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Field trials of calciferol combined with warfarin against wild house-mice (*Mus musculus* L.)*

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SUMMARY

A combination of calciferol (vitamin D_2) and warfarin, each at 0·025 % in medium oatmeal bait, failed to control six of seven house-mouse (*Mus musculus* L.) populations infesting urban and farm buildings. In three further treatments with both calciferol and warfarin at 0·05 % in dehusked canary seed bait plus 5 % corn oil, mortality, estimated from the consumption of pre- and post-treatment census bait, ranged between 94·2 and 97·4 %. Finally, among sixteen treatments done with calciferol at 0·1 % and warfarin at 0·025 % in various cereal baits, the best results (97·0–100 %) were obtained in six treatments where the bait-base was whole canary seed; this was so whether the poison bait was applied directly or after a 3-day pre-baiting period. It is concluded that calciferol at 0·1 % plus warfarin at 0·025 % is an effective combination against house-mice, especially when used with whole canary seed. The role played by warfarin in the poison mixture needs to be investigated further.

INTRODUCTION

The difficulty that has been experienced in controlling rodent infestations in Britain during the past decade owing to the development of resistance to the anticoagulants has been referred to by Greaves, Redfern & King (1974). In an earlier study on the response of wild mice (*Mus musculus* L.) to various anticoagulants (Rowe & Redfern, 1968) attempts were made to render suspected warfarin-resistant animals more susceptible to poisoning by including either sulphaquinoxaline, SKF525-A or vitamin A acetate in 0·025 % warfarin bait. Only vitamin A acetate, in excess, was found to bring about a high mortality rate in the test animals but since its presence greatly lowered the acceptance of the warfarin bait the efficacy of the combined bait was not determined in the field.

More recent laboratory tests (Greaves *et al.* 1974) have shown that bait containing calciferol (vitamin D_2) alone and in combination with warfarin is both toxic and acceptable to wild mice, including warfarin-resistant animals. The present paper describes the results of poison treatments using calciferol and warfarin in combination, against free-living *M. musculus*. The treatments were carried out simultaneously with work on finding improved bait-bases for use against mice and this influenced the course of the tests to be described.

METHODS

The treatments were carried out in urban and rural areas but the majority were done against mice infesting farm storage buildings and animal-rearing houses. Although some of the chosen premises had histories of persistent mouse infestation, despite prolonged or frequent treatment with warfarin, no laboratory tests were carried out before the poison treatments were begun, to confirm that the mice inhabiting them were resistant to warfarin.

Three series of poison treatments were carried out. In the first (Series A), calciferol and warfarin were both included in bait at 0·025 %, the concentrations suggested for field evaluation by the manufacturers. The poison bait was prepared by thoroughly mixing nineteen parts of medium grade oatmeal with one part of a proprietary master-mix comprising 0·5 % calciferol and 0·5 % warfarin in fine oatmeal.

In the second set of treatments (Series B) the concentration of calciferol used was 0·05 % and since at this time the compound was still only available in the form of the above master-mix, the concentration of warfarin had also to be increased to 0·05 %. This is double the strength normally recommended against mice. In each of the treatments the poison bait used was prepared by mixing one part of the proprietary master-mix to nine parts of dehusked canary seed treated with 5 % corn oil.

In the third set of treatments (Series C) one part of 2 % calciferol dissolved in corn oil was mixed with eighteen parts of bait and then one part of a 0·5 % warfarin master-mix was added while stirring continuously, to give a final formulation containing 0·1 % calciferol and 0·025 % warfarin.

With one exception the effectiveness of each Series A poison treatment was assessed by comparing the amounts of poison bait eaten, the number of visits made by mice to the baiting points and the level of activity (footprints and tail-swipes) revealed by strategically placed and regularly smoothed patches of an inert dust.

Percentage success in one of the Series A treatments and in the Series B and Series C treatments was estimated from the total amounts of plain bait that were eaten by mice at pre- and post-treatment censuses. Each treatment was conducted in the following manner. On day 1 the infested area was surveyed thoroughly and the pre-treatment census begun. For this purpose a plain cereal bait, the total weight of which was known, was laid in small, wooden trays distributed a few feet apart throughout the infested area. At sites frequented by birds the trays were protected by metal covers. The total amount of census bait eaten was measured daily for 4 days or, occasionally, at the end of the 4-day census period. In either case, each baiting point was inspected daily to ensure that surplus bait was always available.

At the end of the pre-treatment census the trays and surplus bait were removed and 3 days later the poison treatment was begun. The bait-base used for poisoning and the poison baiting sites were different from those employed in the census baiting; the poison bait was maintained in excess at each site throughout the

Table 1. *Consumption of poison bait in treatments against mice, using* 0·025 % *calciferol and* 0·025 % *warfarin in various bait-bases* (*Series A*)

Treatment no.	Days	Bait-bases	Consumption of poison bait (g.)
1	1–7	Medium oatmeal	111
	8–15	Medium oatmeal	19
2	1–7	Medium oatmeal	144
	8–15	Medium oatmeal	76
3	1–7	Medium oatmeal	111
	8–14	Medium oatmeal	37
	15–25	Medium oatmeal	28
	26–35	Pinhead oatmeal/corn oil/sugar	31
4	1–11	Medium oatmeal	39
	12–19	Dehusked canary seed/corn oil	18
5*a*	1–7	Medium oatmeal	195
	8–14	Medium oatmeal	106
Interval	15–18	Census baiting (plain)	
	19–21	No bait	
	22–25	Choice of plain baits	
5*b*	26–32	Wheat/porridge oats/dehusked canary seed/corn oil/sugar	756
	33–39	Wheat/porridge oats/dehusked canary seed/corn oil/sugar	336
	40–50	Wheat/porridge oats/dehusked canary seed/corn oil/sugar	409
6	1–7	Medium oatmeal	409
	8–14	Medium oatmeal	186
	15–46	Medium oatmeal	368
7	1–7	Medium oatmeal	638
	8–14	Medium oatmeal	96
	15–46	Medium oatmeal	210

treatment period and the total amount eaten was measured daily during the early stages of the treatment and then at less frequent intervals. At each visit the number of baiting points visited by mice was recorded and the dust patches were inspected for mouse signs. The treatment was terminated either when the poison bait was no longer being eaten or visited and the dust patches were undisturbed or when, after the first 7 days of the treatment, there was a regular, albeit small, take of poison bait over several days but little or no decline in activity in the dust patches.

At the end of the treatment period the poison bait and trays were removed and 3 days later, a post-treatment census, conducted in the same manner as the pre-treatment census, was begun.

RESULTS AND DISCUSSION

Series A treatments

Table 1 gives the amount of poison bait consumed during the seven treatments. In each case the greatest amount of medium oatmeal poison bait was eaten on

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either day 2 or day 3 and, as Table 1 shows, there was a marked decline in consumption after day 7. In Treatments 1 and 2 however mice were active in the dust patches throughout the 15 days that poison bait was laid. This was also the case in Treatment 3, which was continued for 25 days. Here the dust patches were also crossed daily during a second 10-day poison treatment when pinhead oatmeal plus 5 % corn oil and 5 % sugar was used as bait. Inadequate control also resulted in Treatment 4 in which poisoned medium oatmeal was laid for 11 days. No poison bait was eaten after day 7 and a second treatment was carried out using bait comprising dehusked canary seed plus 5 % corn oil. More poison bait was consumed as a result but some mice still survived.

Treatment 5 was conducted in two parts. The take of treated medium oatmeal bait (301 g.) in an initial 14-day treatment period (5*a*) seemed low judging from the abundant signs of mice that were evident at the survey and this was confirmed by the amount of whole canary seed eaten (462 g.) in a 4-day post-treatment census baiting. After an interval of 3 days the mice were given a choice of three different plain baits over a 4-day period. A second 25-day poison treatment (5*b*) was then done using the most preferred bait (a mixture of equal amounts of whole wheat, porridge oats and dehusked canary seed plus corn oil and sugar, each at 5 %) as the carrier for the two poisons. The take of this bait during the first 14 days (1092 g.) was considerably greater than in the same period of the first treatment when medium oatmeal was used. However, although a further 409 g. of the mixed bait were eaten during the remaining 11 days of the treatment and 20 dead mice were found, the dust patches indicated that a considerable number of animals had survived. In a post-treatment census baiting 216 g. of whole canary seed were eaten in 4 days and the estimated kill in the second treatment therefore was only 53·2 %.

In Treatments 6 and 7 medium oatmeal poison bait was laid unchanged for 46 days. After day 2 in Treatment 6 the amount of poison bait eaten, the numbers of baiting points visited and activity in the dust patches all decreased as the treatment progressed. In census baitings carried out before and after the treatment, 252 g. and 6 g. of bait were eaten respectively (estimated treatment success 97·6 %). In Treatment 7 most poison bait was also eaten during the first 2 days and thereafter feeding occurred at a much lower level. Twenty-nine dead mice were found, the first on day 2, but despite the low consumption of poison bait at the end of the treatment period, the heavy traffic in the dust patches indicated a residual population of some size. The amount of canary seed eaten (291 g. in 4 days) in a post-treatment census baiting confirmed this conclusion.

Series B treatments

The seven treatments in Series A indicated both that the concentrations of calciferol and/or warfarin employed (0·025 %) were too low and that medium oatmeal was insufficiently attractive. Treatments 4 and 5*a* particularly emphasized the latter point. Effective control was obtained in Treatment 6 where limited alternative food was available, but even here the treatment lasted 46 days. In the three treatments comprising Series B therefore, the calciferol concentration

Table 2. *Consumption of poison bait in treatments against mice using* 0·05 % *calciferol and* 0·05 % *warfarin in dehusked canary seed/corn oil bait* (*Series B*)

Treatment no.	Pre-treatment census: consumption of pin-head oatmeal (g.)	Consumption of poison bait		Post-treatment census: consumption of pin-head oatmeal (g.)	Esti-mated success (%)
		Days	Quantity (g.)		
8	929	1–7	397	24	97·4
		8–14	77		
		15–17	8		
9	104	1–7	42	6	94·2
		8–14	10		
		15–18	1		
10	1246	1–7	367	48	96·1
		8–14	78		
		15–21	98		
		22–25	48		

was increased to 0·05 % and, for reasons explained above, so was the concentration of the warfarin. At the same time the bait-base was changed to dehusked canary seed, which laboratory tests had shown to be preferred to medium oatmeal.

The results of the three treatments in Series B are given in Table 2. Most poison bait was eaten during the first 2 days and, as also in the Series A treatments, consumption fell markedly after 7 days. Contrasting with all but Treatment 6 in Series A, however, the fall in consumption and number of visits to the baiting points that occurred as the treatments progressed was paralleled by a decline in activity in the dust patches. Even so, Column 6 of Table 2 shows that individuals survived each of the treatments, which were fairly prolonged (17–25 days).

In the next series of trials, therefore, it was decided to increase the concentration of calciferol further, to 0·1 %. The advisability of this was confirmed by results obtained later by Greaves *et al.* (1974) in the laboratory, and because a master-mix of calciferol separately in corn oil was now available, it was possible to include warfarin at 0·025 %.

Series C treatments

The first three treatments of Series C (11, 12 and 13 in Table 3) were again done with dehusked canary seed as the bait-base and estimated control figures of 91·0, 91·8 and 91·5 % were obtained. In the next five (14–18), the bait-base used was pinhead oatmeal, which also had shown up reasonably well in laboratory palatability tests. As in previous treatments, most poison bait was eaten during the first 7 days, but, as Table 3 shows, success varied from 29·6 to 91·6 %. Two further tests with pinhead oatmeal as the bait-base gave poor results over 17 days of feeding (Treatments 19*a* and 20*a*): but when, in 17-day follow-up treatments at the same sites, dehusked canary seed in one case (19*b*) and dehusked canary seed and mixed cereal in the other (20*b*), were substituted for the oatmeal, good control was then obtained (97·0 and 97·8 %, respectively). Unfortunately wet weather prevented meaningful monitoring of the course of feeding.

The final six treatments in Series C (Table 4) were done with whole canary seed

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Table 3. *Consumption of poison bait in treatments against mice using* 0·1 % *calciferol and* 0·025 % *warfarin in various bait-bases* (*Series C*)

Treatment no.	Census bait	Consumption of pre-treatment census bait (g.)	Bait-base	Consumption of poison bait		Consumption of post-treatment census bait (g.)	Estimated success (%)
				Days	Quantity (g.)		
11	Pinhead oatmeal	2122	Dehusked canary seed	1–7	1072	192	91·0
				8–18	234		
12	Pinhead oatmeal	1366	Dehusked canary seed	1–7	595	112	91·8
				8–18	54		
13	Pinhead oatmeal	189	Dehusked canary seed	1–7	86	16	91·5
				8–21	79		
				22–27	25		
14	Whole canary seed	52	Pinhead oatmeal	1–7	28	10	80·8
				8–12	16		
15	Whole canary seed	155	Pinhead oatmeal	1–11	*	26	91·6
16	Whole canary seed	89	Pinhead oatmeal	1–7	17	27	69·7
				8–12	2		
17	Whole canary seed	26	Pinhead oatmeal	1	12	9	64·4
				2–12	*		
18	Whole canary seed	162	Pinhead oatmeal	1–7	50	115	29·6
				8–17	19		
19*a*	Whole canary seed	392	Pinhead oatmeal	1–7	76	280	28·6
				8–17	*		
19*b*	Whole canary seed	280	Dehusked canary seed	1–17	*	4†	97·0
20*a*	Whole canary seed	617	Pinhead oatmeal	1–7	87	389	37·0
				8–17	*		
20*b*	Whole canary seed	389	Wheat/porridge oats/dehusked canary seed	1–17	*	3†	97·8

* Unrecordable. † Porridge oats.

Table 4. *Consumption of poison bait in treatments against mice using* 0·1 % *calciferol and* 0·025 % *warfarin in whole canary seed bait (Series C)*

Treatment no.	Census bait	Consumption of pre-treatment census bait (g.)	Consumption of poison bait (g.)		Consumption of post-treatment census bait (g.)	Estimated success (%)
			Days	Quantity (g.)		
21	Pinhead oatmeal	670	1–7	1135	25	97·0*
			8–14	607		
			15–21	765		
			22–28	56		
			29	19		
22	Pinhead oatmeal	331	1–7	361	4	98·8*
			8–14	20		
			15	0		
23	Pinhead oatmeal	115	1–7	122	0	100*
			8–14	0		
			15	0		
24	Pinhead oatmeal	707	1–7	270	8	98·9
			8–11	5		
25	Pinhead oatmeal	376	1–7	213	0	100
			8–9	0		
26	Pinhead oatmeal	423	1–7	485	0	100
			8–14	0		
			15	0		

* Poisoning preceded by pre-baiting.

as the bait-base. This bait had shown up as well as the dehusked seed in palatability tests but it has the disadvantage that mice tend to dehusk it, and probably discard at the same time part or all of any poison adhering to it. Preliminary tests showed however that whole canary seed mixed with the 2 % solution of calciferol in corn oil and treated with warfarin master-mix was toxic to mice, presumably because enough calciferol found its way below the husks to significantly increase the toxicity of the formulation to mice. Poison bait prepared in this manner was used in Treatments 21–26.

In Treatments 24, 25 and 26 the poison bait was laid directly. In the other three treatments poison baiting was preceded by 3 days of 'pre-baiting' with a mixture of 90 % whole canary seed, 5 % corn oil and 5 % wholemeal flour.

Table 4 shows that five of the six treatments gave excellent results in from 9 to 15 days. In Treatment 21 good bait-takes in the first week did not result in much reduction in activity and adequate control was obtained only after 29 days. A contributory factor may have been that certain bait points were so well protected from farm stock that they were also largely ignored by the mice: for when on day 8 they were re-located and increased in number, feeding increased.

CONCLUSIONS

House-mice are most difficult to control in places where food and cover coincide and are extensive. The success of a poison treatment is then as much dependent on having an attractive bait-base as it is on using an effective poison. The results of the poison treatments reported here showed that, provided baiting problems can be overcome, calciferol at 0·1 % plus warfarin at 0·025 % can give effective control of *M. musculus*. The excellent results of the treatments using whole canary seed mixed with calciferol in corn oil and with warfarin also indicate that this formulation is most likely to succeed in controlling mice living in 'difficult' environments.

The present work however does not show either the extent to which warfarin contributes to the effectiveness of bait containing calciferol or how far calciferol penetrates below the husks when it is added in corn oil to whole canary seed. Both subjects are being investigated further.

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