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Wood, B.J. and Chung, G.F., "WARFARIN RESISTANCE OF *Rattus tiomanicus* IN OIL PALMS IN MALAYSIA AND THE ASSOCIATED INCREASE OF *Rattus diardii*" (1990). *Proceedings of the Fourteenth Vertebrate Pest Conference 1990*. 81.
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WARFARIN RESISTANCE OF Rattus tiomanicus IN OIL PALMS IN MALAYSIA AND THE ASSOCIATED INCREASE OF Rattus diardii

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ABSTRACT: Rattus tiomanicus is a serious pest of oil palm plantations in Peninsular Malaysia, feeding on the ripening fruit. R. diardii is a rat of human habitations and has been only an occasional field species, presumably because it cannot compete with better adapted species. A widely used control for R. tiomanicus of proven effectiveness uses maize-based baits containing warfarin at 0.05% in 2 cm³ (14.5 g) wax-bound cubes. These are applied in campaigns at one per palm, replaced on 4 daily rounds, until acceptance has declined to 20%. This "standard method" is applied at about 6-month intervals, and rat populations remain low. In the early 1980s resistance to warfarin began in R. tiomanicus, and from about the same time R. diardii was found more often in oil palms, apparently in the same localities.

This review is of studies of these phenomena. Rat population studies by mark, release, recapture (MRR) confirmed that warfarin baiting was failing against R. tiomanicus, or required prolonged application, whereas second-generation anticoagulants (brodifacoum, bromadiolone, and flocoumafen) were effective. Physiological resistance was confirmed in the laboratory. Direct substitution of second-generation compounds increased the cost of control considerably, and ways to reduce costs were investigated. Smaller bait size presented problems in monitoring bait acceptance, and longer intervals between replacements did not reduce consumption. Half the active ingredient concentration had some promise, but a first-round application of one bait per two palms was most practicable. Bromadiolone baits at 0.005% are now used at half density of one bait per two palms on the first application in areas of warfarin resistance.

Where the problem was first noted, rat populations were compared in areas with and without continued baiting. In an 81-ha plot left unbaited, R. diardii gradually increased and replaced R. tiomanicus during 1982-84. R. diardii then remained predominant until 1989. In the baited plot, once bromadiolone was used (from early 1984) both species were controlled. It is postulated that R. diardii became able to out-compete R. tiomanicus because the genotype of the latter had somehow been weakened in the rapid selection for warfarin resistance.

Proc. 14th Vertebr. Pest Conf. (L.R. Davis and R.E. Marsh, Eds.)
Published at Univ. of Calif., Davis. 1990.

INTRODUCTION

Rattus tiomanicus and R. diardii were formerly both classified as subspecies of R. rattus. They are quite easily separable on characteristics that can be observed in live animals in the field (Harrison 1964). Although laboratory hybrids can be produced, they are of low fertility (Dhaliwal 1963), and the two can be accepted as separate species. R. tiomanicus (formerly called R. jalorensis) has been recognised as the predominant and usually the sole rat species in oil palms in the Malaysian Peninsular, more or less since the palms were first planted (Harrison 1957). Occasionally, R. argentiventer (a major rice field pest) populations increase to become a pest of young mature oil palms in certain localities (Wood 1969a).

R. diardii is most commonly found in human habitations, and according to records they were previously restricted to plantings just around dwellings. Extensive studies of oil palm rats in recent years have not recorded R. diardii. In a 10-year study, Wood (1984) trapped several thousand R. tiomanicus but less than 30 individual rats of other species, and those only where control measures had virtually eliminated R. tiomanicus. No R. diardii were included. From the late 1970s there were some records of R. diardii in oil palms well away from dwellings, particularly in some coastal estates in West Selangor and Lower Perak (Mohd Mat Min 1985).

Before about 1968, rat control attempts in oil palm were sporadic, comprising distribution of loose warfarin baits without considering evidence of the surviving population. During the late 1960s more systematic and effective measures

were devised (Wood 1969b). The technique involved application of warfarin baits at 0.05% in maize-based paraffin-wax bound cubes (2 cm³, 14.5 g). In a campaign, these are applied at 1 per palm, and missing baits (assumed accepted by rats) replaced at 4-day intervals until acceptance declines to 20%. The quantity of bait used is thus "self-monitored" to the size of rat populations. Studies after such campaigns indicate that immigration from outside to initiate reinfestation can be minimised by treating large areas (preferably whole estates) at one time. Build-up by survivors then is slow for about 6 months (unless there is resistance), so control campaigns at 6-month intervals maintain plantings reasonably rat-free at optimum costs (Wood and Nicol 1973, Wood and Liau 1984a).

Most oil palm estates practised this method (with modifications to suit local circumstances or inclinations) throughout the 70s. Despite occasional control problems, physiological resistance to warfarin was not confirmed until 1982 (Wood et al., in press). At about the same time, a study showed an increasing incidence of R. diardii as an oil palm field rat, and in one particular location, it became the predominant species, replacing R. tiomanicus (Wood et al. 1988). About the time the resistance problem was noted, second-generation anticoagulants were becoming available (Hadler 1984), including brodifacoum, bromadiolone, and flocoumafen. These were then developed for use in oil palms.

THE RESISTANCE PROBLEM

Geographical Extent

The first signs of warfarin resistance were a breakdown

in the pattern of acceptance of baits and continuing damage even after several rounds of baiting. These had been noted in Peninsular Malaysia in three localities from a range of representative estates spanning extensive oil palm growing areas. The first was in 1982 near Klang, about in the centre of the west coast. By early 1983, similar problems were found in the Rengam area (south) and in late 1984 around Telok Intan (north). Generally, a single estate first had the problem. Others nearby often developed the same difficulties, but not always soon. By late 1989, still only the three localities had reported clear evidence of warfarin resistance.

Field Trials

The typical progress of a bait campaign before resistance (and where there is no resistance) is about as follows: first application at one bait per palm (100%), first four-daily replacement in the range 40-80%, second 20-60%, third 15-35%, and fourth 15-20%. Sometimes more rounds are needed, but rarely do they exceed 7 or 8. In 1982 in the first affected estate, this typical pattern continued on one division, but on another, one particular programme was: 1st application - 100%; 2nd round (1st replacement) - 83%; 3rd - 43%; 4th - 52%; 5th - 34%; 6th - 57%; 7th - 71%. In face of an evident problem, routine baiting was suspended pending investigation.

Earlier work (Wood 1969b) had showed that *R. tiomanicus* generally had a limited home range, spreading only slowly into depopulated areas. This makes possible trials comparing various control measures in plots of a few hectares.

Mark, release, and recapture (MRR) studies before and after treatments were conducted to show their effects.

Population assessments generally involved about 3 nights of trapping. The trapping grid is based on the planting pattern of the palms, on equilateral triangles of 30 ft (9.14 m) apart (138/ha), with trapping in plots of 5 rows x 10 palms, setting 1, 2, or 3 drop-door traps per point. Trapping is sometimes followed by hunting. This entails banging on the palm trunks and pulling apart the frond piles (heaps of old necrotic leaves cut regularly from the palms) where rats spend the daylight hours. Those discovered are killed with a stick. Hunting allows population estimates to be indexed by an independent capture method, and it produces corpses to assess reproductive condition, diet, and so on.

Population size can be estimated (\hat{N}) by the Lincoln index (LI) (Bailey corrected) $\hat{N} = \frac{M(n+1)}{(m+1)}$ where M =

marked and released, n = "index" capture (final night of trapping or hunting) and m = those in both n and M. If numbers are too small to apply the formula realistically for comparative purposes, total seen (M + (n - m)) is used.

Wood et al. (in press) reported 11 trials in the three localities where resistance to warfarin baiting programmes had become apparent. These variously compared campaigns with warfarin and second-generation anticoagulant compounds and developed means to reduce the high costs of the latter if they were simply substituted for warfarin at manufacturer's recommended concentration. Table 1 summarises the results of some comparisons where anticoagulants are used in this "standard" way.

Table 1. Selected results showing the amount of bait used and its effects against rats, comparing warfarin and second-generation compounds, in the three localities with warfarin resistance.

Locality	Date	Anticoagulant compound ^a	No. of rats trapped ^c							
			Baiting rounds ^b		<i>R. tiomanicus</i>		<i>R. diardii</i>		<i>R. argentiventer</i>	
			No.	Completed	before	after	before	after	before	after
Kluang	Mar-May 83	Warfarin	13	not	28	32	0	0		
		Bromadiolone	8	yes	50	5	0	0		
		Brodifacoum	5	yes	26	3	0	0		
	Nov 84-Jan 85	Warfarin	16	not	3	7	41	2		
		Bromadiolone	7	yes	4	0	38	1		
		Brodifacoum	6	yes	1	0	43	0		
Teluk Intan	Aug-Sept 84	Warfarin	8	yes	30	12			11	2
		Bromadiolone	5	yes	30	2			6	0
		Brodifacoum	5	yes	18	1			14	0
Rengam	Dec 84-Jan 85	Warfarin	11	yes	18	4				
		Flocoumafen	6	yes	17	1				

^aUsed at manufacturer's concentration in 2 cm³, 14.5 cm wax-bound maize-based baits.

^bStandard campaigns - i.e., baits applied at one per palm, with later baits replaced, or rounds of 4-day intervals, until acceptance declines to 20% ("not completed" = means applications stopped before this point).

^cIn a 10 x 5 palm trapping grid (= 0.36 ha), in 3 nights of mark, release and recapture. Figures given only for trials where the species was represented. The mean of two replicates is provided in most cases.

It is evident that in Klang from March to May 1983 that the size of the *R. tiomanicus* population was virtually unaffected by warfarin, but the second-generation compounds reduced it markedly. Subsequent results confirmed near elimination by the second-generation compounds. There was less survival of *R. tiomanicus* at Teluk Intan and Rengam with warfarin but still more than the near elimination previously obtained. These trials were done much earlier after baiting problems were noted than had been the case at Klang. This is because it was clearer by then what was probably happening and how to investigate it.

In all the trials, similar results occurred with warfarin, including one case where it was tried variously in an alternative form (warfarin-sodium) and with other bait mixtures. Second-generation compounds at adequate dosages were always effective.

Warfarin Against Other Species

In the trial starting in late 1984 at Klang, *R. diardii* had become predominant. It is clear that warfarin, as well as the second-generation compounds, is effective against this species. The same result was obtained in some other trials in the Klang area. In the Teluk Intan trial area, the site was near a rice field, and *R. argentiventer* was found in patchy distribution. Evidently it too remained susceptible to warfarin.

Development of Cost Effective Alternative Controls

While the second-generation compounds when substituted for warfarin in standard campaigns gave excellent control of resistant *R. tiomanicus*, the cost was very high. A series of trials was carried out to develop better cost-effectiveness.

Comparison of the relative costs of baiting programmes is not easy. Information on the cost of active ingredients is either confused in the overall cost of made-up baits, or is proprietary. The method adopted by Wood et al. (in press) was to assume the proportions of bait and poison cost in the standard (warfarin) bait to be 85:15, based on commercial evidence. The amount of bait in any method can be compared with the amount needed for a "standard" programme and the relative costs of active ingredients then need to be compared. The assumed cost ratio per unit weight of active ingredient based on available information is that brodifacoum is 200 times more expensive and bromadiolone is 70 times more costly than warfarin. No cost information was available for flocoumafen. This still left the question of what was the required amount of bait for a "standard" programme in a given trial locality if warfarin was not effective. In this case, the standard amount of bait in any trial location was assumed to be that needed with a second-generation chemical at the manufacturer's recommended concentration.

It assumes that the amount of bait needed is similar to what would have been needed with warfarin had resistance not occurred. In each trial, this is the "base" treatment and from this a cost factor is calculated as follows:

$$\frac{\text{Amount of active ingredient in the considered treatment}}{\text{Amount of bait in the base treatment} \times 0.05\%} \times \text{cost ratio for that poison}$$

The relative amount of bait and poison cost factor are then added in the ratio 85:15.

Possible ways to reduce costs were aimed at reducing the chance of "overkill" (i.e., continual taking of baits by rats that had already eaten a lethal dose), or of wasting baits (disappearing to other causes than consumption by rats). They included reducing the initial density of baits from one per palm to one per two palms (with replacement only at the same application points), longer intervals between rounds, reduced bait size, reduced concentration, and combinations of these.

Reducing the size of baits was inconsistent and generally unsatisfactory largely because breakdown of baits made self-monitoring difficult. Longer replacement intervals did not reduce requirements, but they prolonged campaigns with no compensating savings. It is likely that both disadvantages would be greater in commercial practice than in carefully executed trials.

Reducing the concentration of the chemicals appeared promising, but was not pursued in the interest in keeping a single "recommended" concentration for each compound. Halving the initial input density generally led to a reduced total bait usage. Combinations of the approaches did not have the desired result and prolonged campaigns without an apparent benefit in bait quantity needed. The commercial baiting procedure recommended, which is now widely adopted in "resistant" localities, is bromadiolone at 0.005% in standard baits with first application at half density. Such programmes have been applied extensively, on the same 6-month intervals as are recommended for warfarin in nonresistant areas, with apparent success. This is illustrated below (The rise of *R. diardii*).

Laboratory Evidence

Earlier work (Wood and Liau 1977) established lethal feeding periods (LFP) for a "baseline" population, with LFP₅₀ at around 0.9 days for 0.01% warfarin fed in banana mash bait, or 1.7 for 0.025% in particulate bait. Only 1 rat of 42 survived a feeding period of 6 days. Lee and Mustafa (1983) found an LFP₅₀ of about 3.5 days with wax baits at 0.075% warfarin, with survival of 4/20 rats at the 6-day feeding period and 1/20 at 8 days. Wood et al. (in press) found that for rats from the "resistant population," LFP_{50s} (at a higher test concentration of 0.05%) were 3.08 for females, and 1.24 for males. Further, both total intakes of warfarin in surviving rats and survival in longer feeding periods were much higher than in the earlier work. This clearly suggests some change.

THE RISE OF *Rattus diardii*

Buildup in a Population Study

When the change in the progress of baiting programmes occurred in the Klang area in 1982, a trial was set up to monitor rat populations in response to control, including a comparison area without control (Wood et al. 1988). An area of 81 ha of 1974 palms was designated a "nonbaited area" (NA), and another nearby planting of 1973 palms (of 101 ha, but itself within a larger estate division under similar treatment) as "baited area" (BA). A trapping plot of 5 rows x 20 palms (about 40 m x 183 m = 0.74 ha), with each palm a trapping point, was set up in each. MRR trapping was conducted for a 4-night interval, intended every 5 (later 7) weeks. The actual time between intervals varied somewhat from this, and during most of 1983 it was discontinued because finding a solution to the problem of resistance had become paramount. Population size at each interval was estimated by Lincoln index (Bailey corrected) (previously

given) using the fourth night of trapping as the "index" catch. These population estimates are graphed in Fig. 1. Sharp successive peaks and troughs in the graphs probably represent the margin of error in the population size estimation, but the trends are quite clear.

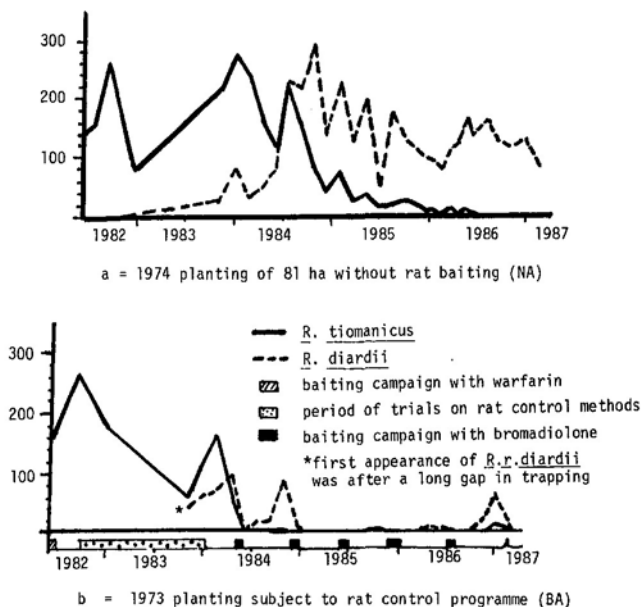


Figure 1. Population of rats estimated by use of Lincoln Index in oil palm usually trapped at 5 to 7-week intervals, but with a large gap from late 1982 to late 1983 (after Wood et al. 1988).

R. tiomanicus is "trap prone" and amenable to investigation by MRR methods (Wood 1984). The edge effect is not very great in the short-term because the home range is relatively small. Based on the earlier study (Wood loc. cit.) which estimated a population by taking into account successive interval "i," using the Jolly-Seber method, the present Lincoln index estimate represents about 70% of the total population. This suggests that the average for the whole period was about 260/ha, well within the range of estimates for this species in oil palm that has been made in many localities over a long period.

The relationship of *R. diardii* trapping figures to actual population has been less thoroughly studied. It appeared to be reasonably trappable, albeit less consistently so than *R. tiomanicus* in respect of "first occasion" trapping in a particular locality. In the study, the total seen in each "i" averaged 65% of the population estimates by LI. The average population estimate was 136/ha in the NA from October 1984 onwards. If the same relationship holds as for *R. tiomanicus*, this implies a total population in the range of 190-200/ha.

At the beginning of the study, the rat population was virtually solely *R. tiomanicus*, as is normal for oil palms, based on previous experience. In the first three trapping intervals (i) starting 1st June, 7th July, and 14th September 1982, respectively, an overall total in the designated NA and BA of 487 rats were trapped, all of them *R. tiomanicus*. In the "i" of 18th December, 2 *R. diardii* were included in the total catch of 78 rats. During this period it was also evident that the population in the BA was not much different from that

in the NA. This is because, as noted above, the trial was laid down when problems with baiting programmes were being noted but the cause was not clearly established. At that time, control attempts were sporadic and unsuccessful. During most of 1983, this study was in abeyance, and when trapping resumed in December the proportion of *R. diardii* in BA was 8 in 62 (including 2 *R. argentiventer*) and in NA, 23 in 49. This continued for 3 more "i" up to 17th April 1984 during which period the proportion of *R. diardii* built up to 22% (NA) and 52% (BA), respectively (Fig. 1a). Then a standard bromadiolone bait campaign was applied in BA and later in the whole estate (except the NA) at 6-month intervals.

From then, in NA, the rat population continued high and the proportion of *R. diardii* rapidly increased until by mid-1985 it was over 80%. This continued until March 1987, the last trapping date recorded by Wood et al. (1988).

In BA, after the successful control over a limited area in April/May 1984, there was a slow buildup of rats, and by 30th October, 26 of the total of 29 rats were *R. diardii*. Some buildup was most probably from slow immigration of rats from the surrounding unbaited palms. When subsequent campaigns were done over the whole estate, in a total of 20 "i" from December 1984 to 3 March 1987, there was a total of only 56 *R. diardii*, 2 *R. argentiventer*, and 13 *R. tiomanicus*. In NA, in the same period there were 1252, 2, and 150, respectively.

The initial baiting campaigns were with standard bromadiolone baits (0.005% a.i.) at 1 bait/palm, and this initial density was later halved (1 bait/2 palms). Details of the campaigns are present in Table 2.

Table 2. Results and details of rat control campaigns with bromadiolone baits (0.005% a.i.).

Date	Baiting density	Number applications (rounds)	Total number baits used	Amount of bait used (kg/ha)
16 April 84	1 bait/palm	7	467	6.8
28 Nov 84	1 bait/palm	6	290	4.2
17 May 85	1 bait/palm	7	488	7.1 ^a
22 Nov 85	1 bait/2 palms	9	397	5.8
7 July 86	1 bait/2 palms	5	172	2.5
17 Feb 87	1 bait/2 palms	3	99	1.4

^aActually 4 more rounds were applied, all less than 20% accepted, due to a misunderstood field instruction. The figure given is up to the 20% acceptance point.

Other evidence

In the trials on control, *R. diardii* was caught in relatively large numbers only in the Klang area. When this began to occur, a patchy distribution was evident, with a preponderance in some plots but very few in others. This probably reflects the rats gradual spread.

It was less easy to catch rats by hunting but results were inconsistent. Considering only 28 plots where "total seen" *R. diardii* before baiting were 10 or more (in 0.35 ha plots), an average of about 33 were trapped (10-72) but only 1.6 (0.5)

were caught by hunting (0.5 of them previously trapped). For comparison, in the same plots an average of 5.6 R. tiomanicus were trapped but 5.9 hunted. However, in one trial, hunting was more successful in three of the six plots, with an average of 31 (17-44) trapped and 25 (10-32) hunted (8 of them marked). In these, the mean relationship of trapped:total seen:Lincoln index estimate ($M:M+(n-m):N$) was 32:47:100. The catch from 3 nights MRR thus represents perhaps about one-third of the total population. For comparison, generally, with R. tiomanicus, about one-third to one-half is assumed, based on more comprehensive evidence.

R. diardii as an Oil Palm Pest

R. diardii is a bigger rat than R. tiomanicus, as was confirmed in the long-term study (mean weight of all individuals irrespective of maturity: 139 g males, 143 g females against 101 and 102 g for R. tiomanicus). The species reached maturity (for 90% of individuals) by a weight of 80 g (male) and 60 g (female) (against 70 and 50 g for R. tiomanicus).

Examination of the stomach contents of rats killed in the field proved that, like R. tiomanicus, most had been feeding on oil palm fruit. In lab trials (Wood et al. 1988) rats of around the mean mature weight that were given a necessary daily supplement of meat to maintain full health consumed an average of 9.9 g fruit per day compared with 4.29 g for R. tiomanicus (which could survive on a meat supplement on only 2 days/week).

Because of its larger average size and higher consumption of palm oil fruit, it appears to have the capability to be a serious pest of oil palms where it occurs.

Possible Reasons for the Buildup of R. diardii

The question remains, why did R. diardii build up and replace R. tiomanicus in the one recorded case and start to appear in small numbers in several other localities? Three generalised postulates were advanced by Wood et al. (1988):

1. The environment had become more favourable for R. diardii.
2. It had become less favourable for R. tiomanicus.
3. Inherent relative competitive ability between the two species had somehow changed.

Initially it appeared possible that a weevil that was introduced (from Africa) in 1982 to effect better pollination might be the reason. The grubs of this weevil develop in the spent male flowers of the palm, and both species of rat have developed the habit of feeding on them (Liau 1985). These, it was thought, might supply the higher meat requirement of the species. Field rats are known to feed on a wide range of insects as a supplement to the diet (Harrison 1954). However, if this were the case, R. diardii ought to have spread further, but by 1989 this had not happened (Wood et al. in press). The present case is the only one reported of it becoming a populous and predominant species in oil palms.

Another possibility considered was that R. diardii may have a greater innate resistance to warfarin, so that it had become favoured by the continual use of the chemical. Lam (1984) reported a somewhat greater tolerance in R. diardii. However, it is now obvious that this was not the reason, as it is still eliminated by the standard warfarin baiting.

The explanation favoured by Wood et al. (in press) is that R. tiomanicus had become less competitive. Harrison (1957) found R. diardii in the field (in scrub vegetation) in Singapore and noted that it was not intrinsically unable to exist in such an environment, only that it appeared less competitive than other species. In this case the change perhaps was because the rapid evolution of resistance had weakened the genotype in other ways. R. diardii appeared in the field in most localities where warfarin resistance had appeared, but only in those localities. It had not generally become predominant in those localities, but the present study is the only one reported where baiting was suspended. Where baiting continued, the warfarin would continue to eliminate other species and so help to maintain the warfarin-resistant population. The weevil grubs may complement this process.

CONCLUSIONS

R. tiomanicus has clearly developed warfarin-resistant populations in the localities indicated, but they can be controlled by second-generation anticoagulant compounds. The best vehicle for their application remains the type of bait developed, after extensive research, for warfarin. Programmes using second-generation compounds in this way tend to be more expensive than programmes using warfarin, but cost can be minimised by appropriate measures, e.g., at a density of 1 bait/2 palms instead of 1/1 palm.

Manufacturers tend to prefer selling ready-made baits rather than concentrates, but unfortunately these have not proved satisfactory, the baits being too small to adapt to the self-monitoring system (through rapid breakdown) or insufficiently attractive to field rats.

The second-generation compounds undoubtedly have a high potency, making uncontrolled handling of concentrates undesirable. Wood et al. (in press) suggest that to overcome this, while meeting the need of effective field rat control in the wet tropics, the involvement of trained professional rat controllers may be useful. Also, manufacturers might make available acceptable reduced concentration "master-mixes."

There is no information upon which to judge the possibilities of resistance arising to the second-generation compounds. Should this happen, there is no obvious solution. Acute poisons as a class have uniformly given disappointing results, as they did in the studies reviewed here. Some give deceptively good results in laboratory tests and clearly kill rats in the field, but they do not control the population. Some biological control possibilities may offer promise (Smal 1989, Wood 1985).

R. diardii seems to arise when R. tiomanicus populations have become warfarin resistant, but continued baiting appears to have generally prevented the buildup that otherwise would have occurred. This could be investigated further by similar trials leaving unbaited areas in other localities with warfarin resistance. It would be interesting to observe, over a longer period, whether R. tiomanicus gradually reassumed dominance in the present trial with the removal of the intensive selection pressure for warfarin resistance.

ACKNOWLEDGMENTS

B. J. Wood is indebted to Unilever Plantations for sponsoring his attendance at this conference. Much of the work reviewed was carried out while he was employed by Sime Darby Plantations in Malaysia. G. F. Chung is grateful to the latter for permission to publish.

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