or added colouring. Bird tables were placed at eight different locations and the results of thirty five observations are summarised below:

FORMULATION	AMOUNT OF BAIT EATEN BY BIRDS AS % OF TOTAL BAIT AVAILABLE
Wax pellet	40%
Non-wax pellet	50%
Whole wheat	71%
Block bait	3%
Crumbled block bait	46%
Crumbled block bait (moist)	43%

The results clearly indicate that block bait is less readily taken by wild birds than any of the other formulations. This lack of attractiveness to birds has been confirmed during environmental monitoring trials based on real use situations in the field.

Human taste deterrent.

In a recent House of Commons Report (Torney 1986) it was revealed that in 1985, of the 2,778 enquiries reported to the National Poisons Information Service, London, involving agrochemicals, 264 (9.5%) concerned anticoagulant rodenticides. Of these 264 suspected poisoning incidents, 169 (64%) involved children under the age of 5, although the vast majority of these cases (92%) showed no symptoms of poisoning.

Storm block baits contain a blue dye which, being a non-food colour, should minimise its attractiveness to humans. In addition, recent formulation studies indicate that the incorporation of very small amounts of a human taste deterrent into the block bait can greately reduce its acceptability to (and therefore its likely ingestion by) humans. Laboratory studies have shown that this taste deterrent does not affect the palatability of the bait to the rodent pest species tested to date, including R. norvegicus and M. domesticus.

Convenience in Use

Traditionally, with the exception of the United States, wax block formulations have been largely confined to use in damp conditions. However, the high potency of the active ingredient, combined with significant advances in its formulation make the flocoumafen block bait suitable for most rodent control operations.

Experimental flocoumafen block baits have been successfully tested in a wide range of operational uses, including farms, grain stores and warehouses, domestic premises and tropical crops. A common observation made by users is that block bait is much easier to handle and apply than the more conventional formulations.

Specific areas in which the block bait is more convenient to use include:

* hole baiting
* laying bait in measured doses
* topping up active bait points
* repositioning bait during the early stages of a treatment
* removing bait from the site at the end of a treatment

CONCLUSION

Effective rodent control continues to rely largely on the use of anticoagulant rodenticides. The earlier and still widely used products, such as warfarin, are characterised by their lower potency (multiple feed poisons) which has tended to limit both their formulation options and method of application. The introduction of the more potent second generation anticoagulant rodenticides has allowed the development of other bait formulations and more cost-effective application techniques.

Flocoumafen is a new highly potent anticoagulant rodenticide with good intrinsic palatability and, used at a bait concentration of 0.005%, has given excellent rat and mouse control in a wide range of operational uses.

The principal bait formulation selected for the commercialisation of flocoumafen is the wax-bound block. Although studies are continuing, recent advances in the development of this formulation, combined with its single-feed potency and convenience, make it a viable alternative for the treatment of rodent infestations.

ACKNOWLEDGEMENTS

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FLUPROPADINE - A NEW RODENTICIDE

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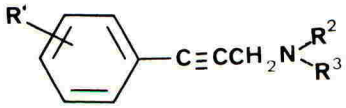
ABSTRACT

The new May & Baker rodenticide, flupropadine, is described. Preliminary free feeding tests on Norway rats (Rattus norvegicus) using a bait containing 0.09% and 0.18% flupropadine are described. The compound acted as a subacute rodenticide. Field trials carried out over four years, by MAFF Tolworth and by May & Baker are summarised. Initial work established that a concentration of 0.18% was essential for adequate control where rodent populations were high. A success rate of between 80-100% was achieved in the majority of trials. Anticoagulant resistant rat populations were controlled equally as well as non-resistant susceptible populations. Trials on house mouse (Mus domesticus) using 0.09 and 0.18% flupropadine baits gave a consistently high level of control. Results are given on palatability experiments using a range of different formulations.

INTRODUCTION

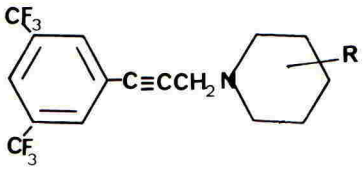
Flupropadine is a novel rodenticide which was discovered in the course of examining the toxicology of a series of phenylpropargylamine derivatives of the general structure below (Fig.1).

Fig.1 General structure of the phenylpropargylamines



A series of compounds was synthesised and it was shown that the compound coded M&B 36,892 exhibited a rather unique kind of toxicology quite distinct from most of the other congeners (Fig.2).

Fig.2 Toxicity of flupropadine related compounds

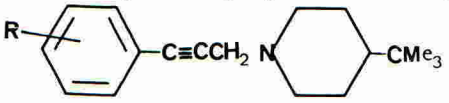


R	Code No.	LD50 (Mouse p.o)
4-Me	35,481	1000 mg/kg
4-Et	35,693	>1000
4-isoPr	36,607	681
4-Bu^t	36,892	68
4-CMe_2Et	40,337	200
4-CMe_2Pr^n	40,350	>1000
2-Bu^t	40,778	>1000
2-Me,4-bu^t	40,858	501

Apart from being more toxic than its analogues, this compound, with either single or multiple prolonged doses, showed a cumulative toxicity resulting in a quiet and humane death, whereas related compounds, apart from M&B 40,337, were only toxic at high doses and rapidly produced convulsions preceding death.

Many other congeners were therefore synthesised, a few of which are shown below (Fig.3).

Fig.3 Relative toxicity of flupropadine and congeners



R	Code	LD50(mouse p.o)
H	37,964	>1000 mg/kg
4-Cl	38,266	>1000
4-CF_3	38,778	584
3-CF_3	38,599	1000
3,5-Cl_2	38,203	1000
3,5-Me_2	38,779	>1000
3.5-CF_3	36,892	68
3,5-NO_2	40,025	>1000
3,5-CN_2	40,246	1000
3-CN,5-NO_2	40,110	>1000

From the LD^{50} values listed it is quite clear that M&B 36,892, subsequently named flupropadine, is unique. None of the analogues approach it in acute toxicity and in fact none of them exhibit the same type of cumulative toxicity with delayed death. Flupropadine was therefore selected for detailed examination of its rodenticidal action and subsequent development for this use.

METHODS

The trial methods employed in the following series of MAFF trials for the control of Norway rats (Rattus norvegicus), have been described by Buckle(1985). Flupropadine was used in the form of the hydrochloride at concentrations of 0.1, 0.15 and 0.2% on medium oatmeal. The trials were monitored daily from Monday to Friday and records were taken of the number of bait points visited by rats and the amount of bait consumed. Daily movements of the rodents were monitored by the use of slag or fine sand tracking patches. The above method was adopted by May & Baker in a number of trials conducted over a three year period using throughout, a prepared bait containing a 9% formulation of flupropadine in edible mineral oil on cracked wheat at 0.18%, (unless otherwise stated concentrations are expressed as active ingredients). Later trials involved the co-operation of Pest Control Officers using the same oil formulation where the effectiveness of flupropadine was monitored with less frequent baiting.

Field testing of flupropadine for the control of house mice (Mus domesticus) has been carried out by both MAFF Tolworth and May & Baker. A description of the methodology employed by Tolworth has been reported by Rowe et al. 1985 in which flupropadine as the hydrochloride on an oatmeal bait was tested at 0.1, 0.15 and 0.18%. In the May & Baker trials the formulation was used at 0.18% on cracked wheat. The trial method used was as described above for rats.

Both rat and mouse trials were conducted against known anticoagulant resistant populations as well as those showing no resistance.

RESULTS

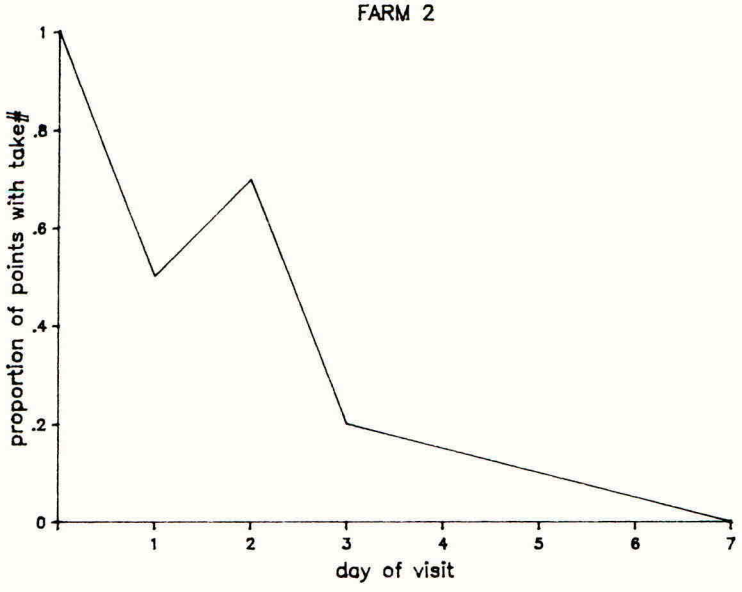
A summary of the MAFF and May & Baker field trials using flupropadine to control Norway rats and house mice are presented.

Initial field trial results as reported by Buckle (1985), show that flupropadine hydrochloride was well accepted at 0.1, 0.15 and 0.2% on medium oatmeal. The amount of bait consumed was shown to be highest during the first week and thereafter declined rapidly to low levels for the duration of the trial. At low rat populations 0.1% flupropadine achieved complete control after nine days (Fig.4).

With high populations bait takes declined during the first week and then fluctuated (Fig.5). Estimates for the level of control were 53 and 72%. A similar level of control at high rat population densities was achieved with flupropadine as the hydrochloride at 0.15% concentration.

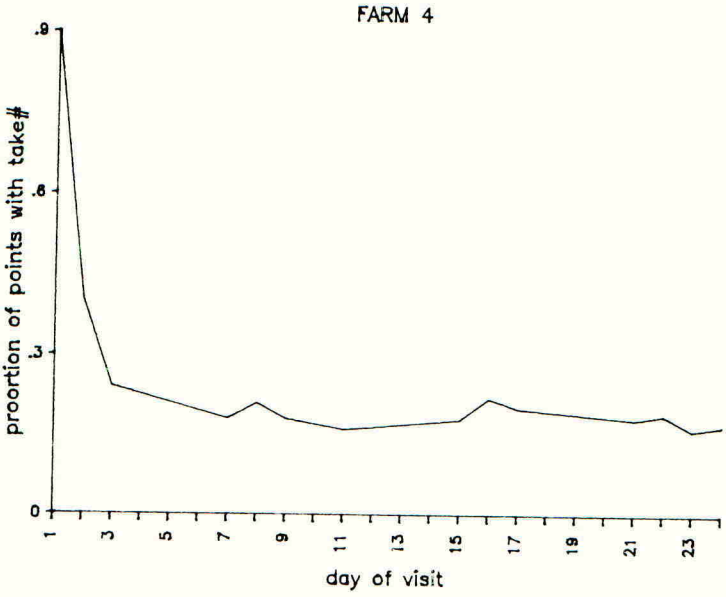
Fig.4 Result of baiting with 0.1% flupropadine on medium oatmeal against Norway rats (Buckle 1985).

Low population density



Decline in the daily proportion of bait points with take. (Number of bait points with take divided by number of bait points with take on day one)

Fig.5 High population density



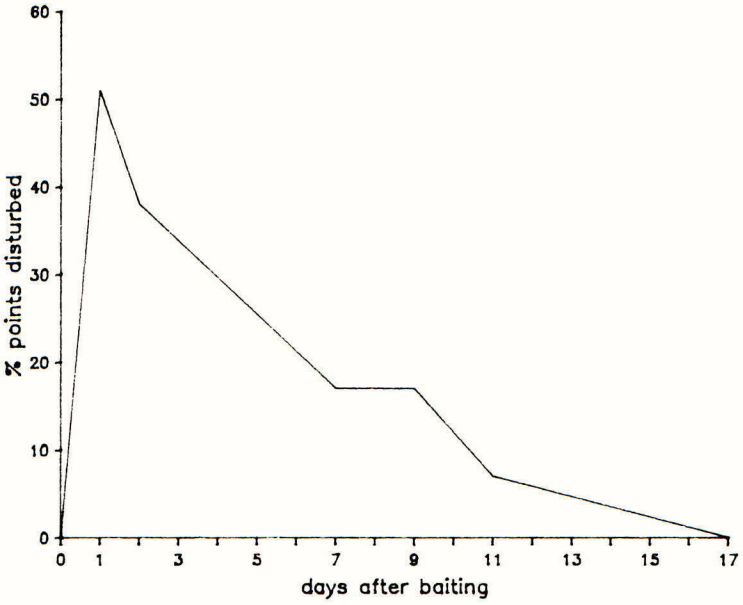
Decline in the daily proportion of bait points with take. (Number of bait points with take divided by number of bait points with take on day one)

At 0.2% complete control was achieved in three out of five trials including two trials with very high rat populations. Incomplete control of the two smallest populations was attributed to an abundant supply of alternative food. Both anticoagulant resistant and non resistant populations were shown to be susceptible to flupropadine.

From these results it was clear that the period of feeding for the rats to consume a lethal dose was short and that to control large populations 0.2% flupropadine as the hydrochloride was necessary. For this reason in trials carried out by May & Baker a 0.18% bait prepared with cracked wheat and 9% flupropadine in edible mineral oil was used. P.C.O's were given the same 9% formulation for use with their usual food source.

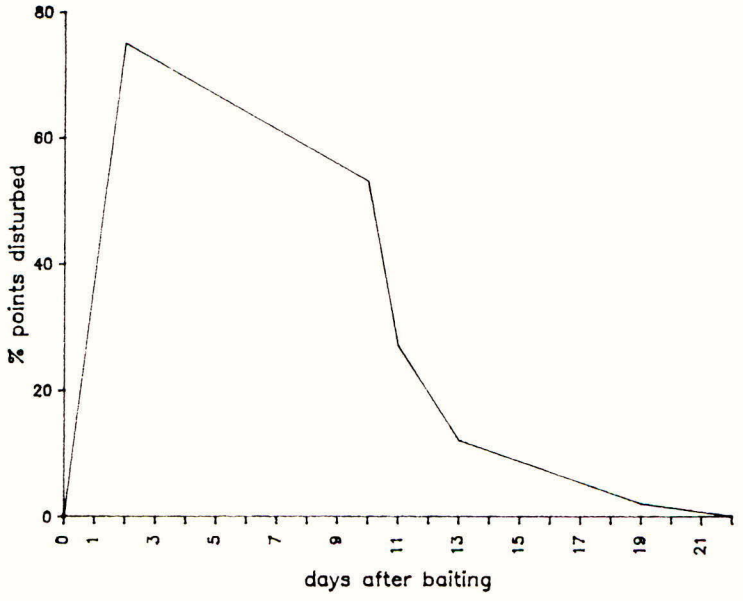
Results from trials against anticoagulant resistant and non resistant rat populations followed the same pattern as described previously. Formulated bait consumption was highest in the first four days and quickly dropped to low levels in the following week, indicating that most of the rats were killed by day 12 (Fig.6).

Fig.6 Results of baiting on low rat population with 0.18% flupropadine prepared on cracked wheat.



However, under high populations of rats and where abundant alternative food is available, the feeding time may be extended, e.g. as in Fig.7.

Fig.7 Results of baiting a high rat population with 0.18% flupropadine prepared on cracked wheat.



This led to the suggestion that there may be some question of palatability of the bait.

Rowe et al.(1985) have reported that in 17 field trials for the control of mice where flupropadine was used at concentrations of 0.1, 0.15 and 0.18% on pinhead oatmeal, most of the bait was consumed in the first seven days. In over half these trials there was no further feeding and the first dead mice were seen on day three.

Sixteen field trials, throughout the United Kingdom, have been carried out by May & Baker over a two-year period on farmsteads infested with mice, using the same cracked wheat bait at 0.18% that was used in the previous rat trials. In this series of trials mouse feeding was shown to be greatest in the first few days and declined rapidly thereafter. The average time to control mice populations was two weeks (Figs.8 & 9).

Fig.8 Control of a moderate mouse population with 0.18% flupropadine prepared on cracked wheat.

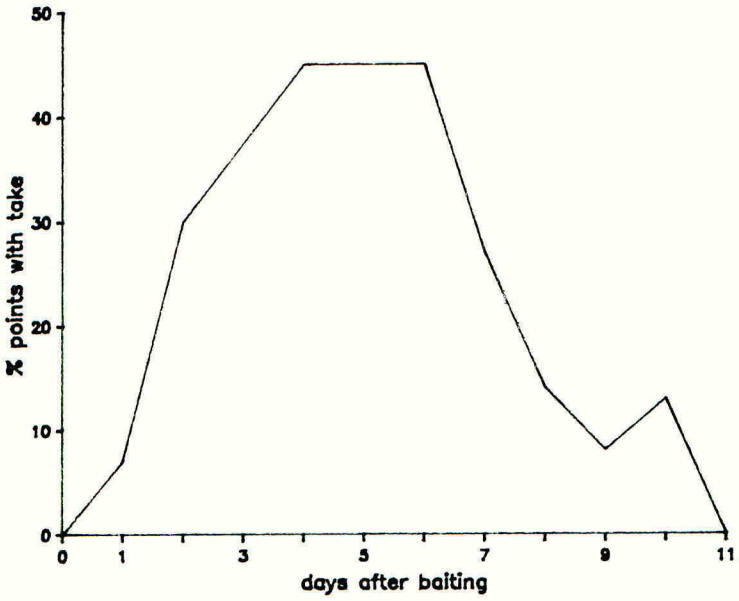
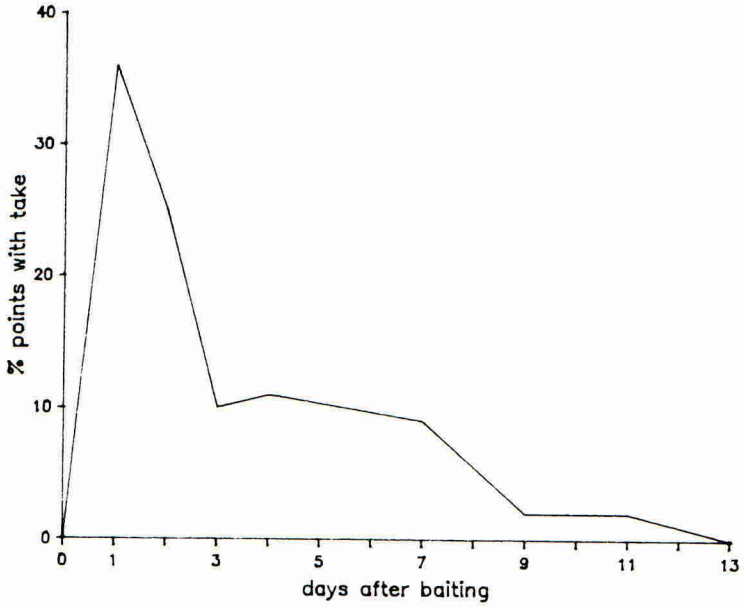


Fig.9 Control of high house mouse populations with 0.18% flupropadine on cracked wheat base.



Aging tests

Flupropadine was originally formulated as the hydrochloride, and then as a concentrate in edible mineral oil but there were indications that palatability, particularly to rats, seemed somewhat erratic. It was therefore reformulated in corn oil which was expected to improve take of bait. Overall palatability to rats and mice was improved. Because of the possibility that developing rancidity might reduce palatability over a period of time, two formulations, one with and one without anti-oxidant were prepared and subjected to comparative tests after storage. The results obtained with fresh concentrate and that stored for three, six and nine months were substantially the same. Only the nine months results are therefore quoted.

The following baits, based on cracked wheat and containing 0.18% flupropadine in corn oil, were used:-

- stored bait without antioxidant
- stored bait with antioxidant
- fresh bait made with stored concentrate without antioxidant
- fresh bait made with stored concentrate with antioxidant

Ten individually caged albino rats (115-239g), and ten individually caged albino mice (15-24g) (five males and five females of each species) were offered combinations of the above baits against each other or, in competition with cracked wheat or oatmeal.

Food consumption was recorded for four days after which baits were replaced by the normal laboratory diet. Hopper positions were alternated daily to avoid place preference and water was available at all times. Deaths were recorded for 21 days. The data collected were subjected to statistical analysis.

Rats

In competition with untreated alternative foods (oatmeal [OM] or cracked wheat [CW]), flupropadine baits made up 17-37% of the total intake (Table 1). In contrast with previous trials, the type of bait made little apparent difference.

In terms of consumption, fresh and stored baits, both without anti-oxidant (F-AO, S-AO), performed best against both alternative foods although they did not always produce complete mortality. F+AO gave the worst overall results in terms of both consumption and mortality. Figures for the intake of active ingredient (mg/kg) indicate that surviving individuals avoided eating the bait rather than being non-susceptible. Times to death varied from two to nine days with means for different treatments of 3.4 to 4.8 days.

TABLE 1 Food consumption and kill of albino rats offered different flupropadine baits (0.18% on cracked wheat) in competition with untreated alternative food. Ten rats per treatment.

# Bait	Alternative	Total Intake (g)	(%)	Mean Intake of a.i. (mg/kg) lived	Died	Kill after 21 days	't'
S-AO	-	109.4	26.3	16.8	131.5	9/10	2.01
-	OM	307.3	73.7				n.s
S+AO	-	111.4	24.9		113.2	10/10	4.13
-	OM	336.0	75.1				**
F-AO	-	126.4	29.5	97.3	136.3	9/10	2.00
-	OM	302.5	70.5				n.s.
F+AO	-	79.6	16.8	54.3	93.5	8/10	5.05
-	OM	394.9	83.2				**
S-AO	-	140.4	36.5	63.7	151.4	9/10	1.57
-	CW	244.3	63.5				n.s.
S+AO	-	107.2	27.3		108.8	10/10	4.40
-	CW	285.1	72.7				**
F-AO	-	129.3	33.8		134.8	10/10	2.54
-	CW	253.8	66.2				*
F+AO	-	75.1	25.1	45.2	91.7	9/10	3.05
-	CW	223.6	74.9				*

# OM = Untreated oatmeal S = Stored bait AO = Antioxidant
CW = Untreated cracked wheat F = Fresh bait

(** = $p<0.01$, * = $p<0.05$, n.s. = not significant)

When the baits were offered in direct competition with each other there was virtually no discrimination between them and they invariably produced a complete kill (Table 2). The greatest observed discrepancy was between baits S+AO and F+AO, but even this was not statistically significant.

TABLE 2 Food consumption and kill of albino rats offered different flupropadine baits (0.18% on cracked wheat) in competition with each other. Ten rats per treatment.

# Bait	Total Intake (g)	(%)	Mean a.i. Intake (mg/kg)	Kill after 21 days	't'
S-AO	94.0	51.5	95.1	10/10	.29
S+AO	88.4	48.5	89.3		n.s.
S-AO	112.4	47.8	113.2	10/10	.62
F-AO	122.8	52.2	132.5		n.s.
S-AO	107.7	50.8	107.9	10/10	.04
F+AO	104.3	49.2	106.7		n.s.
S+AO	97.8	49.7	104.4	10/10	.40
F-AO	99.1	50.3	115.9		n.s.
S+AO	80.4	42.2	91.3	10/10	.97
F+AO	109.9	57.8	120.2		n.s.
F-AO	91.7	48.3	106.9	10/10	.03
F+AO	98.2	51.7	107.8		n.s.

#S = Stored bait F = Fresh bait AO = Antioxidant

Mice

As in previous trials all of the flupropadine baits were better accepted when in competition with oatmeal than when competing with cracked wheat. In all cases there was a significant preference for the alternative food (Table 3). As with rats baits S-AO and F-AO performed best overall in terms of consumption although S-AO produced a rather poor kill in competition with cracked wheat. Bait F+AO gave the worst overall performance in terms of consumption whilst S+AO did very poorly against cracked wheat.

TABLE 3 Food consumption and kill of albino mice offered different flupropadine baits (0.18% on cracked wheat) in competition with unpoisoned alternative food. Ten mice per treatment.

# Bait	Total Intake (g)	(%)	Mean Intake of a.i. (mg/kg) Lived	Died	Kill after 21 days	't'
S-AO	18.6	32.3		182.6	10/10	3.16
Oatmeal	39.0	67.7				*
S+AO	17.7	29.1		168.5	10/10	3.23
Oatmeal	43.1	70.9				*
F-AO	18.5	26.6		165.7	10/10	2.41
Oatmeal	51.0	73.4				*
F+AO	17.5	18.0	105.4	155.0	9/10	3.21
Oatmeal	79.5	82.0				*
S-AO	16.5	16.0	91.0	155.6	7/10	2.71
Cracked wheat	86.8	84.0				*
S+AO	9.7	8.8	87.1	89.4	9/10	4.00
Cracked wheat	100.1	91.2				**
F-AO	17.9	17.2		163.1	10/10	3.48
Cracked wheat	86.3	82.8				**
F+AO	13.0	13.7		98.3	10/10	5.50
Cracked wheat	81.7	86.3				**

S = Stored Bait F = Fresh bait AO = Antioxidant
(** = $p<0.01$, * = $p<0.05$)

The intakes of active ingredient were of the same order of magnitude as for rats, with surviving animals usually showing considerably lower intakes than those that died. Times to death varied from two to seven days - rather shorter than for rats - with means for different treatments of 2.9 to 4.2 days.

When the four flupropadine baits were offered in competition with each other the only significant preference was for bait F-AO over bait S+AO (Table 4). F-AO was also preferred to S-AO and F+AO and so clearly gave the best overall performance.

TABLE 4 Food consumption and kill of albino mice offered different flupropadine baits (0.18% on cracked wheat) in competition with each other. Ten mice per treatment.

# Bait	Total Intake (g)	(%)	Mean a.i. Intake (mg/kg)	Kill after 21 days	't'
S-AO	16.4	34.6	35.4	10/10	2.09
S+AO	31.0	65.4	269.9		n.s.
S-AO	21.7	42.2	187.3	10/10	.91
F+AO	29.7	57.8	257.1		n.s.
S-AO	22.6	45.0	196.2	10/10	.78
F+AO	27.6	55.0	239.4		n.s.
S+AO	15.9	29.2	142.1	10/10	2.38
F-AO	38.6	70.8	348.5		*
S+AO	20.6	34.3	191.5	10/10	2.05
F+AO	39.4	65.7	344.3		n.s.
F-AO	31.0	63.9	268.4	10/10	1.64
F+AO	17.5	36.1	153.3		n.s.

S = Stored bait F = Fresh bait AO = Antioxidant

(* = $p<0.05$, n.s. not significant)

These results show that flupropadine is still effective after being stored for nine months and that baits containing no antioxidant are the best accepted by both rats and mice.

DISCUSSION

Flupropadine's unique chemistry, being neither an acute nor an anticoagulant rodenticide, makes it suitable for the control of both anticoagulant resistant and non-resistant populations of rats and mice. The speed of action of the compound is such that the time available for rats and mice to ingest a lethal dose is much less than for anticoagulants, appearing to be little more than two days, but not so quick that problems of bait shyness occur.

Farmers, in general, do not continue to bait with anticoagulants for sufficiently long periods to achieve complete control of rodent pests and are looking for a treatment which will give an acceptable level of control quickly and economically.

ACKNOWLEDGMENTS

Dr. R. H. Munro, Rentokil Ltd., conducted all the bait aging studies.

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BROMETHALIN - AN ALTERNATIVE TO ANTICOAGULANTS

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ABSTRACT

Bromethalin is a unique rodenticide currently under development by Ciba-Geigy Animal Health. It acts as an uncoupler of oxidative phosphorylation, thus interrupting the vital production of ATP necessary to maintain essential metabolic functions. This mode of action is quite different from that of the anticoagulants, thus bromethalin is effective against anticoagulant-resistant rodents. Data are reviewed that indicate bromethalin's effectiveness against susceptible and anticoagulant resistant rodent strains. Additional laboratory and field efficacy data with various 0.01% bromethalin formulations are also presented.

INTRODUCTION

Since the discovery of warfarin (Link 1945) and the identification of its rodenticidal properties (O'Connor 1948, Schein 1950), anticoagulants have dominated the field of chemical rodent control. However, within a decade of the introduction of anticoagulants as rodenticides, resistance was encountered in Rattus norvegicus in Scotland (Boyle 1960). Subsequently resistance has been well-documented in all three commensal rodent species throughout the world (Meehan 1984, Greaves 1985, Jackson et al. 1985). The development of cross-resistance to other hydroxycoumarin rodenticides and to the newer indanedione compounds has limited the effectiveness of first generation anticoagulants in many parts of the world.

With the spread of anticoagulant resistance, it became apparent that new rodenticides were necessary to control increasingly difficult rodent problems. This need stimulated research that led to the discovery of several potent second generation anticoagulants (Hadler and Shadbolt 1975, Grand 1976). Difenacoum, the first of these new compounds, became available in 1974. Bromadiolone and brodifacoum were available soon after in 1976 and 1978, respectively. Again, within a decade of widespread use, resistance appeared to these second generation anticoagulants. Resistance to difenacoum was encountered in R. norvegicus as early as 1978 (Redfern et al. 1978). The first signs of resistance to bromadiolone were observed between 1980 and 1982 (Greaves et al. 1982, Siddiqi et al. 1982, Lund 1984). Although resistance of practical importance is not widespread to brodifacoum, reports have cited the ability of rodents to tolerate higher than normal doses (Greaves et al. 1982, Siddiqi et al. 1982, Meehan 1984). These results indicate that resistance will in time develop to brodifacoum, as well as to new compounds with similar modes of action such as flocoumafen (Bowler et al. 1984) and LM2219 (Lechevin 1986). For a review of resistance to second generation anticoagulants see Lund (1984). Increased reliance upon anticoagulants will serve to spread the prevalence of resistant rodent populations more quickly. Thus, there exists a need for new rodenticides with modes of action different from that of the anticoagulants.

The discovery of bromethalin (EL-614) as a potent single-feeding rodenticide exhibiting a unique mode of action was first announced in 1979 at the British Crop Protection Conference (Dreikorn 1979). Lilly Research Laboratories subsequently developed bromethalin until U.S. registration was received for uses in and around buildings in 1982. Marketing rights were licensed to

several companies and a pelleted formulation containing 0.01% bromethalin was first introduced onto the U.S. market in 1985. Currently, Ciba-Geigy is developing bromethalin under the tradename DORATID® for all markets outside the U.S.

DISCOVERY OF BROMETHALIN

Dreikorn and O'Doherty (1984) detailed the steps leading up to the discovery of bromethalin's rodenticidal properties. The original interest in diphenyl amines as rodenticides resulted from toxic properties discovered in a highly active compound, trichlorotrinitrodiphenyl amine. This compound was identified in a fungicide screen and was active against downy mildew. However, subsequent toxicological evaluation revealed it to be too toxic to apply as a foliar fungicide. The idea of investigating potential uses as a rodenticide emerged and a structure-activity relation study was initiated to see if related compounds possessed equal or better rodenticidal properties. Systematic substitutions on both phenyl rings and corresponding toxicity tests revealed that bromethalin (Fig. 1) possessed the highest toxicity, while at the same time maintained palatability to rodents.

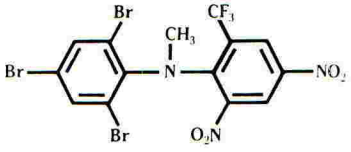

Fig. 1. Chemical structure of bromethalin

TOXICOLOGY

Toxicological aspects of bromethalin have been presented by van Lier and Ottosen (1981), Cherry et al. (1982), Jackson et al. (1982), and Spaulding et al. (1985). A brief review of this information follows.

Acute Toxicity

Acute LD_{50}'s were determined in various target and non-target species by gavage administration of bromethalin dissolved in polyethyleneglycol 200. These data are presented in Table 1 and indicate that similar doses were required to produce lethality in all species tested.

TABLE 1

Bromethalin acute LD_{50} values in target and non-target species

Target Species	mg/kg	Non-target Species	mg/kg
House mouse (male)	5.3	Dog	4.7
House mouse (female)	8.1	Cat	1.8
Norway rat (male)	2.5	Monkey	5.0
Norway rat (female)	2.0	Rabbit	13.0
Roof rat	6.6	Chicken	9.0
		Quail	4.6

Mechanism of Toxicity

Experiments conducted *in vitro* with bromethalin suggest that this agent acts on the central nervous system by uncoupling oxidative phosphorylation. In the central nervous system a large portion of the available energy, in the form of adenosine triphosphate (ATP), is normally utilized to maintain the ion gradient across membranes. Impairment of this process can occur when the levels of ATP are reduced for a long period of time. This impairment is manifested by intramyelinic vacuole formation and an elevation of cerebrospinal fluid pressure (CSFP). In turn, this fluid accumulation probably impedes normal nerve impulse transmission which, following ingestion of a toxic dose, leads to loss of pain and tactile senses, paralysis and death.

Treatment of Bromethalin Poisoning

There is no specific antidote for bromethalin poisoning; however, laboratory studies in rats have shown the toxic effects of bromethalin are reversible using symptomatic treatment. To reverse the toxic symptoms of bromethalin, normal cellular energy production and normal body fluid balance must be restored and maintained. It is likely that humans would have to consume relatively large quantities of final bromethalin bait in order to elicit toxic symptoms. Theoretical extrapolated values from rat acute toxicity studies indicate that a 10 kg child would have to consume 150 g of 0.01% bait to reach an LD_{10}. There have been no human deaths attributed to bromethalin poisoning after widespread use in the U.S. for over one year.

Ingestion by humans would likely result in one or more of the following symptoms: numbness, incoordination, headache, or mild confusion. In extreme (acute) cases, symptoms may include tremors, convulsions or respiratory distress. Symptoms for pets or domestic animals may include incoordination, numbness, loss of appetite and possibly hindleg paralysis. The following steps are recommended in the treatment of bromethalin poisoning:

1. Limit further absorbtion by producing emesis (vomiting). If no convulsions are present, gastric lavage may be administered.

2. Use standard anticonvulsant therapy using a barbiturate, diazepam, or diphenylhydantoin. These treatments have been shown to prolong survival of laboratory rats which have received large doses of bromethalin.

3. Treatment of cerebral oedema and increased CSFP should be conducted in a hospital. Treatment would involve the use of diuretics such as mannitol, and corticosteroid therapy such as dexamethasone. Treatment of this syndrome would largely depend on the severity since rodent studies have shown that these effects are reversible to a large extent within seven days.

Environmental Toxicology and Non-Target Species Hazard

Bromethalin was examined in a series of toxicity tests in both aquatic and terrestrial species (Table 2). LC_{50} values of 210 and 620 ppm were obtained in 5-day dietary tests with juvenile Bobwhite Quail (*Colinus virginianus*) and Mallards (*Anas platyrhynchos*), respectively. These data suggest that these species are less sensitive to the lethal effects of bromethalin than are mammals, since rodents are normally susceptible to 50-100 ppm baits after one to three days of feeding. This discrepancy between mammalian and avian species may be due to a more effective detoxification ability of the birds. LC_{50} values of 120 ppb, between 30 and 80 ppb, and 27 ppb were obtained in bluegill (*Lepomis macrochirus*), rainbow trout (*Salmo gairdneri*), and *Daphnia magna*, respectively. The true solubility of bromethalin in water is less than 10 ppb. Bromethalin should not enter watercourses except if misused; even then, the maximum concentration that could be present should have no effect on aquatic species.

TABLE 2

Bromethalin toxicity tests in aquatic and terrestrial species

Species	LC_{50}	No-effect Level
Quail	210 ppm	6.25 ppm
Mallard	620 ppm	25 ppm
Pigeon	--	250 ppm
Earthworm	--	100 ppm
Bluegill	120 ppm[1]	18 ppb
Trout	>33 ppb < 80 ppb[1]	33 ppb
Daphnia	27 ppb[2]	9 ppb

[1] 96 hr [2] 48 hr

Since dead or dying rodents may be observed after the use of bromethalin bait, it is possible that they might be caught and eaten by predators or domestic carnivores. A secondary toxicity test was therefore conducted to evaluate the possible effects of consumption of poisoned rats by dogs. Rats were fed a lethal dose of bromethalin bait (50 g/kg) and killed after 16 hours when showing signs of toxicity. They were skinned and minced and fed to dogs at up to 600 g daily for two weeks. This exposure was equivalent to eating two or three large rats a day. No adverse effect on the dogs was observed and no histological abnormalities were found in the central nervous system.

EFFICACY

Background Data

Laboratory studies and field trial data required for U.S. registration have been presented by Jackson et al. (1982), Spaulding and Jackson (1984), and Spaulding et al. (1985). Laboratory results indicate bromethalin is effective in both laboratory and wild Mus musculus and R. norvegicus. Choice efficacy tests have resulted in mortality of 90% or greater, even with only one day's exposure to the toxicant. Average days until death ranged between two to four days in both rats and mice. Discrimination against the toxic bait was not evident. Bromethalin was also effective against warfarin-resistant strains of M. musculus and R. norvegicus. Field trials have shown bromethalin to be effective against R. norvegicus and M. musculus in a variety of habitats (Table 3).

TABLE 3.

Summary of U.S. field trials with 0.005% bromethalin bait

Species	No. of trials	Census technique	Avg. % reduction in activity (range)
Rattus norvegicus	8	Feed consumption (7)*	94.9 (80-100)
		Tracking activity (8)	89.7 (56-100)
		Burrow activity (6)	97.5 (91-100)
		Dropping counts (2)	96.5 (95- 98)
Mus musculus	9	Feed consumption (9)*	92.3 (73-100)
		Tracking activity (9)	84.5 (57-100)
		Live-trapping (5)	99.0 (95-100)

* Number of studies using this technique

Average percent reduction in rodent activity following bromethalin treatment ranged from 85-99% using different census techniques. There were no incidents of primary or secondary poisoning to non-target species during the field trial program.

Other reports have indicated successful bromethalin treatments in a wide variety of agricultural and urban environments. Ashton et al. (1984) found greater than 92% reduction in rodent activity following bromethalin treatment against a warfarin-resistant M. musculus population on a poultry farm. Meehan (1983) reported between 80-100% control of R. norvegicus populations following treatments at three farm sites. Jackson (1985) and Carvalho (1985) reported between 87-100% control of R. norvegicus populations in difficult urban environments in Toledo, Ohio and San Paulo, Brazil. Meehan (1983) observed varying degrees of success with bromethalin against populations of M. musculus in extremely difficult and complex urban environments. Crop uses of bromethalin have not been fully explored; however, Soh et al. (1982) reported successful control of R. argentiventer and other species in Malaysian oil palm plantations. Lee and Kamarudin (unpublished data) have successfully used bromethalin in cocoa and coconut plantations in Malaysia. There have been no known incidents of primary or secondary poisoning to non-target species in any of these field trial programs.

Recent Laboratory Studies

Efficacy tests with R. norvegicus and M. musculus are presented in Tables 4 and 5, respectively. These results confirm the efficacy of 0.01% bromethalin whole oat formulations for rats and oat flake and canary seed formulations for mice. With 0.01% baits, mortality ranged between 90-100% within one to three days for both species. Acceptance of the treated diet compared to a placebo diet was excellent ranging from 33 to 88%.

TABLE 4.

Four-day choice tests with various bromethalin formulations against individually-caged rats

Formulation	N	Concn. (%)	Bromethalin Consumption (%)	mg/kg	Mortality (%)	Days to Death
Whole Oat	12	0.002	51	2.7	33	5.5
Whole Oat	12	0.005	46	4.4	75	3.5
Whole Oat[1]	10	0.01	52	7.8	90	2.9
Whole Oat	12	0.01	42	5.1	100	2.8
Whole Oat	12	0.02	52	6.1	100	1.8
Wheat Bonded Block	24	0.01	72	8.0	100	2.1
Maize Bonded Block	12	0.01	47	9.7	100	4.0
Wheat Bonded Block[2]	12	0.01	67	7.0	100	4.1
Oat Bonded Block[2]	15	0.01	82	5.1	100	3.4

1 Survivors of 0.002 and 0.005% treatments
2 Wild Rattus norvegicus

Ten survivors from an exposure to 0.002 or 0.005% bromethalin baits were retested to determine if bait shyness would develop (Table 4). These animals proved susceptible to a second feeding on 0.01% bromethalin baits and 90% mortality was achieved within three days. Bromethalin baits were slightly preferred (52%) to placebo diets.

Preliminary results with a proprietary weather-resistant bonded block appear encouraging (Table 4). Wheat, oat and maize were impregnated with 0.01% bromethalin and bonded together with a polymer in a patented process. Laboratory studies have shown this formulation to be resistant to moisture and heat. Complete mortality was achieved with these formulations within two to four days against R. norvegicus. Acceptance was 47, 72, and 82% with maize, wheat, and oat blocks, respectively. Further laboratory and field evaluations with a variety of formulations are currently underway in the U.K., France, Denmark, and U.S.

TABLE 5.

Four-day choice tests with various bromethalin formulations against individually and group-caged wild Mus musculus

Formulation	Test[1] (N)	Concn. (%)	Bromethalin Consumption (%)	mg/kg[3]	Mortality (%)	Days to Death
Canary Seed[2]	G(9)	0.005	62	9.6	78	4.9
Canary Seed[2]	G(19)	0.01	88	17.2	100	1.4
Oat Flakes	G(9)	0.005	64	14.6	78	2.3
Oat Flakes	G(8)	0.01	88	11.2	100	1.5
Oat Flakes	I(20)	0.01	43	2.5	95	2.4
Canary Seed	I(20)	0.01	33	3.1	95	2.2
Canary Seed[2]	I(20)	0.01	50	2.5	100	1.7

1 I = Individually-caged; G = Group-caged
2 Dehusked
3 Calculation based on average body weight of 25 g

Results from a storage study are presented in Table 6. Baits were stored for three months at 23° and 40°C and evaluated against placebo diets refrigerated for an equivalent period of time at 4°C. Dehusked canary seed baits were used in four-day choice tests with wild M. musculus and whole oat and wheat baits were used with laboratory rats. Mortality of 100% was achieved in all tests within one to three days. Effects of storage and temparature appeared to have minimal impact upon the efficacy and palatability of baits after three months. The study will continue for one year.

Choice tests were conducted to compare the efficacy of 0.01% bromethalin and 0.005 % bromadiolone canary seed formulations against family groups of wild Mus musculus (Table 7). Both formulations were tested independently against placebo canary seed diets and resulted in complete mortality and similar acceptability (bromethalin 43%, bromadiolone 38%). Mice continued to feed on bromadiolone baits throughout the four-day test period, whereas bromethalin consumption dropped dramatically after the first day. Thus the total consumption of bromadiolone bait was nearly three times higher than that of bromethalin bait. Furthermore, mice died sooner after ingesting bromethalin baits, with an average days to death of 1.7 compared to 6.6 for bromadiolone. In a third group, mice were given a choice of feeding on bromethalin or

TABLE 6.

Palatability and efficacy of 0.01% bromethalin baits stored at 23° and 40°C for 3 months (N=12/test)

Species	Formulation	Temp. (°C)	Consumption (%)	Mortality (%)	Days to Death
Mus musculus	Canary Seed	23	33	100	2.7
	Placebo	4	67		
Mus musculus	Canary Seed	40	49	100	2.2
	Placebo	4	51		
Mus musculus	Canary Seed	23	48	100	1.1
	Canary Seed	40	52		
Laboratory Rat	Whole Oat	23	55	100	2.0
	Placebo	4	45		
Laboratory Rat	Whole Oat	40	59	100	2.1
	Placebo	4	41		
Laboratory Rat	Whole Oat	23	56	100	1.8
	Whole Oat	40	44		
Laboratory Rat	Whole Wheat	23	43	100	2.7
	Placebo	4	57		
Laboratory Rat	Whole Wheat	40	40	100	2.9
	Placebo	4	60		
Laboratory Rat	Whole Wheat	23	48	100	1.9
	Whole Wheat	40	52		

TABLE 7.

Choice tests comparing 0.005% bromadiolone and 0.01% bromethalin canary seed baits against groups of wild Mus musculus

		CONSUMPTION (G) Test Days					Acceptance	Mortality	Days to Death
Treatment	N	1	2	3	4	Total	(%)	(%)	
Bromethalin	17	27	2	0	5	34	43	100	1.7
Placebo		28	8	8	1	45	57		
Bromadiolone	18	37	28	20	14	99	38	100	6.6
Placebo		59	46	39	20	164	62		
Bromethalin	19	35	1	1	0	37	58	100	1.8
Bromadiolone		17	4	4	2	27	42		

bromadiolone baits. Complete mortality was achieved within two days. Mice preferred the bromethalin formulation, since 58% of the total consumption consisted of this diet. Feeding patterns over the four day test period and the relatively short time till death (1.8 days) indicated mortality was due primarily to bromethalin.

Recent Field Trials

Trial results with M. musculus in a test pen and under natural

conditions in a swine research unit are presented in Fig. 2 and 3, respectively.

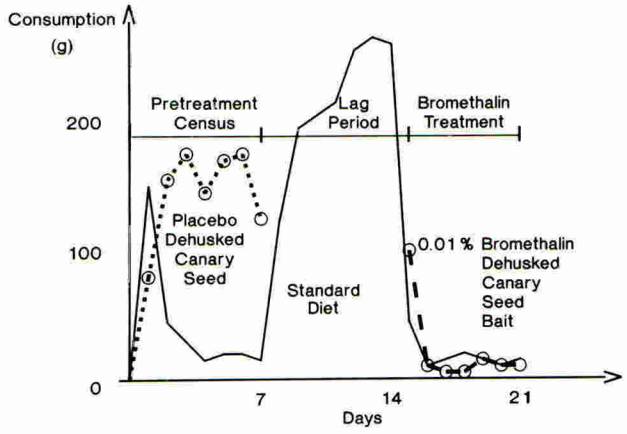

Fig. 2. Field trial with 0.01% bromethalin canary seed bait against an introduced population of Mus musculus in a simulated natural environment.

The first trial was conducted in a 2x4x2 m test pen. Family groups consisting of 20 adults plus offspring were released into the pen and provided a standard laboratory diet plus water. Over the following six-week adaptation period, the population increased to approximately 50 individuals. Mice were offered a choice of placebo dehusked canary seed or standard laboratory diet over a seven-day pretreatment period, during which 78% of total consumption was dehusked canary seed. For the next seven days only standard laboratory diet was available. Dehusked canary seed containing 0.01% bromethalin was then offered along with the standard laboratory diet for seven days. During this treatment period, 59% of the total consumption was comprised of the bromethalin canary seed formulation. Bromethalin bait consumption dropped dramatically after the first day's feeding. Complete control of the mouse population was achieved since no consumption of standard diet was recorded following treatment and a thorough search of the test pen revealed no mouse activity.

The second trial was conducted in a 40x40 m swine research unit that had been infested with mice for nearly 10 years. Repeated attempts with several anticoagulants failed to control the population. Placebo whole grain canary seed was distributed in 50 bait stations for seven days as a pretreatment census. An average of 1043 g per day was consumed over the last three days of this period. This level of consumption would indicate a population of roughly 200-300 mice. Following a seven-day lag period, 50 g of a 0.01% bromethalin dehusked canary seed formulation were placed in each of 50 bait stations throughout the facility. Over 500 g of bait were consumed during the first night. Consumption dropped dramatically to 30 and 35 g the second and third nights, and no consumption was recorded the fourth night. Carcasses of 135 mice were recovered during the four-day period. A three-day lag period was allowed before the post-treatment census. An average of 20 g of whole canary seed was consumed during a three-day post-treatment census, resulting in a 98% reduction of census feed consumption. Nearly complete elimination of the mouse infestation was achieved within four days.

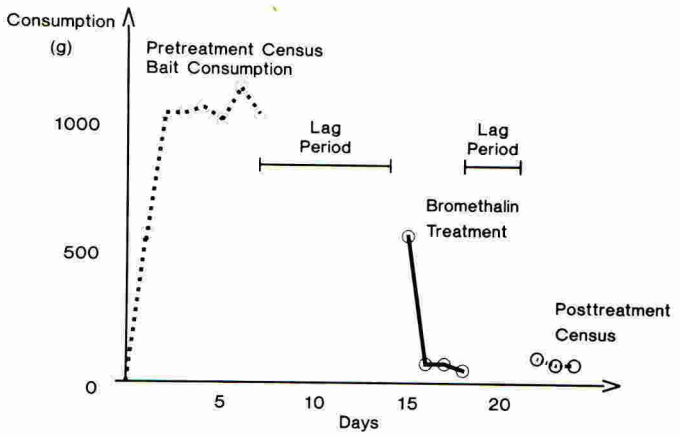

Fig. 3. Field trial with 0.01% bromethalin canary seed bait against an infestation of Mus musculus in a swine research unit.

CONCLUSIONS

Resistance to anticoagulants continues to stimulate searches for new rodenticides. Bromethalin is one such compound exhibiting a unique mode of action different from the anticoagulants. It provides a lethal dose to rodents in a single-feed with death generally delayed two to three days. Excellent bait acceptance is achieved with no pre-baiting and bait-shyness does not appear to occur due to the delayed death. Laboratory and field trial data have shown bromethalin to be effective in a variety of environments and against anticoagulant resistant rodents.

Bromethalin is unique since rodents stop feeding after ingesting a lethal dose, therefore bait quantities necessary to control rodent infestations will be substantially less than those required with anticoagulants. Highest bait consumption will occur on the first day or two after treatment. Thus, bait placements should be inspected after several days to a week and replenished at sites where there is evidence of heavy feeding. Since bromethalin is not a multiple-dose rodenticide, continuous bait exposure is not required, except where rodents from surrounding areas are reinvading premises. Less bait can be utilized over a shorter period of time, thus limiting access by non-target species and the risk of accidental poisonings. Bromethalin's single feed action and quick kill can increase the efficiency of baiting programs.

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4.
Detections of Insect Infestations

Chairman: Professor T. G. ONIONS
Session Organiser: Mr D. B. PINNIGER

RECENT DEVELOPMENTS IN TECHNIQUES FOR THE DETECTION OF INSECT PESTS OF STORED PRODUCTS

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ABSTRACT

Early detection of insect pests in stored products is essential to ensure that pesticides are used only when necessary, but present detection methods remain relatively crude. Significant improvements in detection efficiency should result from recent advances in a wide range of research topics. Progress has been made with the identification of new pheromones, but optimising their use as lures in traps may not be possible until the role of minor components and stereoisomerism are fully understood. Laboratory tests have shown that it may be preferable to base the lures on food aromas rather than pheromones because they are less specific. Progress in deciding which food volatiles are attractive may ultimately help to explain how commodities become infested. There is even greater scope for improvement in non-trapping methods for the detection of immobile stages of insects or for use with commodities in transit. Improvements may depend on research with entirely new methods such as near infra-red reflectance and nuclear magnetic resonance.

INTRODUCTION

Reliable methods for the detection of insect and mite pests in stored cereals and foodstuffs are essential but do not yet exist. They would allow accurate assessment to be made of the extent and degree of an infestation. They would provide a better basis for judging whether a disinfestation treatment is necessary, which treatment would be best (since this might depend on which species are present) and subsequently whether the treatment had been effective. The early detection of very low levels of infestation would enable damage to the commodity to be minimised, and its value and export competitiveness to be maintained. The distribution of pests and possibly pesticide resistance throughout the trade might be avoided, and the use of pesticides reduced. However, stored product pests are small, they are difficult to see with the naked eye and even in an empty store they can easily escape notice, hidden in cracks and crevices.

Until recently, the detection of freely wandering pests in static bulks of stored commodities depended on the traditional method of sampling, using either a spear or vacuum sampler, followed by visual inspection of the sievings. The method is labour intensive, haphazard and largely ineffective, but fortunately is now being replaced by the use of traps of various designs. Those of greatest promise are pitfall traps, which are open-topped jars sunk to position their rims level with the surface of the cereal, and probe traps, which are inserted vertically into the cereal and consist of an hollow tube of downward pointing holes through which insects fall into a collection tube (Loschiavo and Atkinson 1973). In empty cereal stores and food processing locations, detection

can be undertaken with bait bags containing whole wheat, crushed carobs and broken groundnuts (Pinniger 1975).

There is no doubt that traps have brought improved effectiveness in detection (see for example Barak and Harein 1982) but there is still room for further improvement: Cogan and Wakefield (1987) found in laboratory trials that only a few per cent of released insects were trapped. Attempts are therefore being made to improve the effectiveness of traps by the addition of attractive chemical lures based on either insect pheromones or food aromas. These should improve the chances of detecting very low levels of infestation and increase the volume of the bulk sampled by each trap.

Trapping methods are inappropriate for detecting pests which cannot wander freely, for example early stages of weevils developing within cereal grains. They are also inappropriate for commodities in transit, because the detection must be rapid to minimise delay to waiting transport. Under these circumstances heavy reliance is still placed on the sampling and sieving method. The most promising opportunities for developing an alternative method lie with various physical techniques, some of which are well established for other applications.

This paper will review recent research which might help to solve the problem of pest detection and will in turn cover the three areas of pheromones, food attractants and non-trapping methods.

PHEROMONES

There have been several reviews of pheromones identified in stored product insects and their use in control (Burkholder 1982, Burkholder and Ma 1985, Pinniger and Chambers 1987). Broadly speaking, in species with short-lived adults such as Trogoderma, the females produce a sex pheromone which attracts males, whereas in species with longer-lived adults such as Tribolium, the males produce an aggregation pheromone to which both males and females respond. Both types of pheromone can be used to enhance trap catch even though the sex pheromones will not attract females. Recent advances include the identification of pheromones in Prostephanus truncatus (Hodges et al 1984a), Sitophilus oryzae and S. zeamais (Schmuff et al 1984), and Cryptolestes ferrugineus, C. pusillus, C. turcicus, Oryzaephilus surinamensis and O. mercator (Pierce et al 1984). However, optimising the use of a pheromone in a trap depends upon a full understanding of its interaction with the insects and this is rarely if ever straightforward. For example, the pheromones of the five Cryptolestes and Oryzaephilus species mentioned consist of seven closely-related unsaturated macrocyclic lactones, each species producing between two and five of them but responding to only two or three of those that they produce. The relative amounts of lactones may prove to be significant but until the exact role of each component, including minor ones, is established, it may be premature to classify them as aggregation pheromones and impossible to maximise their effectiveness in a trap.

Full biological response to a multi-component pheromone may demand the presence of exact amounts of all the minor components: each component may have a different function (see for example Haines 1976). It is the development of high-resolution capillary gas chromatography columns and more sensitive detectors, generally dedicated mass spectrometers, which has enabled these minor components to be observed, identified and quanti-

tated. However there is a further problem: many pheromone components can exist in more than one stereoisomeric form and the presence of an incorrect form in a lure may inhibit the response. Deciding which is the correct form usually demands stereospecific synthesis and, despite recent discoveries of better synthetic techniques, it may still take several years to do this. Fortunately, with most stored product insect pheromones studied so far, the presence of incorrect isomers does not inhibit the response, although it has been suggested that this may be the case with T. castaneum (Barak and Burkholder 1985).

Even if the exact chemical nature of the pheromone can be described completely, it still does not follow that an artificial lure of identical composition will be effective. A critical factor is the rate at which the pheromone is released: increasing the release rate up to a certain level improves trapping effectiveness in most cases, but with Sitotroga cerealella a reduction in trap catch is observed when the release greatly exceeds just one female equivalent (Vick et al. 1979). Measurement of such small amounts of chemical is not easy but preliminary results indicate that insect release rates can depend on such things as the presence of food and time of day (Burkholder 1982). However it is not yet clear how the ratios of constituents in multicomponent pheromones vary with distance from the source due to relative volatility, oxidation and photoisomerisation. Nevertheless, methodology for release rate measurements is being established (Weatherston et al. 1981) and artificial lures which provide the correct release rate of different components constantly for long periods are now commercially available. Attempts are also being made to understand the dispersal of pheromone vapours in warehouses (Mankin et al. 1980) and their effect upon the orientation of moths (Mankin et al. 1983). Even if release and dispersal can be optimised it is now known that the response of a receiving insect such as T. castaneum, O. surinamensis or O. mercator can depend on factors such as age (Faustini et al. 1981, Pierce et al. 1983).

Despite all these complications it is beyond doubt that lures can be used in field situations to monitor infestations of certain species by bringing a marked improvement in trap catch. There have been several reports of the successful use of pheromones to monitor populations of stored product lepidoptera and coleoptera, and factors which influence the effectiveness of traps, such as placement, have been discussed (Burkholder and Ma 1985). The attractancy of a lure might be influenced by the presence of traces of insecticide vapour which may be repellent, or by manipulated physical conditions, especially low temperature. So it may be difficult to assess the effectiveness of some control treatments by this method.

The use of stored product insect pheromones has usually been restricted to monitoring. Pheromones have been used to achieve a reduction in mating of Ephestia cautella (Haines 1976, Hodges et al. 1984b) and there is a report that pheromone trapping removed such large numbers of male E. elutella that fumigation could be postponed by a year (Reichmuth et al. 1978). However, it has been suggested that the use of higher concentrations of pheromones to achieve control through either mass trapping or mating disruption may be ill-advised since it might contaminate the commodity with pheromones, resulting in subsequent infestation (Burkholder and Ma 1985).

One feature of pheromones which cannot be overlooked is their speci-

ficity, because in a storage situation it is frequently necessary to monitor for any of several species of pest representing different families. Fortunately this is not always a problem. Interspecific responses to sex pheromones of various Trogoderma species have been reported (Vick et al. 1970), it is known that P. truncatus responds to components of the Rhyzopertha dominica pheromone (Hodges et al. 1983), and various Phycitid moths can be caught in traps baited with a single pheromone (Cogan 1983). In most other cases though it will be necessary to use mixed lures. Lindgren et al. (1985) have shown that C. ferrugineus and Tribolium castaneum are caught in traps with a mixed lure to the same degree as in traps baited with their respective pheromones separately. This may not be the case when the pheromones are more similar in chemical structure. There is some evidence that the presence of 9Z,12E-tetradecadienyl acetate to trap Plodia interpunctella may reduce the efficiency of 7Z,11E-hexadecadienyl acetate in trapping S. cerealella (Vick et al. 1979). Thus it is quite possible that in the case of the five Cryptolestes and Oryzaephilus species mentioned earlier, the attractiveness of a lure containing the seven macrolide lactones could be compromised by inhibition. This has been reported to be the case with a mixed lure for insect pests of fruit trees (Yushima 1984). Even if inhibition did not occur it is difficult to see how a lure of mixed lactones could achieve effective ratios of components for all five species or, in view of the complex syntheses involved, could be obtained at a reasonable cost. The only viable alternative might be to use a lure based on food attractants.

FOOD ATTRACTANTS

Over the years there have been many literature reports of laboratory studies demonstrating promising responses of various stored product insects including T. confusum, T. castaneum, Sitophilus zeamais, S. granarius and O. surinamensis to food components usually based on wheat or oats (eg Levinson and Kanaujia 1981, Seifelnasr et al. 1982, Mikolajczak et al. 1984). The first successful field use of food baits was reported by Strong in 1970. However although the use of food attractants in control strategies has been proposed (Levinson and Levinson 1977) there has been much less published about field trials than might have been expected. This is even more surprising given that the few reports which have been published clearly show the ability of food lures to attract several species simultaneously (eg Torriani 1984, Hodges et al. 1985, Sinclair and Haddrell 1985).

For the earliest possible detection of a developing infestation it has never been particularly promising to use a lure based on a single food which is very similar to the commodity being infested : this will do little more than achieve an equilibrium population in competition with the commodity itself, and at the very low levels of infestation which must be detected to avoid subsequent problems, this is likely to result in no catch in the traps. In contrast, the use of a lure based on material which is known to be an alternative foodstuff for the insects but is different to the commodity at risk is likely to be much more successful.

Kibbled (crushed) carobs appear to be a particularly effective source of attractants for insects which infest cereals: the addition of carobs to bait bags containing wheat and groundnuts greatly increases the number of insects found (D. B. Pinniger, personal communication). Volatiles

isolated from carobs have been shown in laboratory tests to elicit attractive responses from several species including O. surinamensis, S. oryzae, S. granarius, T. castaneum, C. ferrugineus, Ahasverus advena and Lasioderma serricorne (C P Morgan, personal communication).

For a food lure it would be possible to use a food extract directly, but it might be preferable to use only those components known to be attractive. This would save the need for isolation from the foodstuff, be easier to stabilise from degradation if this were necessary, be easier to formulate and would not itself sustain pests. Deciding which of the many components are important can now be undertaken by electro-antenogram (EAG) techniques. This method has been well established for a number of years but only recently has it been applied successfully to Silvanid beetles such as O. surinamensis (Chambers et al. 1986). The EAG method reduces to a manageable size the number of components which have to be studied but it is still necessary to discover which of them elicit an attractive response by behavioural bioassay in the laboratory.

Logically the choice of bioassay method must reflect the type of trap in which the components are ultimately to be used. This approach has shown that it is not always the same components of a foodstuff which show the greatest promise in the different types of trap. Thus, to improve the effectiveness of bait bags, results from laboratory bioassays using flat arenas suggest that acidic volatiles would be the best carob components (Stubbs et al. 1985). Other investigations with the same bioassay method show that the attractancy to O. surinamensis is due to degradation products of long chain fatty acids, principally linoleic acid (O'Donnell et al. 1983). It is of interest that linoleic acid is thought to be the biosynthetic precursor of the three lactones which comprise the aggregation pheromone of O. surinamensis (Pierce et al. 1984). However, behavioural studies on the potential of carob components to act as lures in probe and pitfall traps are best conducted using a laboratory pitfall bioassay. These studies show, in contrast to the above results, that it is the neutral volatiles which would be the best carob components for this use. In future it may be possible to use video techniques and image analysers to give a full description of insect behaviour in responding to lures and the relative importance of each microbehavioural step, for example exactly how, why and when an insect falls into a pitfall trap. It may then be possible to make further improvements in the effectiveness of lures and traps.

The relationship between an insect and its food is probably as complex as that with its pheromone. It depends on whether the adults eat (even if they do not, they may need to lay eggs near food for their larvae), when they eat, what they eat and why. It may be that what they eat varies with both dietary needs (Levinson and Levinson 1978), maturity and time. Understanding these points could elucidate how food is located (Barrer 1983) and establish the importance of aromas from damaged or mouldy commodities. If it is possible to learn how and why particular commodities become infested, it might be possible to prevent infestation occurring.

NON-TRAPPING METHODS

The two primary requirements for a detection method which is not based on trapping are (i) that it should work not only for freely

wandering insects but also for immobile stages especially those developing within grains, and (ii) that it should be rapid so that it can be used for grain in transit. The term "in transit" is used here to refer to any cereal, whether in a lorry, auger, blower etc, which is not stationary long enough for trapping methods to be used.

Detection methods based on ninhydrin-staining, flotation, X-radiography, and measurement of sound, uric acid or carbon dioxide are well known, but for use in practical situations all have serious limitations due to slowness, ineffectiveness, cost, or small sample size. To achieve further improvement it may be necessary to develop a completely new method. Ideally this would use equipment which is already widely distributed throughout the trade, for example near infra-red (NIR) analysers. These are in regular use for measurement of oil, protein and moisture in cereals (Williams 1975) and once a suitable calibration has been established the analysis is quick with low running costs. However, establishing the calibration can be very difficult, requiring detailed research with an advanced scanning NIR spectrometer and a large allocation of computer time to interpret the data obtained. Furthermore most applications of NIR are for the analysis of a relatively evenly distributed bulk parameter, such as protein, rather than for the detection of a small number of pests.

Despite this unpromising background, it has proved possible to correlate NIR reflectance spectra recorded on a research spectrometer with manually assessed numbers of flour mite Acarus siro in samples of pig feed (Wilkin et al. 1986). The basis of the response appears to be that in infested samples mite haemolymph causes the absorbance maximum for water to be shifted from 1934 to 1928nm. The results suggest that the NIR method has the potential to quantify the presence of mites in feed before reaching economically significant levels (about one million mites per kg) and that it should be possible to use this calibration in existing routine analysers in the trade. However there might be further problems in trying to detect individual insects within whole grains. The mite calibration was obtained from reflectance spectra of radiation which barely penetrates the sample and might miss a hidden insect. Spectra can be obtained by transmittance through a sample but there is a great loss in energy, and therefore sensitivity, at the longer wavelengths which include the absorption at 1928nm which is so significant for the mite calibration. It might be possible to detect insects with greater accuracy by obtaining reflectance spectra of ground samples but clearly the method would then be destructive, at least for whole grains.

Penetration through whole grains would be less of a problem for a method based on nuclear magnetic resonance (nmr) and routine low resolution analysers are already used for determining such things as the oil content of rapeseed. Most of their applications are based on the ability to distinguish liquid in the presence of solid, which is precisely the situation in trying to detect an insect (at least partly liquid) in a solid commodity. Work with a high resolution laboratory spectrometer has shown that it is possible to detect a S. granarius larva within an individual wheat kernel from about 15 days after the egg was laid i.e. about 25 days before the adult emerges and becomes visible to the naked eye (Chambers et al. 1984). The nmr response is due mainly to water. Preliminary work with a low resolution analyser showed that in a single scan taking 8s it is possible to detect ten grains infested with

S. granarius larvae in a batch of 500 grains. Although the method is nondestructive, it is unlikely to achieve the required sensitivity with "continuous wave" analysers, such as the one used for this study, because they are unable to distinguish between the water in the insect and the moisture in the grain. It might be possible to do this with low resolution "pulsed" nmr machines which are commercially available. One particular advantage of both this and the NIR techniques is that they have the potential to work unsupervised on a continuous flow basis.

CONCLUSIONS

It is clear that the detection of insects and mites in stored products, especially at low levels of infestation, is very difficult. The work reviewed in this paper shows that painstaking research on pheromones, food attractants and non-trapping methods is bringing significant advances in all three of these areas. If the ability to detect pests can be further improved, it might become possible to use the results not only to quantify the infestation and to select the most appropriate control strategy as early as possible,but to predict with confidence the consequential damage which might otherwise result, this of course being the key problem in successful pest control.

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FURTHER DEVELOPMENTS IN TRAPS USED TO DETECT LOW-LEVEL INFESTATIONS OF BEETLE PESTS IN BULK STORED GRAIN.

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ABSTRACT

Trapping beetle pests using pitfall and insect probe traps in bulk grain floor-stores has been shown to be at least 10 times as effective as conventional sampling methods. Laboratory and field trials have shown that these trapping methods can be made more sensitive by using both modification to the trap and by enhancement with a carob food lure. Pitfall traps were found to be more effective when a mesh cover and a carob lure were used. Insect probe traps were more effective when the collection tube was coated with Fluon® to prevent beetles escaping. Observations on the results of using unmodified and unenhanced traps to monitor floor-stored grain bulks are presented.

INTRODUCTION

For the past 50 years detection of insect pests in bulk grain in the UK has relied upon sampling; grain spears or vacuum samplers are used to obtain a sample of grain which is then sieved and the sievings examined for insects. These methods are labour intensive and rely heavily on the experience of the operator. They are also extremely unlikely to detect low-level beetle infestations.

Recently laboratory and field trials were conducted to evaluate these sampling methods and various trapping techniques (Cogan et al. In Press). This work demonstrated that two trap types, an insect probe trap and a pitfall trap, when used together were at least ten times as sensitive for the detection of beetle pests in bulk floor-stored grain as conventional sampling methods.

The sensitivity of the two traps, although much greater than conventional techniques, was relatively low in the laboratory trials. The pitfall traps performed best against Tribolium castaneum but only 3.4 per cent of the 450 insects released were trapped in the 3 day period. With probe traps, the highest level of catch was with Oryzaephilus surinamensis although only 1.6% of the insects released were recaptured.

Ways to improve this low trapping ability were therefore investigated. Increase in the sensitivity of the two traps was desirable for at least two reasons. Firstly, it may enable a greater area to be monitored by each trap, thus allowing fewer more reliable traps to be used. Secondly, the detection of an infestation at a lower insect density would lead to earlier detection of potential problems. Earlier detection would allow more options on the future of the grain to be open to the management. These options would of necessity be governed by the intended market of the grain, the condition of the grain, and the season when the infestation was discovered. An increase in sensitivity of the traps was sought in two ways.

MODIFICATION OF THE TRAPS

Trap performance is governed by the ability of traps to catch and retain insects and when this was investigated it was found that the insect probe traps were inefficient in retaining O. surinamensis. In the laboratory, 5 replicates of 20 adults were placed in the detachable collection tubes of the trap. The insect probe traps were placed upright into a block of plasticine on the base of empty glass tanks 32 x 22 x 38 cm high. Each tank was coated with Fluon® for 10cm below the inner rim to prevent insects escaping. Within 24 hr, 90 per cent of the insects had escaped from the collection tubes and traps into the tanks. When the top 3 cm of each collection tube was coated with Fluon and the experiment repeated no escapes occurred. These findings were discussed with the UK manufacturer of the probe traps and as a result the traps are now sold with a Fluon-coated collection tube.

To investigate and exploit the natural tendency (positive geotaxis) of many stored-product beetle pests to climb up a gradient, a batch of insect probe traps were made with the holes angled at 45° upwards, rather than downwards as originally designed. The modified traps were compared with both UK and USA manufactured, commercially available, probe traps. The laboratory trial was conducted using the 2301 plastic tanks and method used in previous experiments (Cogan et al. In Press). The tanks were filled with 150 kg barley and 450 insects of one of 3 species; Sitophilus granarius, O. surinamensis and Cryptolestes ferrugineus were released.

TABLE 1.

Comparison of 2 insect probe designs, 45° upward holes and normal 45° downward holes, using 3 beetle species released at 3/kg into barley (9 replicates).

Trap type	Mean number trapped (±S.E.) after 3 days		
	S. granarius	O. surinamensis	C. ferrugineus
Upward 45° holes	8.4 ± 2.0[a]	19.1 ± 7.1[a]	0.4 ± 0.2[a]
UK normal (downward 45°)	9.0 ± 2.1[a]	17.1 ± 5.4[a]	3.1 ± 0.9[b]
USA normal (downward 45°)	9.8 ± 2.0[a]	14.9 ± 5.2[a]	3.1 ± 1.3[b]

Means followed by the same letter in each column do not differ significantly, P>0.05 (Two sample t-test).

The results (Table 1) show that the traps with inverted 45° holes did not differ significantly (P >0.05, using two sample t-test) compared with UK and USA normal traps when trapping S. granarius and O. surinamensis but performed poorly against C. ferrugineus when compared with normal traps (P = <0.05, two sample t-test). As the original design of the probe was the most effective means for trapping C. ferrugineus in the field (Cogan et al. In Press) this modification was deemed not successful and therefore not pursued further.

The results (Table 1) also showed an approximate 10-fold increase in each species trapped by the probe traps compared with the previous trial (Cogan et al. In Press). Both trials were conducted under the same conditions of temperature and humidity. The reason for the variation in catch between the trials is not yet understood but may relate to variations in behaviour of the insects due to undetected changes in the physical state of the grain such as grain compaction, surface moisture content and/or carbon dioxide levels.

ENHANCEMENT OF TRAPS

Most beetle pests of grain are attracted to carob (Ceratonia siliqua L.), which also forms one-third of the constituents of bait bags (Pinniger 1975, Pinniger et al. 1984). Bait bags were found in laboratory trials in grain to be the best means for trapping O. surinamensis and second most effective trap for C. ferrugineus and T. castaneum but proved ineffective in field trials due to interference by mice (Cogan et al. In Press).

Preliminary laboratory trials indicated that pitfall traps with an 8 gauge (2mm) brass mesh cover (Fig 1a) did not reduce trap catch of either

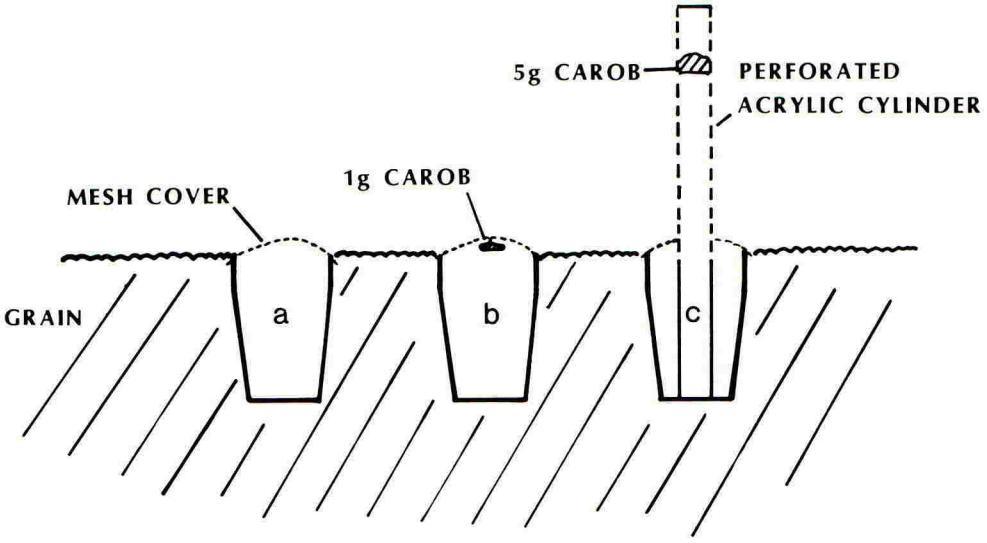

Fig 1. Pitfall traps used in laboratory and field trials :
a) with mesh cover b) with carob beneath the mesh cover and
c) acrylic tube with carob (shown at 15 cm) above the pitfall rim.

S. granarius or O. surinamensis compared with empty traps. In the laboratory pitfall traps enhanced by carob lures were then evaluated against O. surinamensis. Traps were baited in two ways; firstly with a 1 g piece of kibbled carob held by a paperclip beneath an 8 gauge brass mesh cover, domed to 1.5 cm at the centre above the pitfall rim (Fig 1b).

Secondly, with 5 g kibbled carob placed in a 2.6 cm diam, 35 cm long, perforated acrylic tube either 0, 7.5 or 15 cm above the pitfall rim (Fig 1c). The acrylic tube passed through the centre of the mesh cover, which also held the tube upright, and rested on the base of the pitfall trap. The trial was conducted in 230 l plastic tanks filled with 150 kg barley as in previous trials. For each of 4 replicates for each trap evaluation 450 O. surinamensis were released.

TABLE 2.

Laboratory comparison of carob lure height and trap catch in pitfall traps using O. surinamensis at 3/kg in barley.

Pitfall trap	Mean ± SE* trapped in 3 days
Empty with mesh	1.46 ± 0.46 ac
With 1 g carob under mesh cover	3.58 ± 0.48 b
With 5 g carob height 0 cm	1.39 ± 0.15 ac
7.5 cm	2.09 ± 0.19 bc
15 cm	2.08 ± 0.55 bc

* Data skewed, therefore transformed to square roots.
Means followed by the same letter in each row do not differ significantly, P>0.05 (t-test).

The results presented in Table 2 show that significantly more (t-test, P< 0.05) O. surinamensis were trapped in traps with 1 g carob under the mesh cover than in either empty pitfall traps or those with carob at 0 cm in the acrylic tube. There was no significant difference (P> 0.05) between the numbers trapped in 7.5 and 15 cm carob-baited traps and those with 1 g under the mesh cover.

When preliminary field trials were conducted, mice caused a serious problem by entering the traps with carob under the mesh cover and eating the lures. Traps with carob placed in a perforated cylinder were not attacked.

A field trial was undertaken in a bulk floor store housing 15000 tonnes of wheat lightly infested with O. surinamensis and S. granarius. Five trapping positions were used in the form of a X with a central trap 1 metre from 4 other positions. Each trap was checked for insects at weekly intervals and then moved in rotation to the next position until each position had been occupied twice. Three of the traps were empty pitfalls, one trap was covered with an 8 gauge mesh cover and the fifth trap was covered with a mesh plus perforated acrylic cylinder as described in the laboratory trials. Approximately 5 g kibbled carob was placed within the cylinder 15 cm above the pitfall rim. This carob was renewed weekly.

The results are presented in Table 3. Analysis of the data by two sample t-test for S. granarius showed no significant difference (in numbers trapped) between empty traps and those with mesh covers (P> 0.05) or

containing carob in the acrylic tube ($P > 0.05$). From the limited data obtained for O. surinamensis it was observed that the addition of a mesh cover appears to increase capture.

TABLE 3.

Mean number of S. granarius and O. surinamensis trapped in a field evaluation of carob-enhanced pitfall traps.

	Mean number trapped/trap/week (± SE)	
Pitfall trap	S. granarius	O. surinamensis
Empty	3.14 ± 0.70	0.25 ± 0.02
With mesh cover	3.08 ± 0.95	0.83 ± 0.10
With carob in acrylic tube	2.83 ± 0.95	0.83 ± 0.07

From the results obtained both in the laboratory and field, the addition of an 8 gauge mesh cover to pitfall traps is worth considering. The mesh cover also prevents entry of large spiders which produce webbing and may eat the trapped insects. It also prevents grain from falling into the trap which may occur if the trap is sited on a slope or near a walkway.

A distillate of carob and hexanoic acid, found by Stubbs et al. (1985) to be a major constituent of the carob distillate were evaluated in the laboratory as possible lures for the pitfall and probe traps. The traps and attractants were evaluated as in the other tests in 230l plastic tanks filled with 150 kg barley. Using a syringe, 0.5 ml of each attractant was put into a cotton wool plug placed in a 2 x 0.6 cm diam. polythene tube. This tube was positioned at the top of the insect probe trap attached to the removable lid. With pitfall traps the lure was tied to the top of a length of stiff wire so that the lure was level with the rim of the trap. The other end of the wire was inserted into a small cork block which acted as a stand and was positioned centrally in the pitfall trap. Five replicates of 3 species; O. surinamensis, C. ferrugineus and S. granarius were used at a density of 3/kg in barley.

The results (Fig. 2) show the considerable variance present in the data which can be seen in the standard errors and this influences a two-way analysis of variance on the data which showed no significant effect on trap catch ($P < 0.05$) by any of the attractants. Despite the variation it may be seen that carob distillate and hexanoic acid in both pitfall and probe traps seem to improve the capture of O. surinamensis and S. granarius. Secondly, carob distillate in probe traps increased trapping of C. ferrugineus over unbaited traps.

Although this work has shown that in the laboratory, carob and to a lesser extent carob distillate enhance the capture of O. surinamensis and C. ferrugineus, no field trial as yet has clearly demonstrated this. Further field trials are necessary to fully evaluate the role of carob and carob volatiles and exploit their potential for trap improvement.

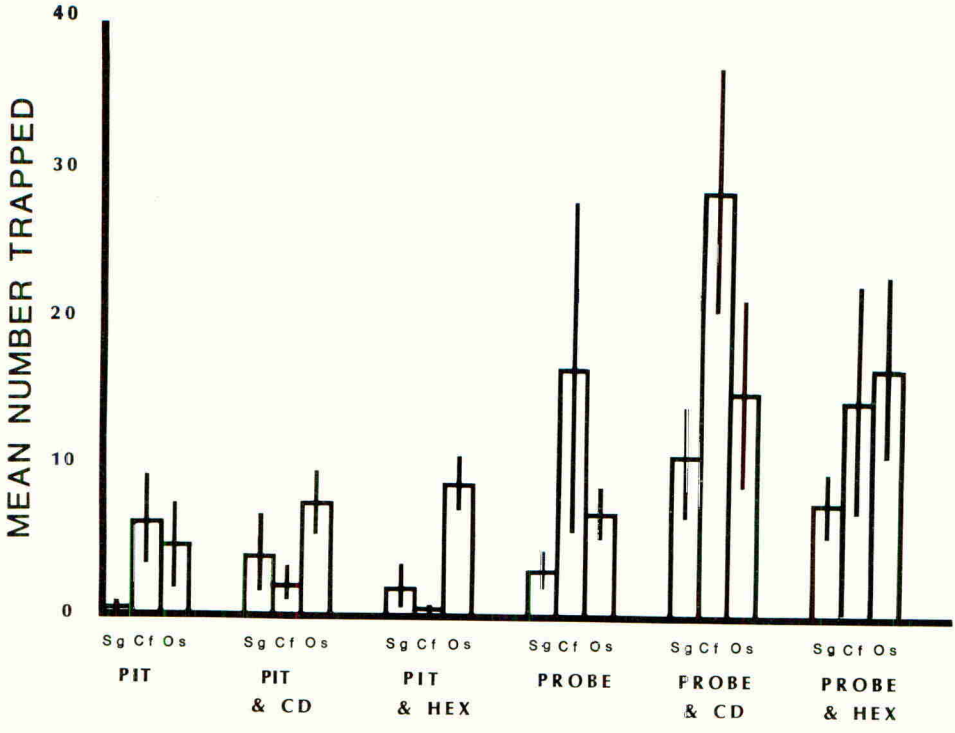

Fig. 2. Mean number, with standard error bars, of insects trapped at a density of 3/kg using pitfall and probe traps, empty or baited with carob distillate (CD) or hexanoic acid (Hex).

MONITORING BULK FLOOR-STORED GRAIN

Unmodified and unenhanced probe and pitfall traps have been used to monitor large bulks of floor-stored grain in 12 stores. Each store was monitored using one probe trap and one pitfall trap, placed in pairs, at 4 m intervals across the grain surface to form a grid. The traps were checked weekly.

From the data obtained from the 12 infested stores, the following observations were possible. Firstly, beetle pests were found in the probe and pitfall traps long before their presence was detected by conventional sampling (grain spear and/or vacuum sampler). In two stores, the traps detected beetles approximately 12 months before they were found by sampling. Secondly, at low levels of trapping (below 6 beetles per trap, per week) a more rapid build up of infestation was noted when beetles were trapped in fewer than 25 per cent of the trap positions. This suggests that, at low levels of infestation, the frequency with which the beetles were trapped was more important than the number of beetles trapped. Finally, a trapping level above 6 beetles per trap, per week, indicated that the infestation would demand drastic remedial action within a year unless remedial action was taken earlier.

This interpretation is however, based upon data obtained from just 12 infested floor-stores using unmodified and unenhanced traps, and must therefore be viewed with caution. In future the use of modified, enhanced traps should enable detection at lower infestation levels. Using this data, plus information on the consequence of remedial action, coupled with ambient and grain conditions, should lead to a greater understanding of the relationship between trap catch and infestation problems. It should then be possible to produce a reliable and detailed plan of remedial action based upon trap catch, for use in all trap-monitored grain.

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COMMERCIAL DEVELOPMENT OF PHEROMONE-BASED MONITORING SYSTEMS FOR INSECT PESTS OF STORED PRODUCTS

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ABSTRACT

Problems involved in the commercialisation of pheromone-based monitoring systems for insect pests of stored products will be looked at from both the producer and the consumer point of view. The consumer's principal worries regarding such products relate to ease of use, efficacy of capture and reliability; there is often a period of testing that every consumer goes through before confidence in the product is built up. Perceived value of the product is also often neglected in attempts at commercialising such devices. Problems encountered by the producers of such products, on the other hand, include the rather small size of the market, the lack of good patent protection, legislative control on the use of the product and possible competition from the larger pesticide manufacturers in response to reduced pesticide usage arising from the successful operation of pest monitoring systems.

INTRODUCTION

Since the characterisation of the first pheromone components over 20 years ago, much effort has been put into the development of products which make use of these highly biologically active compounds. The principal use for these components to date is as baits in highly specific trapping devices for insect pests. Other papers presented at this Symposium describe the development of both attractants (Chambers 1987) and trapping devices (Cogan & Wakefield 1987) used to monitor insect pests of stored products. This paper on the other hand will concentrate on the commercial considerations which have to be fulfilled before such devices can be successfully adopted on a wide scale as tools for integrated management of stored product insect pests.

MONITORING SYSTEMS CURRENTLY AVAILABLE FOR STORED PRODUCT INSECTS.

Moth pests

The most common moth pests of stored products (*Ephestia* spp. and *Plodia interpunctella*) share a common major component in their sex pheromone blend. As a result it has been possible to develop trapping devices which can be used for monitoring simultaneously all the major moth species normally encountered in stored product environments with the exception of *Sitotroga cerealella* which requires a different pheromone major component. These traps were first introduced into the U.K. in 1978 and have become increasingly used since that time. Two types of traps are used; one which uses a sticky base as an entrapment mechanism while the other employs a funnel system leading to a retaining chamber where the insects caught are killed by an insecticide or water and detergent (Reichmuth *et al.* 1978, Cogan 1983). Choice of trapping device is often related to practical use. Sticky traps can catch more insects in low dust environments but funnel traps perform better in high dust environments (Cogan & Hartley 1984).

Beetle pests of bulk grain

Two trapping devices are currently available in the U.K. to detect low level populations of species such as Oryzaephilus surinamensis, Cryptolestes ferrugineus and Sitophilus granarius in bulk grain.

(i) Probe trap. This trap was first designed by Loschiavo & Atkinson (1973) but was later developed into a more practical device by Burkholder (1984) in the U.S.A. for commercial exploitation. The probe is a hollow tube left buried vertically in bulk grain. Insects active in the grain work their way through diagonal holes drilled through the walls of the probe. Once inside the central cavity the insects fall down to the base through a funnel which helps prevent their escape.

(ii) Pitfall traps. Simple pitfall traps pushed into the grain until the lip is flush with the grain surface can be very effective at catching insects active on the surface of the grain. The inside rim is coated with PTFE to prevent insects climbing out.

Both these traps can be used with attractants but even without such lures they have been shown to be at least 10 times more effective at detecting low levels of insect infestations than more conventional techniques such as spear or vacuum sampling followed by visual inspection of samples (Cogan et al. 1987).

Beetle pests of processed commodities

For monitoring beetle pests such as Tribolium spp. Stegobium spp. or Lasioderma serricorne three trapping devices are currently available.

(i) Storgard.™ This trap exploits attraction to a sex or aggregation pheromone, a food extract and the crevice seeking behaviour of stored product beetles. The insects entering the trap fall into a small plastic pitfall containing an oil which both contains the attractant and kills the insects caught. This trap was developed by Barak and Burkholder (1985) and is now produced commercially in the U.S.A.

(ii) Bait Bags. An attractive food bait, wheat, broken groundnuts and kibbled carobs, contained in a plastic mesh bag developed by Pinniger & Wildey (1979) has been shown to give a general broad spectrum attraction for a wide range of pest species in food stores. After 2 to 7 days, the bait bag is retrieved and shaken over a tray to collect the trapped insects. Jacobson & Pinniger (1982) describe in detail the use of this device to monitor populations of O.surinamensis after treatment of a farm grain store.

(iii)Serrico™ traps and Lasiotraps™ for Cigarette beetles. These two traps produced in Japan and West Germany use the sex pheromone of Lasioderma serricorne to attract males onto a sticky entrapment surface. Both are used extensively in monitoring this important pest in tobacco storage and processing.

These various devices have been accepted in the market place to varying degrees and in an attempt to explain these differences, pheromone based monitoring devices will be considered from both the producer and the consumer's point of view.

THE END-USER'S PERSPECTIVE OF PHEROMONE-BASED MONITORING DEVICES.

Product Performance

Before a product becomes accepted as a valued tool in pest management the ultimate end-user has to be convinced that the product is capable of fulfilling the role for which it was intended. Trapping devices for stored

product insects must for instance:

(i) detect low levels of infestation which would otherwise go unnoticed,

(ii) perform consistently,

(iii)reflect accurately any population changes,

(iv) catch all the species which it is intended to sample.

If the monitoring devices used to detect moth pests are compared using the above criteria, with a product such as the corrugated cardboard trap for beetle pests of processed foods, then it is possible to see why the former have found greater acceptance than the latter at the warehouse-keeper level. The sex pheromone traps for warehouse moths, for instance, will often detect moth infestations at levels below which visual observations would not be reliable (Cogan 1983). The beetle trap on the other hand is only as sensitive as visual inspection by an experienced observer.

Ease of use

Product design at the research laboratory level is often a result of availability of materials, empirical testing of designs used already for other species, or it may in some cases be brought about from an attempt to accommodate some aspect of the target insect's behaviour. By the time the product reaches the commercial production stage it may have features which makes it difficult to use by an untrained person or it may be expensive to produce without further modifications which themselves may reduce the efficacy of the product. Ideally producers and researchers need to combine their efforts from an early stage in the development of the product. In this way, the end product can be optimised for both efficacy and ease of use.

Product testing by end-user

As far as the end-user is concerned, pheromone based trapping systems constitute new technology, and to be fully convinced of their value a period of testing and evaluation is often required before any firm commitment can be made to the product. Whereas this is entirely reasonable on the part of the end-user, it can be discouraging to the producer especially if the period of testing is long and protracted, sometimes lasting many months or even years. This makes it very difficult to plan ahead for production and sales. This problem can be addressed to some extent by having comprehensive field data to support claims made for a product though many consumers will not accept other than their own evaluations.

Perceived Value

Having satisfied all the requirements of efficacy, ease of use, etc. a new product will not be successful unless it has an adequate perceived value in the purchaser's view. Pitfall traps provide very good examples in this context. If these traps are made from familiar off-the-shelf materials such as plastic drinking glasses and the only novel feature to it is a band of PTFE coated around the lip, then it will not sell well if it is priced several times higher than the material costs. The addition of the PTFE is essential to the effective performance of the trap but it has not given the device sufficient perceived added value over its household counterpart which is probably available from most hardware stores at a much lower price. If on the other hand, a sophisticated pheromone or food lure is sold with it to attract the insects to the trap, then the product begins to have a greater perceived value.

Cost effectiveness

This criterion, above all others, is probably the main determining factor in the success or failure of a product. If it can be proved to the end-user that by investing in a particular device he or she will benefit directly from reduced insecticide costs, reduced incidence of infestations or reduced numbers of complaints from customers then the decision to purchase is made much easier. Fortunately in the case of many pheromone based products, such evidence is now becoming available, albeit in very few cases. Table 1 shows the results of continued use of warehouse moth monitoring systems in a chocolate factory and government flour stores. In both cases, substantial reductions in pest infestations were achieved as a direct response of improved pest monitoring which in turn more than compensated for the initial outlay in purchasing the products.

TABLE 1

Evidence of effectiveness of warehouse moth monitoring practices

Sticky Traps: In Chocolate Factory

	Year	Complaints	Complaints/100 tonnes of chocolate
Pre	1978	450-500	15-20
	1978-80*	440	10-15
	1981	100	4
	1982	10	0.3
	1983	8	0.3

* Gradual introduction of monitoring systems.

Funnel Traps: In Government Flour Stores (3 traps/flour store)

	Year	% with traps	% Infested
Pre	1978	0	24
	1979	29	41
	1980	59	29
	1981	76	24
	1982	100	6

Data from Cogan and Hartley (1984)

THE PRODUCER'S PERSPECTIVE OF PHEROMONE-BASED MONITORING SYSTEMS.

Market size and margins

Since pheromone-based monitoring systems are not designed to replace conventional pest control products, but merely to aid in their successful implementation, it is generally agreed that the market for such devices will not be of sufficient size to be of interest to large corporations. Consequently it is only the small to medium sized company that will consider marketing such products. The market for these products is also highly specialized and requires a relatively high degree of technical support. A major consequence of these facts is that the financial margins which a company must have to commercialise such products successfully have to be high in order to cover the costs of promotion, product education and technical back up facilities.

Patent protection

Since pheromones are natural products, it is often difficult, if not impossible, to make them into proprietary products through patenting. This has discouraged many companies from entering the market and will probably continue to do so unless there are major changes in the law governing their patentability. Those companies that are active in the market have overcome this problem in many cases either by using patented controlled release devices for the pheromone, by using registered designs or patented trapping devices, or through building into the product as much know-how and technical expertise as possible so as to make it difficult for potential competitors to copy the devices.

Legislative control over product use

Registration requirements for pheromone-based monitoring systems are currently very lenient because of their biorational status under the U.S. Environmental Protection Agency (EPA) classification (Zweig et al. 1982). Under the EPA guide-lines, pheromones used for monitoring insect pests are not subject to any requirements under the Federal Insecticide, Fungicide and Rodenticide Act or its several amendments - the legislation which regulates the use of pesticides in the U.S.A. Should this situation change however, such that pheromones need to be registered for use as monitoring devices then it would pose great financial restrictions on the companies commercialising such products with the net result that the technology would advance no further and probably go into a catastrophic decline.

CONCLUSIONS

Several pheromone-based products are currently available for monitoring insect pests of stored products. A number of such products have now established an important role in pest management strategies. A number have not been so successful however and this may have been due in part to insufficiently developed technology, consumer resistance or lack of promotion. It is clear that in the future, researchers, government authorities and private industry must work together to develop this technology since in many cases in the past lack of success has arisen through having the individual parties working in isolation.

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5.
Chemical Control of Insect Infestation

Chairman: Dr P. I. STANLEY
Session Organiser: Dr T. J. LAWSON

INSECTICIDE RESISTANCE IN STORED PRODUCT BEETLES AND ITS CONSEQUENCES FOR THEIR CONTROL

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ABSTRACT

Insecticide resistance needs to be identified at the earliest opportunity and the resistance tests described are intended to achieve this. Data presented show the changes in the proportion of malathion resistant populations among seven species of stored product beetle between 1971 and 1986. Increases are recorded for four species. New discriminating doses have been used to examine the frequency of populations of Oryzaephilus surinamensis resistant to malathion, pirimiphos-methyl and chlorpyrifos-methyl, and details of cross or multiple resistance to these compounds in field strains are given. Over 90% of the populations were resistant to chlorpyrifos-methyl and this resistance is inherited separately from those to pirimiphos-methyl and malathion. 29% of populations were resistant to pirimiphos-methyl. The importance of low levels of resistance is stressed and strategies to combat resistance are discussed in relation to the stored product environment. It is concluded that the treatment of storage structures offers the best hope of controlling resistance and reducing pesticide residues.

INTRODUCTION

There is a continuing need to provide insect-free grain and other foodstuffs for trade on the world market, and to ensure that losses to stored commodities are kept to a minimum. Throughout much of the world pesticides appear to be the simplest and most cost-effective means of achieving these aims (Champ 1986). This is certainly true of the situation in Great Britain and a recent survey (Taylor & Sly 1986) reported that 56% of farms storing grain used pesticide in association with the grain, while a 1982 survey of commercial grain stores showed that 76% of storekeepers admixed pesticide with at least some of the grain they stored (Wilkin et al. 1983). Although the use of chemical control is widespread the number of insecticides which can be used to control stored product pests is limited to those which appear to present the least hazard to the consumer. Snelson (1986a) was able to list only sixteen compounds which are being used, or which appear to fulfill the FAO criteria (Anon. 1982) for approval, as grain protectants. Of these compounds six, all organophosphorus insecticides, are currently cleared for use on grain in the United Kingdom. A total of seventeen compounds may be used in the United Kingdom to treat the structure of the grain store.

Resistance to any one of the available compounds will greatly restrict the choice of alternatives, the more so as resistance to any one compound may be accompanied by cross-resistance to another. Cross-resistance is especially important when, as is the situation in the United Kingdom, all the compounds cleared for application on to grain come from one group of chemicals. Internationally the problem has been recognised since the early 1970s when the FAO commissioned a world-wide survey of resistance in stored grain pests. The survey took place in 1972 and 1973 and investigated the resistance of eight species of beeetle to malathion and lindane (Dyte 1974)

and the findings were subsequently published by the FAO (Champ & Dyte 1976). The data from Great Britain showed that resistance to malathion was present in Tribolium castaneum, T. confusum, Rhyzopertha dominica, Sitophilus granarius, S. oryzae and S. zeamais, but absent in Oryzaephilus surinamensis and O. mercator. The current world situation has recently been reviewed by Champ (1986). Since the FAO survey, published reports of resistance in stored product beetles in Great Britain have been sporadic. This account sets out the current situation in Great Britain and records the changes in resistance that have taken place between 1971 and the present time. I shall also consider, briefly, the consequences of these resistances and the strategies which might be used to contain them.

THE IDENTIFICATION OF RESISTANCE

There are a number of different definitions of the term 'resistant' and it can appear that the definition depends on the direction from which the problem is being approached. The two extremes are, on the one hand to describe as resistant a population which contains a single individual capable of surviving a laboratory dose of insecticide which would kill all (or least 99.9%) of a laboratory susceptible strain or, on the other hand to use the term resistant to describe only a field population which could not be controlled by the field dose properly applied. I would use the former definition for the reason that, as I have explained elsewhere (Muggleton 1984), if resistance is to be managed it is important that it is detected at the earliest stage. I would not, however, use the term 'resistant population' but would describe the population as one containing resistant individuals.

Resistance in stored product beetle pests is detected by the use of discriminating dose tests following the methods recommended by the FAO (Anon. 1974). The discriminating dose is determined by exposing adult beetles on filter papers which have been impregnated with technical grade insecticide in a solvent carrier such as 'Risella 17' oil. Beetles from susceptible strains are exposed to a range of doses of the appropriate insecticide and the numbers 'alive' and 'knocked-down' are counted at the the end of a set time period. The response can then be plotted against the dose and a line fitted to the plot using the probit transformation. The dose required to kill 99.9% of the susceptible population can be estimated from the fitted probit line and used as the discriminating dose. Fig. 1 illustrates the determination of a discriminating dose of chlorpyrifos-methyl for O. surinamensis and shows the probit lines for two laboratory susceptible strains not known to have exposed to organophosphorus insecticides, together with the probit lines for two field strains collected in 1979/80. The fact that one of the field strains has a response very similar to that of the laboratory strains indicates that the level of susceptibility shown by the laboratory strains can still be found amongst field populations and thus the laboratory strains provide a realistic base line against which field strains can be compared. Using this method discriminating doses have been) obtained for malathion with S. oryzae, S. zeamais, S. granarius, R. dominica, T. castaneum, T. confusum, O. surinamenis and O. mercator (Anon. 1974). We have also determined discriminating doses for O. surinamensis against pirimiphos-methyl, 0.6% in oil (J. Waller & J. Muggleton unpublished), and chlorpyrifos-methyl, 1.0% in oil (J. Muggleton & L. Sharrock unpublished), and revised the dose against malathion to 0.3% in oil (J. Muggleton unpublished). The exposure period for each of these tests is five hours. A discriminating dose of malathion against Cryptolestes ferrugineus has also been developed (Anon. 1981) as have doses for a number of other species and insecticides (Champ 1986) but most of these latter tests are for species or insecticides which are not of major importance in

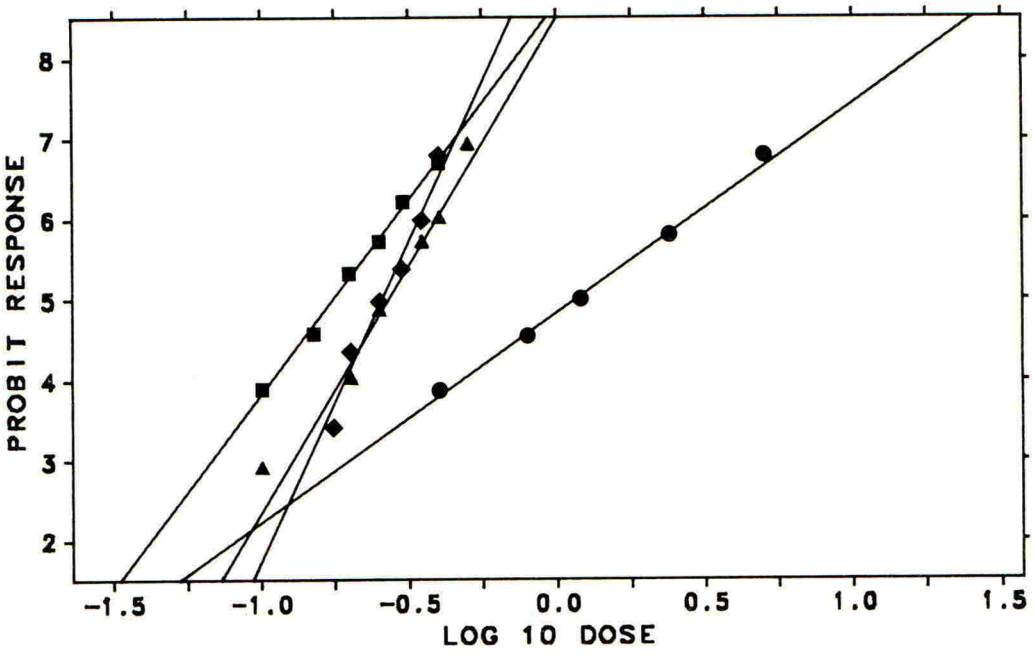

Fig. 1. Dose-response lines, against chlorpyrifos-methyl, for two laboratory susceptible strains of O. surinamensis (■) (◆) and two field strains collected in 1979 (▲) and 1980 (●). The dotted line shows the LD 99.9 of the strain used to set the discriminating dose. The dose finally adopted was rounded-up to 1.0% (0.0 on the logarithmic scale).

the United Kingdom. An exception is the discriminating dose of fenitrothion against O. surinamensis (Collins 1986) which is discussed below.

To confirm that what we detect is an inherited resistance, rather than the tail of the normal distribution of a population's natural tolerance to the insecticide, the progeny of the survivors from a discriminating dose are bred and tested for resistance. If the ability to survive the discriminating dose is inherited then there will be an increase in the proportion of resistant individuals in the progeny compared to that in the population from which the parents were derived.

RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES IN GREAT BRITAIN

The frequency of malathion resistance in beetle pests of stored products has been monitored at the Slough Laboratory since the 1970s. Initially all the testing and collating of results was done at Slough, but in 1983 the responsibility for testing passed to the Wildlife and Storage Biology Discipline of the then Agricultural Science Service. Recently resistance testing of field strains of O. surinamensis has resumed at the Slough Laboratory as part of an experimental programme aimed at identifying and classifying the different types of resistance present in British grain stores. Table 1 shows the proportion of malathion resistant samples for each of the six beetle pests, for which discriminating dose tests were available, in four year periods from 1971 to 1986. The malathion discriminating dose test for C. ferrugineus was not available until the end of the 1970s and there-

fore data for this species are only available for the last two periods. It is evident that for some species, S. granarius and S. oryzae, there has been no significant change in the frequency of resistant populations over the entire period. T. castaneum, T. confusum and O. surinamensis have shown significant ($P < 0.05$) increases in the frequency of resistant populations during the period 1971-1986. The number of samples of R. dominica available is too small for any valid comparison to be made. The low number of resistant samples for the two Sitophilus species is puzzling. Champ and Dyte (1976) noted that the Sitophilus species did not seem to be so prone to developing malathion resistance as did species from other genera. They did give evidence, however, that in Australia the intensive use of malathion over a number of years had eventually led to resistance appearing in S. oryzae. It may be that the use of malathion in Great Britain has never been sufficiently intensive to select for widespread resistance in these species.

TABLE 1

The percentage of United Kingdom populations of each of seven stored product beetles found to be resistant to malathion in four year periods between 1971 and 1986. The numbers of populations tested are given in parenthesis.

	Percentage resistant			
Years	1971-74	1975-78	1979-82	1983-86
Species				
C. ferrugineus	-	-	33.6(107)	25.0(28)
O. surinamensis	0.4(254)	3.4(801)	6.0(585)	23.7(135)
R. dominica	16.7(6)	0(1)	61.5(13)	66.7(3)
S. granarius	5.0(60)	6.7(45)	2.8(109)	5.3(38)
S. oryzae	11.8(17)	0(2)	5.7(87)	0(13)
T. castaneum	76.7(43)	94.1(17)	91.4(58)	84.2(19)
T. confusum	43.2(37)	50.0(2)	68.8(32)	75.0(8)
Total tested	417	868	991	244

Although the frequency of resistance is increasing the number of populations of pest species tested in the last four years has fallen to about a quarter of the 1975-82 totals. This is the result of a reduction in the amount of time available to collect samples and carry out the tests rather than less frequent occurrences of the insects. Clearly our picture for all the species would be greatly improved if a larger number of populations could be tested. It should also be noted that the insects tested are from premises where advice has been sought from ADAS as the result of a problem. It is therefore likely that the results will be biased towards finding resistance. A further bias concerns the type of premise from which the beetles have been collected. Table 2 shows the number of each type of premise examined in the period 1983-86, and it will be seen that most of the data refer to infestations of farm grain stores; we have very little current information on the extent of resistance in commercial grain stores, mills or other premises, where selective pressures and species involved may be very

different. Only a truly random resistance monitoring exercise would give us a real picture of the extent of resistance in Great Britain today. Another

TABLE 2

The number of each type of premise from which the populations sampled in 1983-86 (see Table 1) were collected

Type	Number	Type	Number
Arable farms	112	Livestock farms	6
Commercial grain stores	6	Warehouses	5
Feed Mills	29	Other	1
Flour mills	3	Total	162

weakness of the data in Table 1 is that it concerns only malathion, a compound whose use has greatly decreased during the period under consideration. Malathion accounted for an estimated 67% by weight of the insecticide used in farm grain stores in 1971/72, 58% of that used in 1976/77 but only 5% of that estimated to have been used in 1983/84. On the other hand the use of pirimiphos-methyl increased from an estimated 4% by weight in 1976/77 to 80% in 1983/84 (Taylor & Sly 1986). To some extent testing for malathion resistance is still valid as it can indicate the presence of non-specific organophosphorus resistance, but in some species (T. castaneum and C. ferrugineus) a malathion specific resistance can be found and the existence today of this type of resistance is of little importance. The frequency of populations of T. castaneum and C.ferrugineus with malathion resistance did not increase in 1983-86 compared with 1979-82 and this may reflect the reduction in use of this compound.

The development, at the Slough Laboratory, of discriminating dose tests to detect resistance to pirimiphos-methyl and chlorpyrifos-methyl in O. surinamensis means that for that species at least we have some information about resistance to the three most frequently used contact insecticides. Populations of O. surinamensis collected during advisory farm visits since August 1984 have been tested against these three compounds and the results are shown in Table 3. Since a discriminating dose for fenitrothion (0.6% in

TABLE 3

The proportion of populations of O. surinamensis collected from inland premises between August 1984 and December 1986 and found to be resistant to four organophosphorus insecticides.

Insecticide	Number resistant	Number tested	% resistant
Chlorpyrifos-methyl	33	37	91.7
Fenitrothion	2	31	6.4
Malathion	28	95	29.5
Pirimiphos-methyl	16	54	29.6

oil) has been developed in Australia, information on the number of our populations which this dose would classify as resistant is also included in Table 3. It has been possible to test thirty-seven of the populations to each of the three compounds, malathion, chlorpyrifos-methyl and pirimiphos-methyl, and the combinations of resistances found in these strains are shown in Table 4. It will be seen that a very high proportion of O. surinamensis

TABLE 4

The combinations of resistances present in populations of O. surinamensis collected from inland premises between August 1984 and December 1986 and tested with each of three organophosphorus insecticides.

Insecticide	Combinations of resistances							
Malathion	R	R	R	R	S	S	S	S
Pirimiphos-methyl	R	R	S	S	R	R	S	S
Chlorpyrifos-methyl	R	S	R	S	R	S	R	S
Number of populations with each combination	6	1	3	0	7	0	18	2

R = resistant, S = susceptible

are resistant to chlorpyrifos-methyl and, of those populations, nearly half possess resistance to chlorpyrifos-methyl only. It may, therefore, be deduced that in these latter populations, at least, chlorpyrifos-methyl resistance is conferred by a mechanism separate to that involved in resistance to pirimiphos-methyl and malathion. Given that chlorpyrifos-methyl has been in use in the world grain trade only since 1978 (Snelson 1986b), compared with 1960 for malathion and 1969 for pirimiphos-methyl, and its apparently low usage in England and Wales (an estimated 5% by weight of the insecticides used in farm grain stores in 1983/84 (Taylor & Sly 1986)) then within the limitations of our survey this high frequency of resistant populations is surprising. It may be that the resistance mechanism involved is one which the insect had already evolved to cope with some other compound either naturally occurring or man-made. As yet there is no evidence that other stored product beetles have developed a similar frequency of resistance to chlorpyrifos-methyl. No populations of O. surinamensis have been found that are resistant to only malathion or pirimiphos-methyl, however some of the chlorpyrifos-methyl resistant strains are either resistant to pirimiphos-methyl and susceptible to malathion or are resistant to malathion and susceptible to pirimiphos-methyl. Such strains may have specific malathion or pirimiphos-methyl rersistance as well as the separate mechanism giving specific chlorpyrifos-methyl resistance. There is one strain which is resistant to both malathion and pirimiphos-methyl and six strains which are resistant to all three organophophorus compounds tested. We do know, from work on an indigenous strain collected in 1981, of a resistance mechanism in O. surinamensis controlled by a single gene which gives resistance to both malathion and pirimiphos-methyl as well as to other organophosphorus compounds (except chlorpyrifos-methyl) and to compounds from unrelated groups such as carbamates, organochlorines and pyrethrins. At this stage it is not possible to say whether the multi-resistant populations detected since 1984 are truly cross-resistant or whether they possess multiple resistances.

It is worth noting that only two out of the thirty-seven populations tested show no evidence of any organophosphorus resistance.

I have excluded from the remarks above the small amount of data we have about fenitrothion resistance. Of the thirty-one populations we have tested, only two appear to be resistant to fenitrothion. In view of the widespread occurrence of organophosphorus resistance described above and the expectation from the 1973 FAO survey of a high degree of cross-resistance to fenitrothion from malathion resistant strains (Anon. 1975), resistance to fenitrothion should be frequent. There may, however, be a problem with the discriminating dose for this compound. In particular the dose of 0.6% in oil used by Collins (1986) , and developed in a region where resistance to fenitrothion is at a high level (see Collins 1985), may not be adequate for detecting low levels of fenitrothion resistance. A better picture may emerge once we have developed a discriminating dose base upon British populations. However, it is probably safe to conclude that the level of resistance to fenitrothion in Great Britain, both in terms of frequency of resistant populations and resistance factors, is far less than in Australia.

RESISTANCE, ITS RELEVANCE AND CONTROL

The resistances described above have been identified in the laboratory and it would be reasonable to ask what relevance they have to the manager of a grain or other food store. The first point to be recognised is that resistance is likely to have an effect well before control failure occurs. Each occurrence of resistance represents a population containing individuals which are able to survive a substantially higher dose of insecticide than those from a susceptible strain. While this does not necessarily mean that the individuals will be able to survive a correctly applied treatment at the full recommended dose, it may mean that they can survive in situations where, for one reason or another, the dose reaching the insects is less than that recommended. There are many such situations and thus the presence of even low frequencies of resistance is likely both to increase the need for complete treatments at the maximum permissible dose and to result in repeated treatments in locatios where a complete treatment has not, or cannot, be applied. Thus the amount of insecticide in the environment and the cost to the store manager will increase.

Very little is known about the relationship between failure of control in the field and the resistances we have identified. Conclusive data are very hard to obtain and this remains one of the largest gaps in our knowledge. In 1985 we carried out a field trial at a farm in the north of England which had a long history of control problems with O. surinamensis (Muggleton et al. 1986). The population was found to be resistant to a wide range of organophosphorus insecticides including pirimiphos methyl. A fabric treatment was carried out by MAFF staff using the recommended dose and this resulted in a heavy mortality among the adult beetles. However the frequency of resistant individuals in the surviving population increased, as did these individuals' ability to survive higher doses of pirimiphos-methyl. The beetles were able to reinfest the grain from the next harvest and despite further treatments by the farmer, are still present in the grain store. As mentioned above, all the resistances listed in Tables 3 and 4 are from premises where ADAS advice had been sought because the usual control measures had failed. Six of the farms found to have organophosphorus resistant O. surinamensis in 1984-85 still had resistant populations a year later; we have no information on the situation at the remaining premises where resistance was found. Taken together these examples suggest that there is

at least circumstantial evidence that resistance can cause field control problems in O. surinamensis in Great Britain.

If it is accepted that resistance is a problem, then what can be done to slow or even halt its spread while at the same time controlling the pest populations? It might be worth considering first where the resistant insects, indeed any beetle pests of stored food, come from. Without exception the species that are pests in food stores in this country are not pests of the growing crop. At the point of entry into the store, or on to transport to the store, the grain or other commodity will be free of storage pests. The beetle pests come from either the structure of the store, the vehicles used for transport or the machinery used for processing. It follows that control of resistance needs to concentrate on these areas. While alternatives to insecticides exist for the treatment of bulks of foodstuffs they are not so readily applied to the fabric of the store. Fumigation and cooling by aeration may deny the beetles access to the greater part of the bulk of the stored commodity but the ability to survive on food residues for long periods of time seems to be a characteristic of these beetles. It must be said here that ensuring that no grain pockets or spillages are left when the commodity has been removed from the store is a major step towards control.

In the past resistance to one compound has been overcome by switching to another (e.g. malathion to fenitrothion in Australia or to pirimiphos-methyl in the United Kingdom) but we must now ask whether this is still possible. The costs of development of a new compound are high (Taylor 1986) and the stored product market may not be sufficiently large to make the marketing of a specialised compound worthwhile. An additional problem is that infestations are often made up of more than one species and the susceptibilities of each species may be different. Also, as mentioned above, a single genetic mechanism exists in at least one strain of O. surinamensis which gives resistance to a wide range of unrelated compounds. The mechanism of this resistance is not known and its elucidation would obviously help predict what alternative insecticides might be useful. However it seems unlikely that a new compound drawn from those groups already available would be successful. Some alleviation of the problem might be sought by turning to the 'novel' compounds such as juvenile hormone mimics and other insect growth regulators. However the potential for the development of resistance to these compounds exists, and the first report of resistance to a juvenile hormone mimic was from a stored product pest, T. castaneum, (Dyte 1972).

Many models have been put forward suggesting strategies that might delay the spread of resistance. In a world which demands insect free grain and processed food, integrated control and the release of insecticide susceptible insects, sterile males, or of strains that will produce sterile progeny when mated with the resistant population (Comins 1977, Wool & Manheim 1980, Curtis 1981), would all seem unacceptable. The use of an alternation of pesticides (Pimental & Bellottl 1976) would seem to be an attractive proposition for the treatment of storage structures although, as Prickett (1987) has pointed out, it requires considerable cooperation between those applying and those supplying the insecticides. It also requires that the pest being treated has a different resistance mechanism for each of the insecticides. Thus the alternation of an organophosphate, a carbamate and a pyrethroid is unlikely to have prevented the selection of the multi-compound resistant O. surinamensis described above. Nevertheless in at least one situation, involving field crops, the strategy of using alternating compounds has been operated on a voluntary basis and the results appear promising in that selection pressure from the insecticide, to which resistance had

arisen, has been reduced (Daly & McKenzie 1986). Strategies which involve leaving 'refugia', that is places where insects are deliberately allowed to escape treatment (Georghiou & Taylor 1977, Wood & Mani 1981) , would seem particulary applicable to grain stores where the inability to treat all the pest population is often unavoidable (Muggleton 1986). Prickett (1987) points out that the use of refugia will leave live insects and would therefore be as unacceptable as the release of live insects. If, however, the refugia strategy was confined to the fabric of the store, then the escapees could be dealt with by a prophylactic treatment of the commodity in store. Finally, one cannot escape the fact that as Wood & Mani (1981) have pointed out, the use of a high dose to ensure the recessiveness of the resistance gene and the susceptibility of the heterozygote is essential if insects carrying the initial mutations conferring resistance are to be killed. Nothing is likely to spread resistance more quickly than an inadequate or uneven treatment. Dosages must, of course, be kept within the prescribed limits, but by concentrating the strategy on the control of resistance in the fabric of the store it should be possible to use high doses while at the same time reducing the amount of insecticide applied to the stored product. In this way both residue and resistance levels might be reduced. To end, two notes of caution should be added. The first is that none of these strategies has been tried in a real grain store nor in any other storage situation, and some way must be found to do this. The second is that all the strategies mentioned above need to be put into operation when resistance is at a very low, indeed almost undetectable, level; otherwise they cannot work efficiently. This again emphasises the importance of detecting resistance long before it has become a control problem and the need to put strategies for controlling resistance into operation as a matter of course.

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REPELLENCY OF INSECTICIDE FORMULATIONS TO RUST-RED FLOUR BEETLES (TRIBOLIUM CASTANEUM)

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ABSTRACT

The response of an insecticide resistant strain of rust-red flour beetle to two commercial formulations of fenitrothion and pirimiphos-methyl was assessed in arenas half treated with insecticide and in fully treated arenas. In the former, emulsifiable concentrate formulations were more effective than wettable powder formulations. In the latter the efficacy was reversed. This was shown to be due to a strong repellent effect of the wettable powder carrier component of the formulation. In arenas with halves treated with two different doses of wettable powder carrier, beetles avoided the higher dosed area. The practical implications of this repellency are discussed.

INTRODUCTION

The behaviour of stored product pests in the presence of an insecticide has been shown to be an important factor in their survival of an insecticide treatment. It has been shown that untreated refuges placed in insecticidally treated arenas can increase the time to 100% mortality by a factor of up to 20 x. (Pinniger 1974, 1975, Wildey 1977.) Differences in the degree of refuge seeking were noted between susceptible and insecticide resistant strains of insect and it was suggested that increases in refuge seeking may result from an avoidance of the insecticidally treated surface as a protective mechanism. When avoidance of treated surfaces is detected the surfaces may be considered repellent (Dethier 1947) and according to Kennedy (1947) a repellent surface may be defined as one on which "insects are found to spend less time and so occur in smaller numbers on it than on other available and comparable surfaces." To determine whether stored product insecticide formulations were repellent to the rust-red flour beetle - Tribolium castaneum - adult beetles were placed in arenas half treated with commercial formulations of storage insecticides and their distribution and mortality recorded daily.

MATERIALS AND METHOD

Test Arenas

Whatmans No. 1 filter papers of 27 cm diameter were attached to 30 cm x 30 cm polystyrene ceiling tiles and an aluminium ring of 21 cm diameter placed centrally on the paper and attached using instant polyfilla smeared round the outside of the ring. A black filter paper was placed on top of all arenas to exclude light and the arenas were kept in a dark cabinet at 25°C; 70% RH. Replicates of 8 arenas were used in each test and the treated sections of part treated arenas were each orientated differently (0°, 45°, 90° etc) one from the other to overcome any preference or spatial bias occurring in the arenas.

Test Insects

30 unsexed adult beetles, 3-5 weeks old, were placed in the centre of each test arena. The beetle strain used (S634) had been in culture at the

Slough Laboratory since 1973 having been collected in an American food store for the FAO worldwide survey of resistance in stored product insects (Champ and Dyte 1976). The strain had been shown to be resistant to a number of organophosphorous insecticides with resistance factors of 10 x to malathion and 3 x to fenitrothion.

Insecticides

Two commercially available wettable powder and emulsifiable concentrate formulations of both fenitrothion (dimethyl 3-methyl 4-nitrophenyl phosphorothioate) and pirimiphos-methyl (2-diethylamino - 6-methylpirimidin-4-yl dimethyl phosphorothioate) were tested in both fully treated arenas and half-treated arenas. Both fenitrothion formulations contained 40% a.i. and were applied at either 500 mg/m^2 or 250 mg/m^2 a.i. The pirimiphos-methyl formulations contained 25% a.i. and were applied at either 500 mg/m^2 or 250 mg/m^2 a.i. The higher doses represented recommended field application rates for the control of stored product pests using a residual insecticide applied to storage structures. In addition, blank formulations of pirimiphos-methyl wettable powder and emulsifiable concentrate and fenithrothion wettable powder were applied to half-treated arenas at rates to deposit an equivalent amount of formulant carrier to that present when the arenas were treated at full field rates of 500 mg/m^2 a.i. Test papers for all arenas sprayed with active ingredient were analysed using gas-liquid chromatography and only results from arenas treated within ± 8% of intended dose are reported in this paper.

Test Counts

All half-treated arenas were examined each morning and the number and position of live insects noted, whether on treated or untreated surfaces, and any dead insects removed. Each test was run for 7 days. In fully treated arenas the number of live and knocked down or dead beetles was noted every 2 hours for 8 hours.

TESTS AND RESULTS

Half-treated Arenas

Mortality was assessed in arenas half treated with fenitrothion wettable powder (WP) and emulsifiable concentrate (EC) and pirimiphos-methyl WP and EC (Figs. 1 and 2). For both active ingredients the mortality on the EC formulation was greater than on the WP formulation. Over the first two days of test both EC formulations killed approximately 70% more beetles than their equivalent WP formulation and this difference was maintained to the end of the test period. This result was contrary to the expected superiority of WP's reported in fully treated arenas by a number of workers. (Singh and Sarup 1978, Anon 1981, Wildey 1984.) Accordingly, the two formulations of both insecticides applied to half-treated arenas were compared against S634 beetles in fully treated arenas.

Fully Treated Arenas

Eight replicates of 30 adult S634 beetles were exposed to wholly treated arenas dosed at 500 mg/m^2 and 250 mg/m^2 a.i. with each insecticide formulation. The latter dose was chosen to represent the same amount of insecticide as present in an arena with one half-treated with 500 mg/m^2 a.i. Mortality (knocked down or dead beetles) was recorded every two hours and results are represented in Figs. 3 and 4. The standard errors of the mean percentage mortalities were calculated but were too small to be represented on the graphs. In all cases the WP formulation was more effective than its equivalent dose of EC formulation. Indeed, the 250 mg/m^2

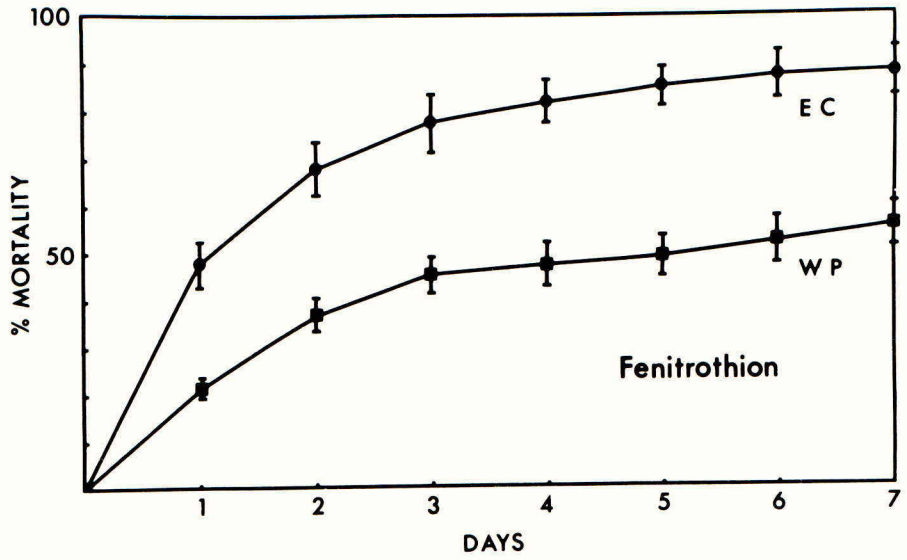

Fig. 1. Mortality of S634 I. castaneum in arenas half-treated with 500 mg/m² fenitrothion emulsifiable concentrate (EC) or wettable powder (WP) on the treated area.

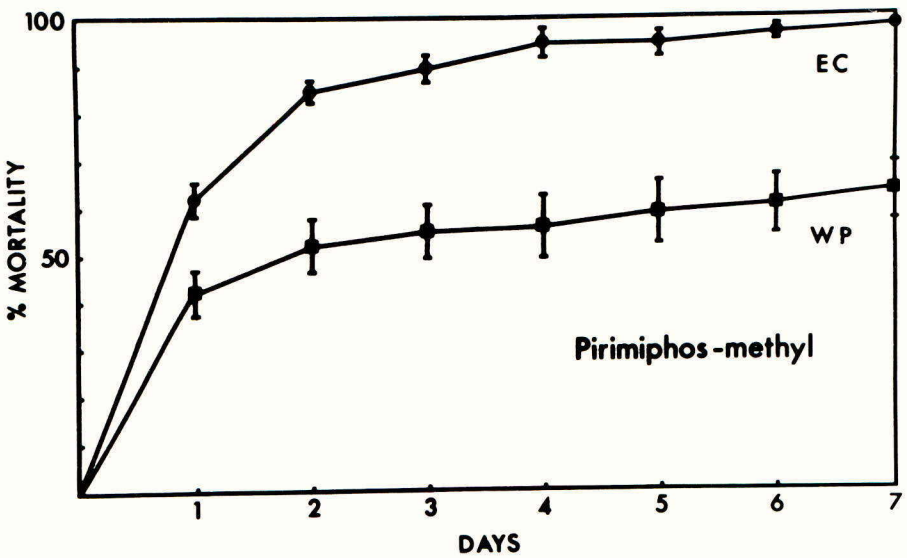

Fig. 2. Mortality of S634 I. castaneum in arenas half-treated with 500 mg/m² a.i. pirimiphos-methyl emulsifiable concentrate (EC) or wettable powder (WP) on the treated area.

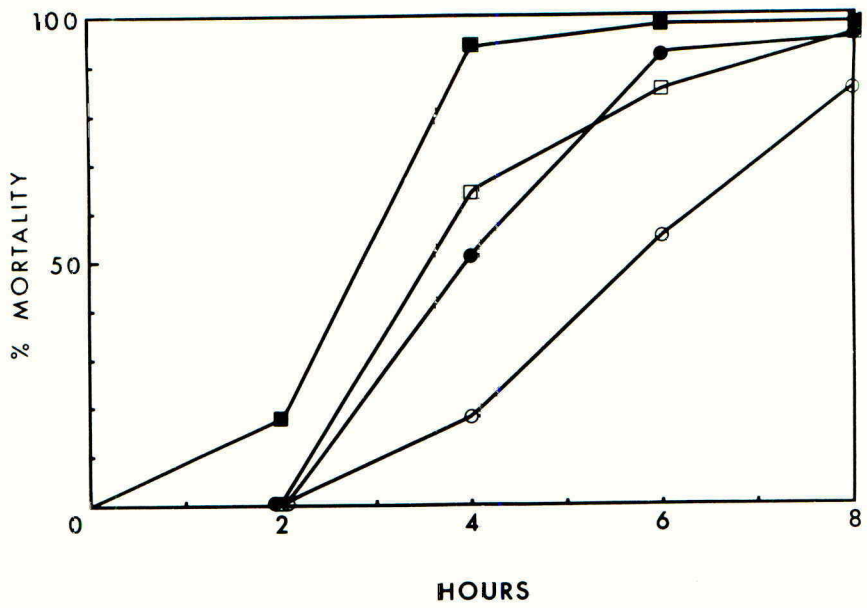

Fig. 3. Mortality of S634 T. castaneum continuously exposed in arenas fully treated with fenitrothion EC or WP at 500 mg/m² or 250 mg/m² a.i.
500 mg/m² WP ■ 250 mg/m² WP □ 500 mg/m² EC ● 250 mg/m² EC ○

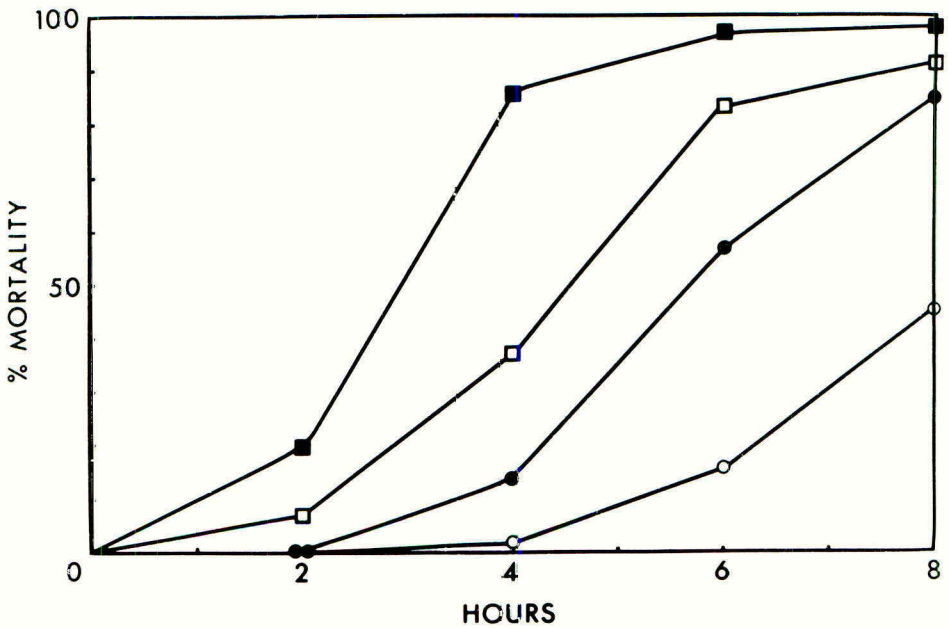

Fig. 4. Mortality of S634 T. castaneum continuously exposed in arenas fully treated with pirimiphos-methyl EC or WP at 500 mg/m² or 250 mg/m² a.i.
500 mg/m² WP ■ 250 mg/m² WP □ 500 mg/m² EC ● 250 mg/m² ○

WP was at least as effective as the 500 mg/m² EC in both tests. This evidence, in conjunction with other published work, demonstrated that WP formulations were inherently more effective insecticides when applied to filter papers.

Blank Formulations

The reversal of formulation efficacy in half-treated arenas could have resulted from the WP formulations being more repellent than their equivalent EC, allowing some insects to avoid lethal contact in the 'choice' arenas. Thus the repellency of the two formulations of pirimiphos-methyl was assessed in arenas half-treated with 'blank' formulations identical to the insecticide formulations but lacking the active ingredient. In addition, untreated controls were set up and marked arbitrarily as having one side 'treated'. The results are shown in Figs. 5, 6 and 7. The repellency of the WP blank (Fig.5) is significant at $P<0.001$ over the entire test period; for example, on day one only 17 beetles out of a total of 240 beetles were observed on the treated halves of the arenas. A theoretical distribution of a total of 120 beetles on either half of the treated arenas was the null hypothesis given that the treated halves of the filter paper were not repellent. The deviation from this distribution under chi² analysis based on a ratio of segregation into two categories, predicted the upper and lower limits of numbers of beetles to be found on either side of the arenas, (e.g. 146 and 94 respectively for a sample of 240 beetles). For ease of visual assessment the area within which the segregation would fall 999/1,000 times, given no repellency, was represented by the hatched area in the middle of the graphs. The central hatched area was adjusted downwards as appropriate to account for any mortality within the arenas. If the number of insects on the untreated half was above the hatched area then the formulation was repellent. If the number of insects on the treated half was above the hatched area then the formulation could be considered attractant. In the control arena (Fig.7) neither half of the arenas was attractant or repellent. The EC blank formulation (Fig.6) was not significantly repellent over the first two days of test but became more repellent towards the end of the test.

Choice Between WP Blank Formulations Concentrations

Having observed a strong repellency of pirimiphos-methyl WP blank the test was repeated with fenitrothion WP blank applied at a range of dosages and, in some tests, at different rates to two halves of the same arena. Tests of the following dosages of fenitrothion active ingredient (mg/m²), in terms of the WP blank equivalent only, were carried out - 0/250; 0/500; 0/1,000; 250/500; 500/1,000. The results of the 0/500; 250/500 and 500/1,000 tests are represented in Figs 8, 9 and 10. In arenas where one half was untreated, all applied dosages of WP blank were significantly repellent and repellency increased with increasing dose of WP blank carrier. In the 250/500 and 500/1,000 treated arenas a significant number of beetles were present on the lower treated half over at least the first two days of test; for example, at day one in the 500/1,000 treated arenas 222 out of 240 beetles were observed on the 500 dosed halves - despite other tests confirming that this dose itself was highly repellent. Control mortality was greater on the fenitrothion WP blank than it had been on the pirimiphos-methyl WP blank and was particularly obvious in tests where both sides of each arena had been treated. At the end of 7 days the mortality in the 500/1,000 arenas was 72%. The toxicity of wettable powder carriers is considered in more detail by Wildey (1984).

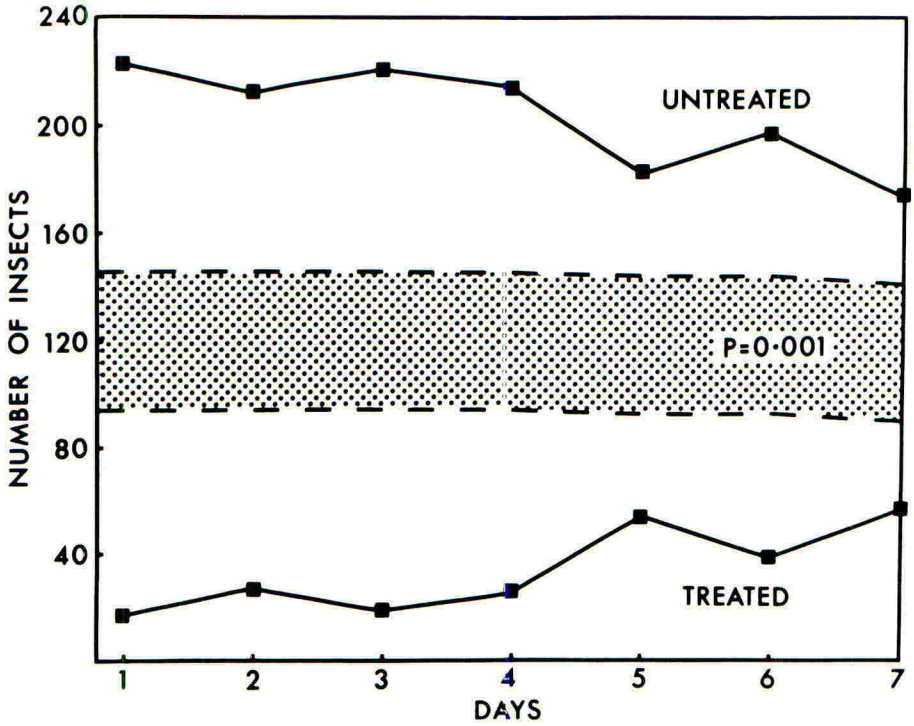

Fig. 5. Distribution of S634 T. castaneum on treated and untreated halves of arenas half treated with pirimiphos-methyl wettable powder blank at 500 mg/m².

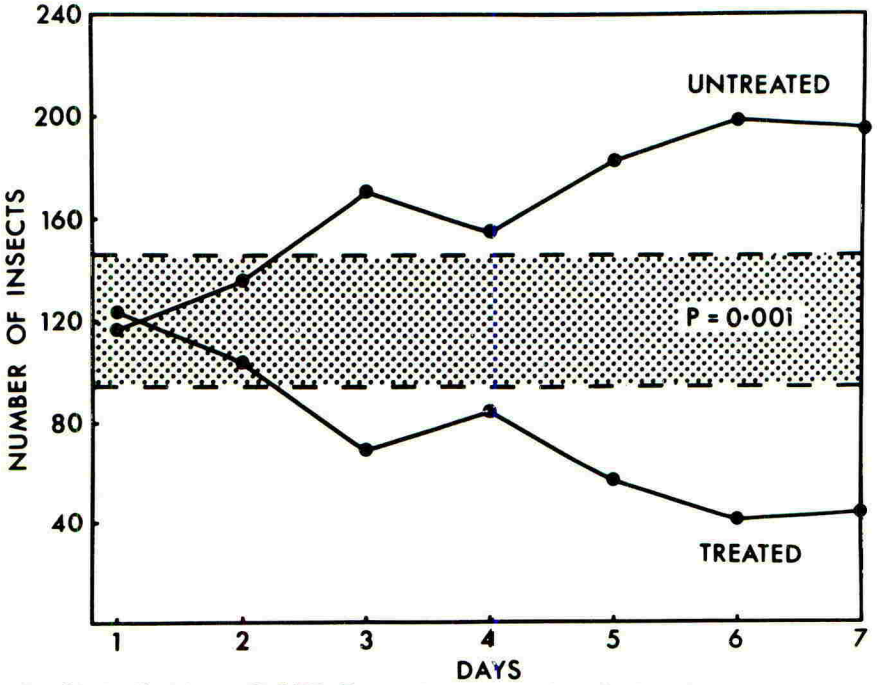

Fig. 6. Distribution of S634 T. castaneum on treated and untreated halves of arenas half treated with pirimiphos-methyl emulsifiable blank at 500 mg/m².

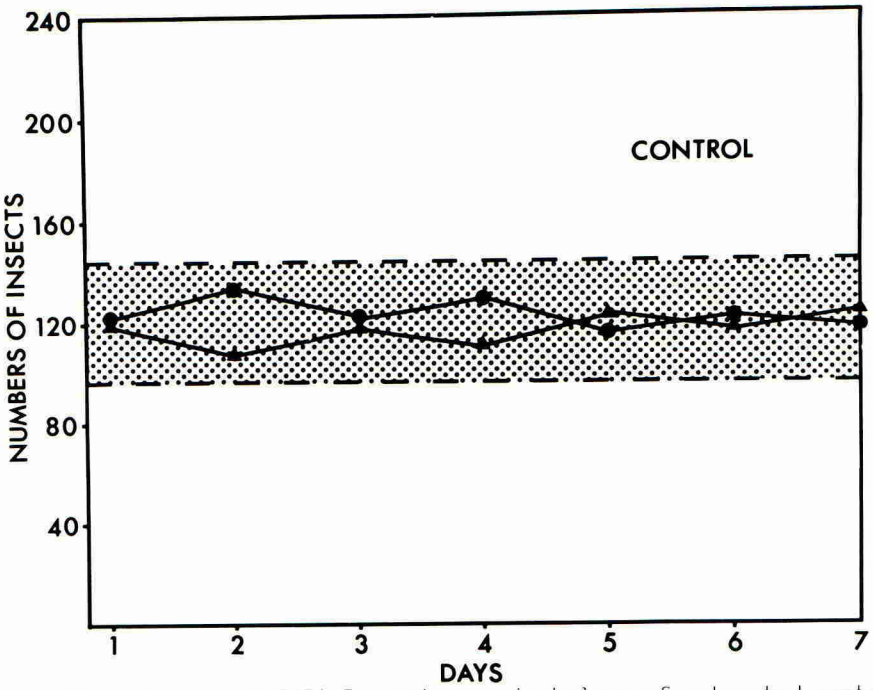

Fig. 7 Distribution of S634 I. castaneum in halves of untreated control arenas.

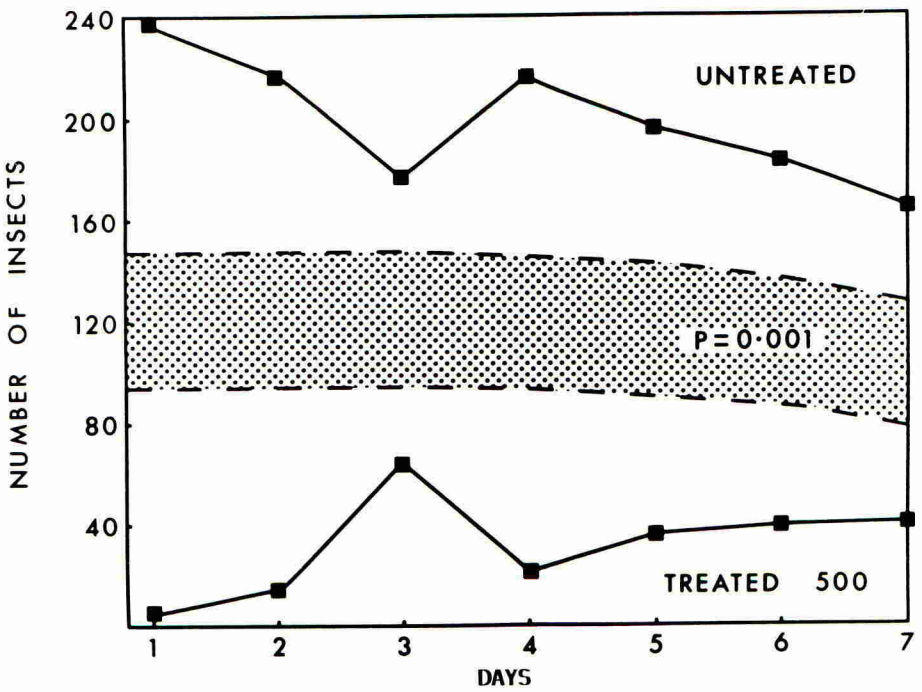

Fig. 8 Distribution of S634 I. castaneum in half treated arenas with 500 mg/m² fenitrothion wettable powder blank on the treated arena.

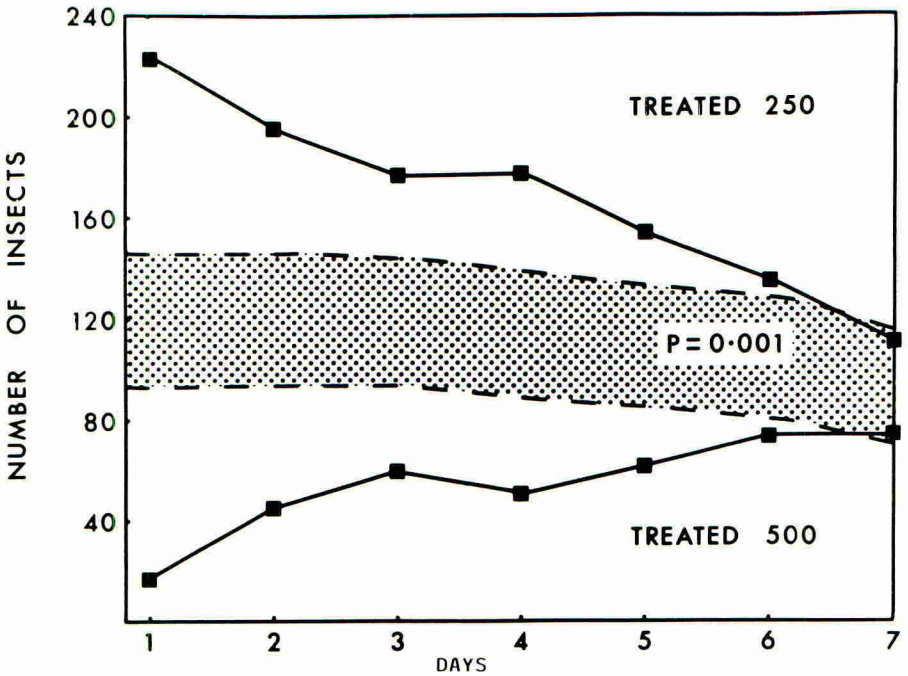

Fig. 9. Distribution of S634 I. castaneum in arenas with 250 mg/m² and 500 mg/m² of fenitrothion wettable powder blank on each half of the arena.

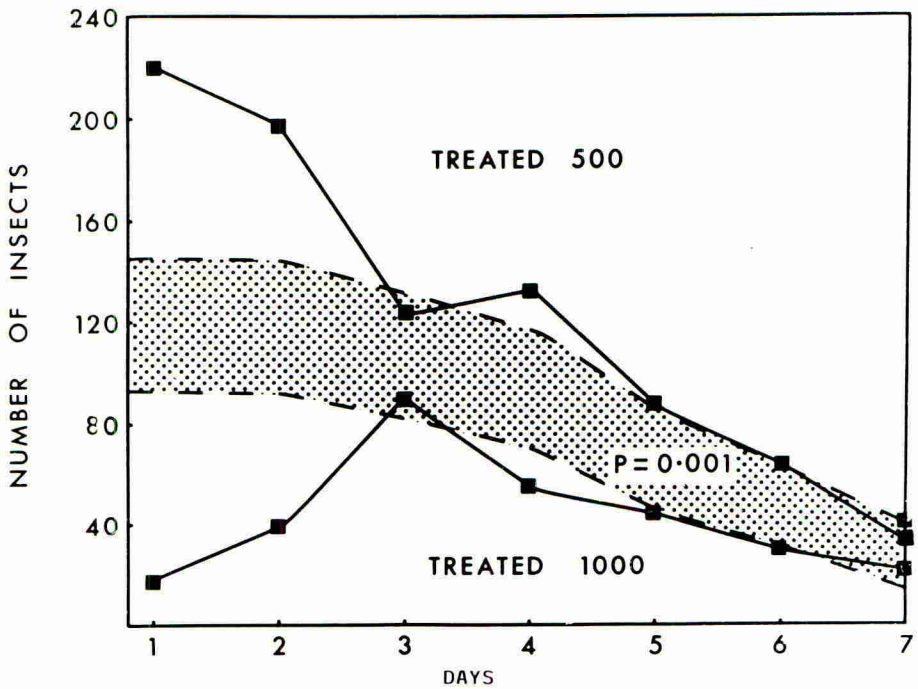

Fig. 10. Distribution of S634 I. castaneum in arenas treated with 500 mg/m² and 1,000 mg/m² of fenitrothion wettable powder blank on each half of the arena.

DISCUSSION

The reversal of WP formulation superiority over EC formulation when insects were tested in half-treated arenas would appear due to the strongly repellent effect of WP formulant carriers. Since repellency was shown to be carrier dose dependent, WP formulations with a higher concentration of active ingredient could be less repellent, dose for dose, than those containing lower concentrations of active ingredient, since the former contain less carrier per unit a.i. In situations where complete treatment of all surfaces was not possible and insects therefore had a chance to avoid contact with the treated surface, insect survival could be greater. Obviously, many factors must be taken into consideration when designing a WP formulation to promote formulation stability and persistence on treated surfaces and the effects of reducing carrier content in the formulation on other aspects of performance would have to be carefully monitored. The ability of insects to differentiate between higher and lower dosed WP blank treated areas would suggest that in practical situations they could differentiate between actual higher and lower dosed insecticide treated areas. This would also tend to lead to increased survival in storage premises.

In the constant exposure (no-choice) arena tests reported, 100% mortality on field doses of two storage insecticides was noted after 8 hours exposure. In half-treated arenas up to 44% of the test insects survived for 7 days when contact with the WP treated surface could be avoided. The indication of escape-by-choice would reinforce the ability of these resistant beetles to survive for long periods in insecticidally treated environments. Indeed, the level of insecticide resistance may well determine their ability to escape - in addition to the influence of the degree of repellency of the formulation. In any conditioned avoidance response, the time of exposure to the repellent stimulus could be greater for insecticide resistant insects than for susceptible insects, thus increasing their likelihood of escape.

Future part-treated arena studies of newer formulations and individual components of formulations may improve stored product control recommendations.

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NEW APPROACHES TO THE PROTECTION OF STORED PRODUCTS USING AN INSECT JUVENILE HORMONE ANALOGUE

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ABSTRACT

The potential of the insect juvenile hormone analogue fenoxycarb to protect wheat against 4 major beetle pests of stored grain has been investigated. Tests were carried out under semi-practical conditions, whereby treated grain was periodically infested with known small populations of Tribolium castaneum, Oryzaephilus surinamensis, Rhyzopertha dominica and Sitophilus granarius. Following treatment and the first introduction of insects, grain samples were examined at three month intervals for the presence of insects, the quality of the grain and the levels of pesticide remaining in the grain. The efficacy of fenoxycarb was assessed by comparison with results obtained with untreated grain and with grain treated with a conventional insecticide (chlorpyrifos-methyl). Results obtained after one year of storage indicate that fenoxycarb has considerable potential as a practical protectant of stored products.

INTRODUCTION

The presence of insect infestation in stored products has always posed unique problems. This is because, although such infestation can cause serious damage to the infested commodity, preventative measures utilizing conventional insecticides can result in the presence of chemical residues (Wilkin and Fishwick, 1981) in foodstuffs that are frequently destined for human consumption. In addition, there is evidence of an increasing incidence of resistance to conventional insecticides among the major insect pests of stored products (Dyte, 1974; Champ and Dyte, 1976;Champ, 1986). Recently, these problems have been exacerbated by increases in the total quantity of grain stored in the U.K., the size of bulks in individual stores, and in the length of the storage period. These changes in grain storage practice have been accompanied by the introduction of regulations requiring the complete absence of insect pests from grain destined for some export markets or for intervention storage. In practice, these standards are very difficult to achieve and maintain. Even insect-free grain can become infested from small residual populations present in vehicles or grain stores. Initially, such low levels of infestation (which may be below the levels of detection) rarely cause serious damage or significant economic loss. However, if such populations are able to increase unabated, serious damage can occur in a relatively short time.

All of these factors have combined to underline the urgent need to develop new methods for the protection of stored products against infestation by insects and mites.

Insect juvenile hormone analogues produce lethal disruption of insect development, and in some species, reduce the reproductive capacity of

adults. However, such compounds generally have negligible toxicity to vertebrates, and disrupt hormonal-based systems only in insects and other arthropods. For this reason, they have considerable potential as insect control agents especially in stored products. This potential was recognised and investigated more than 10 years ago (Strong and Diekman, 1973) but as yet the practical use of these compounds in storage pest control has been limited by a number of factors. In particular, a major problem has been the insensitivity of beetles of the genus Sitophilus (grain weevils) to the effects of hormone analogues. This problem, together with the poor stability of some juvenile hormone analogues, has been paramount in hindering the otherwise valuable contribution that such compounds could make to the protection of stored products (Edwards and Menn, 1981). Recently however, a new compound (fenoxycarb) has been shown in laboratory tests to provide effective protection against Sitophilus spp. at levels between 5 and 10 mg/kg in grain (Dorn et al., 1981; Kramer et al., 1981; Edwards and Short, 1984). Moreover, this compound is highly active against all other major coleopteran pests of stored products (Kramer et al., 1981; Thind and Edwards, 1986). These encouraging laboratory studies led us to examine the effectiveness of fenoxycarb as a grain protectant under semi-practical conditions, and in this paper we present some preliminary results from these large-scale experiments.

MATERIALS AND METHODS

The insects used were from standard Laboratory strains of Tribolium castaneum, Oryzaephilus surinamensis, Rhyzopertha dominica and Sitophilus granarius. With the exception of T. castaneum (where we used the CTC12 multi-resistant strain, Champ and Campbell-Brown, 1970) all other insects were insecticide-susceptible strains.

English milling wheat was treated with fenoxycarb emulsifiable concentrate (125g a.i./l) or with chlorpyrifos-methyl (cpm) emulsifiable concentrate (500g a.i./l) by spraying moving grain as it was augered from the holding bin into 1 tonne capacity polypropylene bins. For both treatments, we used a N.F.S. Ltd. model FTS 50 pressure sprayer equipped with a hollow-cone nozzle (type 60/8). The sprayer was calibrated to deliver 1.5l/tonne at a grain flow-rate of 22 tonnes/hour). Grain was nominally treated with either 5.0mg/kg fenoxycarb or with 4.5mg/kg cpm. Actual (mean) dosages achieved were 4.8mg/kg fenoxycarb and 3.9mg/kg cpm. Four replicate bins were each loaded with approximately 2/3 tonne grain treated with either fenoxycarb or with cpm, and 4 bins were loaded with untreated grain.

The top inside edge of each bin was painted with a double band of 'Fluon', and covered with a ventilated polypropylene lid. Immediately after treatment, and again after 6 months, each bin was 'seeded' with 300 adults of each of the 4 species described above. Following treatment, and subsequently, at three-month intervals, two 1kg samples of grain were removed from each bin with a suction sampler and examined for the presence of insects, levels of cpm or fenoxycarb present, and for the quality of grain (see below). Ambient air temperature (which was nominally maintained at 25°C by thermostatically operated heaters), and the temperature of grain in each bin were recorded continuously by thermocouples linked to a data recording system. No attempt was made to control ambient humidity. At each 3-month assessment point, the following

parameters were measured:

Insect populations

Insect infestation levels were estimated by sieving the grain and counting the numbers of adult insects of each species that were removed from the grain samples.

Grain and bread quality

Grain quality was assessed by measurement of moisture content, germination rate, percentage damaged grains (number of damaged grains apparent on visual inspection of 100 randomly-selected grains) and weight loss after sieving a known weight of grain over a 1mm mesh (aperture) sieve. Moisture content was measured using the British Standards Institute method BS 4317 part 3 (1980) and germination rate was determined using the procedures described by Pixton et al. (1964). Bread making quality (loaf score, specific grain weight and flour yield) was assessed by the Flour Milling and Baking Research Association (F.M.B.R.A) Chorleywood, Herts, using standard procedures.

Chemical analysis

Residues of fenoxycarb and chlorpyrifos-methyl were measured in the grain samples taken from each bin. Chlorpyrifos-methyl was analysed by gas chromatography (flame photometric detector equipped with phosphorus filter) of methanol extracts of grain (Anon, 1980). Fenoxycarb residues were analysed by reverse-phase HPLC (Waters Radial-Pak (105mm, 5mm i.d.) C-18 disposable column; U.V. detector wavelength 228nm; mobile phase - 0.05M aqueous KH_2PO_4/acetonitrile, 1:1). Hexane extracts of grain were partially purified by silica gel column chromatography (solvent - 3% ethyl acetate in toluene) before HPLC.

RESULTS

Insect populations

Insect populations in the control (untreated) bins increased during the first 9 months of the experiment (Table 1) after which time the populations of insects were so high, and the grain so badly damaged, that the grain in these bins was discarded. The high level of insect activity in the control bins was reflected by temperature rises in these bins. The average monthly temperatures in control bins were consistently more than 5°C above those in treated bins, and reached a maximun of 33.8°C after 8 months. Temperatures in both cpm-treated and fenoxycarb-treated grain, remained close to or lower than ambient throughout the period, and never exceeded ambient by more than 2.5°C. Populations of O. surinamensis (68 adult insects per kg), R. dominica (843 adults per kg) and S. granarius (75 adults per kg) were highest at the 6-month assessment point and declined thereafter (Table 1). In particular, the numbers of S. granarius dropped markedly between 6 and 9 months. By contrast, the population of T. castaneum continued to increase upto the 9-month assessment point (Table 1).

In the bins containing grain treated with cpm, insect populations were generally low (i.e. less than 1 adult per kilogramme) during the first 6 months of the trial (Table 1). At the 6-month assessment point, neither S. granarius nor T. castaneum were detected in the cpm-treated bins, although small populations of both O. surinamensis and R. dominica were present (Table 1). Between 6 and 12 months, populations of R.

TABLE 1.

Effect of fenoxycarb or chlorpyrifos-methyl (cpm) treatment on insect numbers (adult insects/kg) in stored grain.

Species	Treatment	initial population	TIME AFTER TREATMENT (MONTHS) 3	6	9	12
	control	0.5	7.3	97.8	120.8	-[a]
Tribolium castaneum	cpm	0.5	0.0	0.0	0.7	0.88
	fenoxycarb	0.5	0.7	0.9	1.7	0.99
	control	0.5	24.2	843.0	646.0	-
Rhyzopertha dominica	cpm	0.5	0.0	1.3	0.7	0.0
	fenoxycarb	0.5	0.3	0.0	0.1	0.0
	control	0.5	13.2	68.1	22.5	-
Oryzaephilus surinamensis	cpm	0.5	0.0	0.9	4.9	49.1
	fenoxycarb	0.5	0.0	0.0	0.5	0.1
	control	0.5	21.7	75.5	1.5	-
Sitophilus granarius	cpm	0.5	0.0	0.0	0.0	0.0
	fenoxycarb	0.5	2.4	7.5	3.9	10.1

[a] Control grain discarded after 9 months.

dominica declined, and this species was not detected at the 12 month assessment point. Sitophilus granarius was not found in cpm-treated grain throughout the trial period. By contrast, the populations of T. castaneum and O. surinamensis increased - the latter reaching about 50 adults per kg after 12 months (Table 1).

In the bins containing fenoxycarb-treated grain, insect populations also remained generally low during the first 6 months (Table 1). At the 6-month assessment point, both R. dominica and O. surinamensis were not found, and the T. castaneum population remained low. By contrast, the population of S. granarius showed some increase (Table 1). Between 6 and 12 months, fenoxycarb treatment continued to prevent the development of populations of R. dominica and O. surinamensis. During this period, the population of T. castaneum remained relatively constant at about 1 adult insect per kg. However, there was a continued increase in the population of S. granarius which reached 10.1 adults per kg after 12 months.

Grain and bread quality

Grain quality, as indicated by several parameters (Table 2) deteriorated with time and with increasing levels of insect infestation. The untreated grain was severely damaged by 9 months (Table 2) and subsequently was discarded. This deterioration of untreated grain was also reflected by the progressive loss of milling and baking quality (Table 3).

TABLE 2.

Effect of treatment with fenoxycarb or chlorpyrifos-methyl (cpm) on various aspects of grain quality in insect-infested wheat.

Grain quality factor	Treatment	initial quality	TIME AFTER TREATMENT (MONTHS) 3	6	9	12
% weight loss (sieved)	control	0.0	0.3	6.3	10.4	-[a]
	cpm	0.01	0.1	0.32	0.2	0.55
	fenoxycarb	0.02	0.1	0.32	0.3	0.22
% damaged grain (visual)	control	8.7	10.2	29.2	39.2	-
	cpm	7.2	10.2	11.0	9.0	11.2
	fenoxycarb	7.7	11.2	11.2	10.2	11.5
Moisture content (%)	control	15.9	15.3	14.6	14.5	-
	cpm	16.3	15.2	14.8	14.8	14.5
	fenoxycarb	16.1	15.0	15.0	15.1	14.6
Germination rate (%)	control	88.7	82.0	12.2	2.0	-
	cpm	92.5	86.5	52.0	34.7	28.7
	fenoxycarb	89.0	82.0	48.5	28.5	26.2

[a] Control grain discarded after 9 months.

Treatment of grain with cpm or with fenoxycarb prevented deterioration in grain condition (Table 2) and in milling and baking quality (Table 3). During the 12 months of storage, there were no marked differences in the performance of the two compounds in terms of maintaining grain quality.

Chemical analysis

Table 4 shows the average levels of fenoxycarb or cpm found in grain samples removed from the bins. After 12 months, only about 15% (0.5mg/kg) of the applied dose of cpm was detectable on grain samples. By contrast, about 70% (3.3mg/kg) of the applied dose of fenoxycarb was detectable after the same period of storage.

DISCUSSION

These experiments were designed to evaluate the potential of the insect juvenile hormone analogue fenoxycarb as a grain protectant under conditions reflecting those that could be found in practice. When grain is stored it is at risk from any infestation present within the bulk, from infestation present in the fabric of the store and from infestation brought in with fresh bulks of grain. To simulate such periodic introduction of small populations we artificially infested the experimental grain at 6-monthly intervals. The numbers of insects of each species

TABLE 3.

Effect of treatment with fenoxycarb or chlorpyrifos-methyl (cpm) on the milling and baking quality of insect-infested wheat.

Milling & baking quality	Treatment	Intitial quality	TIME AFTER TREATMENT (MONTHS) 3	6	9	12
Specific weight (kg/hl)	control	70.1	74.8	71.9	66.3	-[a]
	cpm	73.1	75.0	73.2	71.1	71.1
	fenoxycarb	72.7	74.8	73.2	70.4	70.6
Flour yield (%)	control	70.9	72.6	75.2	72.8	-
	cpm	71.6	72.8	72.7	72.1	75.2
	fenoxycarb	71.1	71.8	73.6	71.7	75.4
Loaf score	control	38	30	22	10	-
	cpm	36	32	29	25	29
	fenoxycarb	35	32	34	25	30

[a] Control grain discarded after 9 months

introduced were equivalent to about half the estimated minimum level that could be detected by conventional spearing and sieving techniques (i.e. approximately 1 insect per kg of grain; D.R. Wilkin, pers. commun.). However, when considering the performance of the two compounds, it should be remembered that this level of infestation represents a rather severe test in relation to what might be expected in practice. In addition, the two compounds we have used (fenoxycarb and chlorpyrifos-methyl) have completely different modes of action, so that any conclusions about their comparative effectiveness must be made only in relation to the specific conditions present in these tests. For example, it is likely that cpm killed all insects introduced immediately after treatment, and that the cpm-treated grain was not challenged by insect pests until the next artificial introduction at 6 months. By contrast, fenoxycarb has no direct toxic action at the dose applied, and therefore, fenoxycarb-treated grain was continually challenged by insects from the point of initial introduction until natural mortality had accounted for all introduced adults. This was certainly true in the case of S. granarius, T. castaneum and O. surinamensis - all of which have average adult longevities in excess of 6 months. (Surtees, 1964; Howe, 1956; Back and Cotton, 1926).

As expected, the populations of all 4 species showed a general increase in untreated grain during the first 6 months of storage. Subsequently, although the numbers of T. castaneum continued to increase, the numbers of the other 3 species declined. The reduction in the number of adult insects per kg between 6 and 9 months was about 24% for R. dominica, 67% for O. surinamensis, and 98% for S. granarius. Some of these changes in population levels could well be due to the normal varia-

TABLE 4.

Average residues (mg/kg) of fenoxycarb and chlorpyrifos-methyl in grain samples.

Commodity	Treatment	Initial residues	TIME AFTER TREATMENT (MONTHS) 3	6	9	12
Grain in bins	control	0.0[a]	0.0	0.0	0.0	-[b]
	cpm	3.9	2.3	1.1	0.7	0.5
	fenoxycarb	4.8	4.1	3.7	3.4	3.3

[a] Figures given as 0.0 indicate residues below levels of detection.
[b] Control grain discarded after 9 months

bility inherent in our sampling methods, and this could probably account for the relatively small apparent reduction in numbers of R. dominica. However, such variability could not explain the much larger reductions in numbers of S. granarius and O. surinamensis. In both cases, increases in the temperature of the grain in untreated bins (which occasionally reached 35.5°C during the period spanning 6-9 months) could well have reduced survival and development in both species, especially S. granarius which is less tolerant of temperatures above 30°C than the other species (Howe, 1965). In addition, S. granarius requires relatively undamaged grain in which to develop and, by 9 months, nearly 40% of the grain in untreated bins was found to be damaged.

Treatment of the grain with either cpm or with fenoxycarb generally gave a good degree of protection against the insects upto the 9-month assessment point. Subsequently, cpm showed slightly less efficacy against O. surinamensis - a species apparently less susceptible to this compound than the others (T.J. Binns pers. commun.). Similarly, fenoxycarb was least effective against S. granarius, reflecting the fact that, of the 4 species, this is the least sensitive to this compound (Thind and Edwards, 1986). In the grain treated with fenoxycarb, small numbers of T. castaneum were present throughout the storage period. It is highly unlikely that these were the progeny of the artificially introduced adults since levels of fenoxycarb as low as 0.1 mg/kg are sufficient to prevent development (Thind and Edwards, 1986). Since the average longevity of adult T. castaneum is greater than 6 months at 25°C (Howe, 1956) some of the T. castaneum will be those deliberately introduced. However, the high temperatures (>30°C) in the control bins led to small numbers of T. castaneum escaping from the bins by flying to the gauze-covered ventilation hole and crawling through. It is likely that some of these will have been attracted to, or found their way into, the treated bins. If such movement of T. castaneum also occurred between the control bins and those treated with cpm, then the early (3 to 6 months) absence of live T. castaneum in these bins suggests that the compound was present at sufficiently high concentration to kill them as they were introduced. Similarly, the results obtained at 9 and 12 months suggest that levels of cpm were declining to below the levels required to kill all T. castaneum adults. Some evidence for this comes from the chemical analyses which show that, at 12 months, cpm levels in treated grain were approximately

0.5mg/kg - a concentration that would probably be too low to be completely effective against T. castaneum (CTC 12 strain) on wheat (T.J. Binns, pers. commun.).

Chemical analysis of the treated grain samples showed that fenoxycarb was considerably more persistent than cpm. This was in accord with the results of previous laboratory tests (Edwards and Short, 1984) in which there was no loss of biological activity of fenoxycarb applied to grain which was stored at 25°C for a period of 2 years. However, any further marked decrease in the levels of fenoxycarb in the grain would probably result in a noticeable increase in numbers of S. granarius. Analysis of cpm-treated grain showed a steady loss of insecticide upto 12 months, by which time only 0.5mg/kg remained. This level is probably only sufficient to maintain control of S. granarius, should the grain be stored for longer periods. In previous studies (LaHue, 1977) S. granarius was shown to be more susceptible to cpm than both T. castaneum and R. dominica.

In conclusion, both compounds gave a good degree of protection during the 12 month storage period. However, the greater persistence of fenoxycarb suggests that this compound could give protection (at least against T. castaneum, R. dominica and O. surinamensis) for much longer than one year. In addition, the low vertebrate toxicity and insect-specific action of fenoxycarb makes it an ideal candidate for application to grain and other foodstuffs. Moreover, the fact that cpm is especially effective against S. granarius, whereas fenoxycarb is particularly effective against the other 3 species, suggests that a combination of the two compounds (possibly at much lower application rates) could give an outstanding level of protection for at least 12 months. Because these preliminary results have been encouraging, we intend to extend and expand this experiment, and a full report will be published at a later date.

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