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VARIOUS PYRETHROIDS ON BEDNETS AND CURTAINS

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Verandah trap huts in a Tanzanian village were used to assess the effectiveness of impregnated bednets and curtains in preventing hut entry and feeding by, and in killing of, Anopheles gambiae and An. funestus. Permethrin, deltamethrin, lambda-cyhalothrin and pyrethrum were used for impregnation of damaged or undamaged bednets, sisal eaves curtains or bed curtains made of polypropylene fibre. The performance of the three synthetic pyrethroids did not differ statistically significantly, except that on a damaged net permethrin was better at preventing feeding. Sisal eaves curtains deterred mosquitoes from hut entry but did not kill those that had entered. In assessing damaged nets and curtains it must be recognised that anything less than the best vector control may have no appreciable impact on holoendemic malaria.

Key words: *Anopheles gambiae* – *Anopheles funestus* – impregnated bednets – verandah trap huts – impregnated curtains – malaria vector control

The idea of impregnation of bednets or other textiles with pyrethroids is now widely recognised as the most important advance in malaria vector control since the introduction of house spraying. The impregnation acts both to improve the functioning of ill-used nets and to kill mosquitoes attracted to the nets, thus reducing the vectorial capacity of the local mosquito population even for people not under nets. There have been numerous demonstrations of the effectiveness of impregnated nets against vector populations (see review by Curtis et al., 1989) and, in some cases, against malaria, though it is recognised that in areas of holoendemic malaria moderate reductions in sporozoite inoculation rate may be insufficient to have an impact on the prevalence of infection.

In some parts of the tropics and sub-tropics bednet usage is already widespread, primarily against nuisance mosquitoes. Unless nets are used very carefully they are insufficient in themselves to control highly endemic malaria, but the treatment of existing nets with a pyrethroid insecticide is a relatively cheap and easy method of improving their functioning. However, there are still questions of the optimum compound

and dosage to be investigated, and there is a tendency for different research groups to make different arbitrary decisions about what to use without having compared the different possibilities.

In countries where bednets are not affordable by most people and/or where they are considered uncomfortable to sleep under, there have been some tests of impregnated curtains over eaves, doorways etc. (Majori et al., 1987; Sexton et al., 1990; Procacci et al., 1991) and the use of locally available fibres as a substitute for imported bednets has been suggested (Lines et al., 1988). Comprehensive tests of these, in comparison with conventional bednets, remained to be carried out.

Rather than attempting to test all the possibilities in village scale trials of malaria control, useful preliminary information can be obtained with experimental huts (Darriet et al., 1984; Lines et al., 1987; Li Zuzi et al., 1987; Lindsay et al., 1991, 1992; Miller et al., 1991; Pleass et al., in prep.) and this may allow some possibilities to be eliminated so that the range of options for full scale testing can be made more manageable.

Experimental huts need to allow the monitoring of any deterrence to house entry or excitorepellency, leading to exiting after touch-

ing the nets, which the pyrethroid may cause. Also its lethal effect needs to be monitored by collecting the dead mosquitoes, without them being taken by scavengers such as ants. The verandah trap hut of Smith & Webley (1969) fulfils these requirements.

THE HUTS AND CHECKS ON HOW THEY FUNCTION

Two verandah huts were built in the village of Kisiwani, near the Ubwari Field Station of the N.I.M.R. Amani Centre at Muheza, Tanzania. For lightness and ease of construction our huts were made of wooden frames covered with hardboard. Fig. 1 indicates how verandah trap huts function. They are built on concrete pillars incorporating a water channel to exclude ants. Between each wall and the roof is a 1 cm wide slit at the eaves. On two sides these slits open to the outside so that wild mosquitoes can enter or escape from the hut. The other two eaves slits, which appear identical from inside the hut, open into verandahs screened with durable fiberglass mosquito screen coated with PVC. Mosquitoes can exit either the way they came in or into the verandah traps. To correct for possible bias in the direction of exiting, the screens are alternated weekly between the north and south sides of the hut or the east and west sides. Thus it is reasonable to assume that, of the mosquitoes exiting through the eaves slits, half are caught and half succeed in escaping, so that the catch in the verandahs can be

multiplied by two to estimate the total which exited through eaves slits. In addition to these slits, an exiting mosquito may also go into window traps, whose catches do not need to be multiplied by two as there are no corresponding open apertures to allow entry of wild mosquitoes.

Two children from the village sleep in each hut from 20.00 to 06.00 h. The same two children always occupy a given hut so that any variation between huts or children in attraction for mosquitoes is confounded. The nets or curtains under test are rotated between huts according to a pre-arranged schedule whereby each one being tested in a given experiment (as well as absence of any protection) is tested in each hut once a week.

The hut door is kept closed except when the children or the collection team are entering or exiting. The huts and traps are searched each morning and the catch is classified by species (mainly *Anopheles gambiae* s.s., *An. funestus* and *Culex quinquefasciatus*), sex and abdominal state. Culicines and anophelines responded differently to the impregnated material. The data on culicines will be presented elsewhere and that shown here include only female anophelines which were unfed, freshly fed or half gravid, i.e. which presumably entered the hut in search of blood. The latter two categories are assumed to have succeeded in feeding at some time during the previous night.

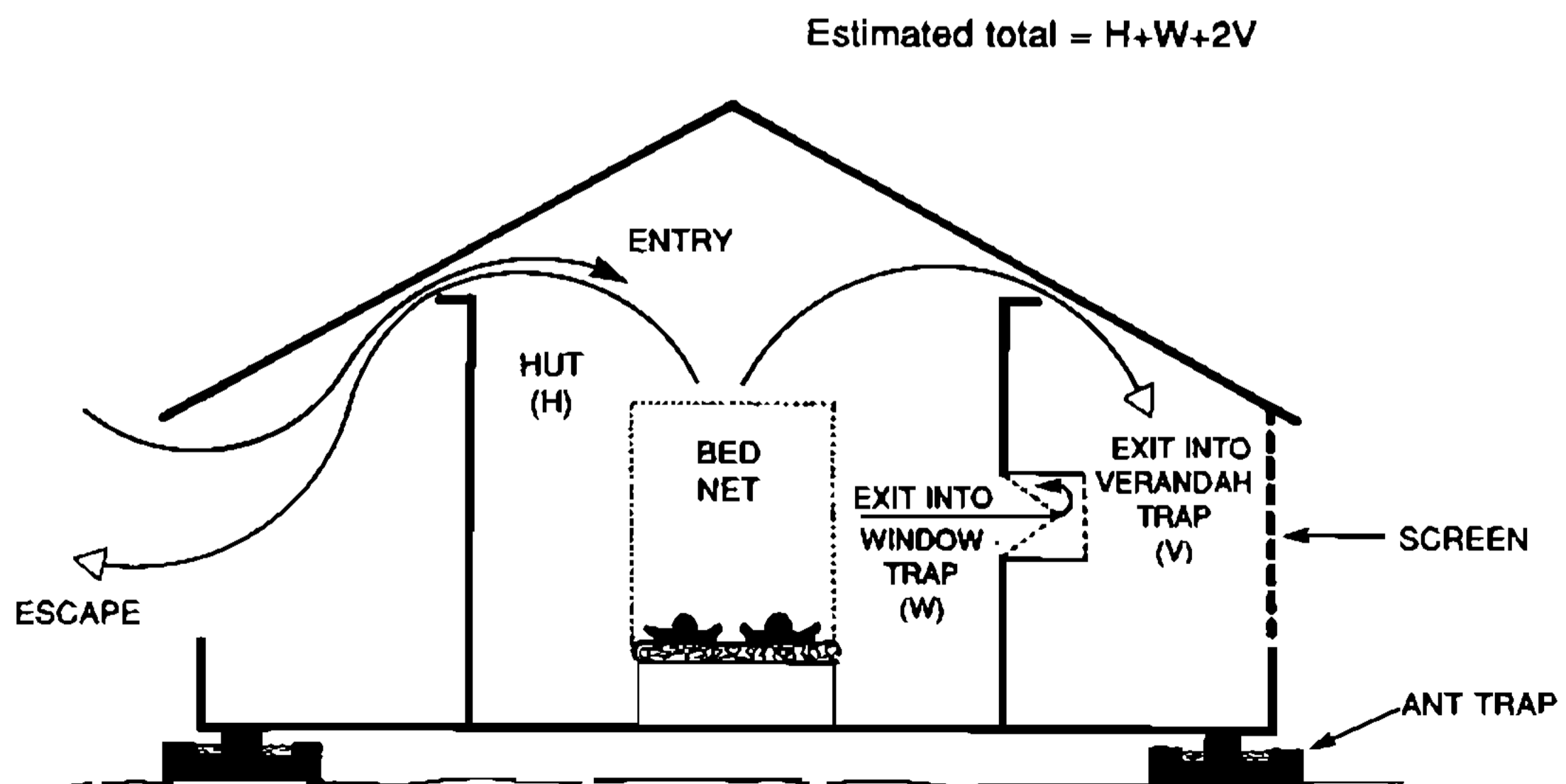


Fig. 1: diagrammatic section through a verandah trap hut to show how it is used to estimate the total number of mosquitoes which entered a hut during the night.

TABLE

Results of releases into the huts

	<i>An. gambiae</i> (lab. reared, released unfed)		<i>An. funestus</i> (wild collected, fed)	
	no net	perm. net	no net	perm. net
No. replic.	4	3	3	2
No. ^a wild mosq. in hut without release	14	23	22	9
No. released	178	150	143	80
No. ^a captured in release hut	142	121	123	48
No. recap. (corr. for wild mosq.)	128	98	101	39
% recap.	71.9	65.3	70.6	48.7

a: after multiplying catch in verandah trap by 2.

The numbers of mosquitoes knocked down or dead were recorded at the time of collection and 24 h later. With treated nets the 24 h mortality was only about 3.5% higher than that at the time of collection and data from controls without treated nets show that this can readily be explained by the effects of handling, not delayed effects of the insecticides and the data reported here refer to time of collection. Checks with dead mosquitoes left in the huts overnight showed that scavengers were being successfully excluded and the observed mortality could be taken as a fair reflection of what happened overnight.

As a check for losses of mosquitoes, releases of known numbers were made at about 22.00 h at seasons when there were few wild mosquitoes. The numbers collected dead or alive the next morning in the verandah traps were multiplied by 2 and added to those in the hut and window traps, as already explained. The results (Table) indicate that only about 71% can be accounted for when there are no nets, and apparently somewhat less when there were permethrin treated nets in use. Every effort has been made to block crevices in which mosquitoes might hide and one possible explanation of the losses is that mosquitoes which have exited on to the verandah traps return to the

hut during the night and attempt to exit again, i.e. they have a second chance of choosing the unscreened eaves.

TESTS OF IMPREGNATED BEDNETS

Nylon bednets were impregnated by dipping in mixtures made from emulsifiable concentrates of permethrin or lambda-cyhalothrin from ICI, or flowable concentrate of delta-methrin from Roussel Uclaf. The mixtures were targeted to give 200 mg/sq m of permethrin or 15 mg/sq m of the other two compounds, taking into account the amount of liquid retained by the nets after dipping and wringing. Absence of any form of protection and untreated nets were used as controls and in some experiments six holes, each 10 x 5 cm, were cut in the nets to simulate damaged nets as are often seen in use in rural houses. In each experiment each treatment was tested once a week in each hut for six weeks, i.e. 12 times in all.

Figs 2, 3 and 4 show data for the malaria vectors *An. gambiae* and *An. funestus* from three successive experiments. The former species represented 60-90% of the collections. In the absence of protection of the sleepers, an average of 10-25 anophelines entered the hut each night and over 80% of them fed. With the

untreated undamaged net (Fig. 2) the feeding rate was reduced to about 17% and many of the unfed mosquitoes died. This high death rate was found with untreated undamaged nets by Lines et al. (1987) and is probably an artifact of using verandah and window traps; without these, mosquitoes prevented from feeding would probably have gone to another house and found a bloodmeal and thus have avoided starvation. With pyrethroid treated nets most of the few blood fed mosquitoes died. However, three-way analysis of variance (by hut, week and treatment) showed that the overall death rate was not significantly higher with the treated than the untreated undamaged nets. In view of the probably artifactual nature of the mortality with the untreated net it would be unwise to conclude that there is no advantage to a community in impregnating undamaged nets in order to kill mosquitoes; Jana (1991) showed in villages in Assam that the *An. minimus* population was suppressed by the introduction of treated, but not untreated, nets.

With the permethrin treatment of an undamaged net the number of anophelines estimated to have entered the huts was significantly less

than without protection. This was shown to be significant by three-way analysis of variance on log transformed data which also showed a highly significant difference in the numbers entering the two huts. Significant deterrence from house entry has been reported before with permethrin treated nets and ascribed by Lindsay et al. (1991) not to the permethrin itself, which has very low vapour pressure, but to components of the formulation.

With the treated nets the number of fed mosquitoes was less than 5% of the number with no protection. This reduction was partly because fewer mosquitoes entered the huts with the treated nets but mainly because few of those that entered were able to feed. It would be interesting to know whether the few mosquitoes which were found blood fed had somehow managed to reach the children under the treated nets or whether they had come into the huts after feeding elsewhere. We are intending to answer this question by applying the DNA "fingerprinting" method to the bloodmeals (Coulson et al., 1990) and comparing the results with those from the blood of the children who sleep in the huts. The observed number of bloodfed mosquitoes was

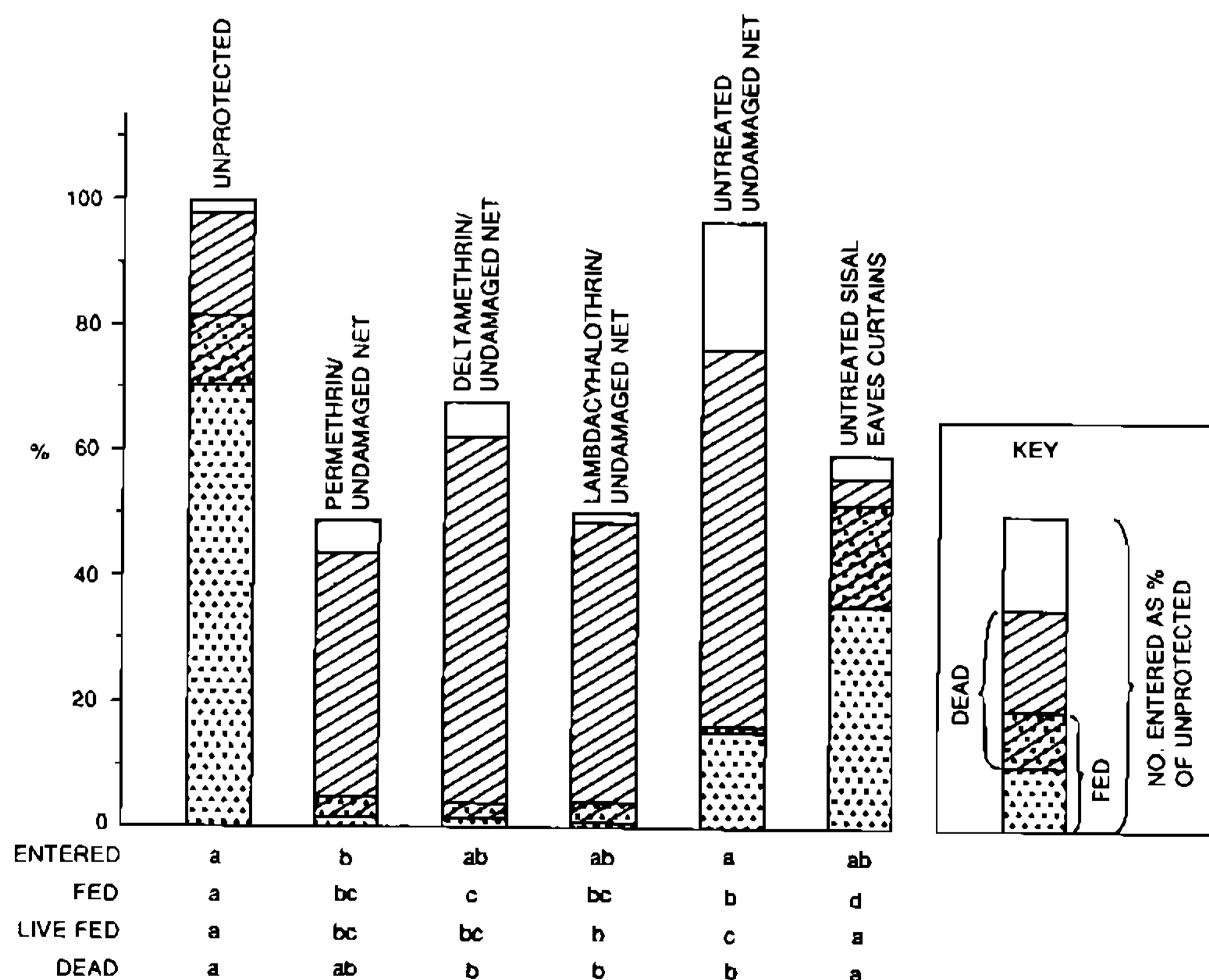


Fig. 2: numbers of *Anopheles gambiae* plus *An. funestus* which entered the huts, fed and died in experiment 1 on undamaged impregnated or unimpregnated bednets or sisal eaves curtains. The letters indicate the statistical significance of differences between treatments - within a row (e.g. for nos. entered the hut) treatments not sharing a letter differed at the 5% level.

considerably less with the treated than with the untreated, undamaged nets. However, the difference was only of borderline statistical significance, P being less than 0.05 only in the case of deltamethrin in comparison with the untreated net.

The chance of transmission of malaria from someone under a net depends on the number of mosquitoes which bloodfeed and survive ("live fed" in the footnotes to the Figs.). The observed numbers were less with the treated than the untreated nets. It was not considered justifiable to use analysis of variance with these data as on many occasions zero scores were obtained. Therefore the non-parametric STP method for multiple comparisons (Sokal & Rohlf, 1981) was used. By this stringent test, the difference in the numbers of live fed anophelines between the treated and untreated undamaged nets did not quite reach

statistical significance, except in the case of the lambda-cyhalothrin versus untreated comparison.

Fig. 3 shows data for tests with the same nets after they had been deliberately damaged. The untreated net gave no significant protection, many fed mosquitoes were found inside it and few died. Significantly more died with the nets treated with the alpha-cyano pyrethroids, deltamethrin and lambda-cyhalothrin, indicating the expected benefit of widespread treatment of imperfect nets.

The number of fed mosquitoes was significantly less with permethrin treated than with the alpha-cyano treated or untreated nets, suggesting that the irritant effect of permethrin has the advantage of rapid interference with a mosquito's search for a way into a net, whereas

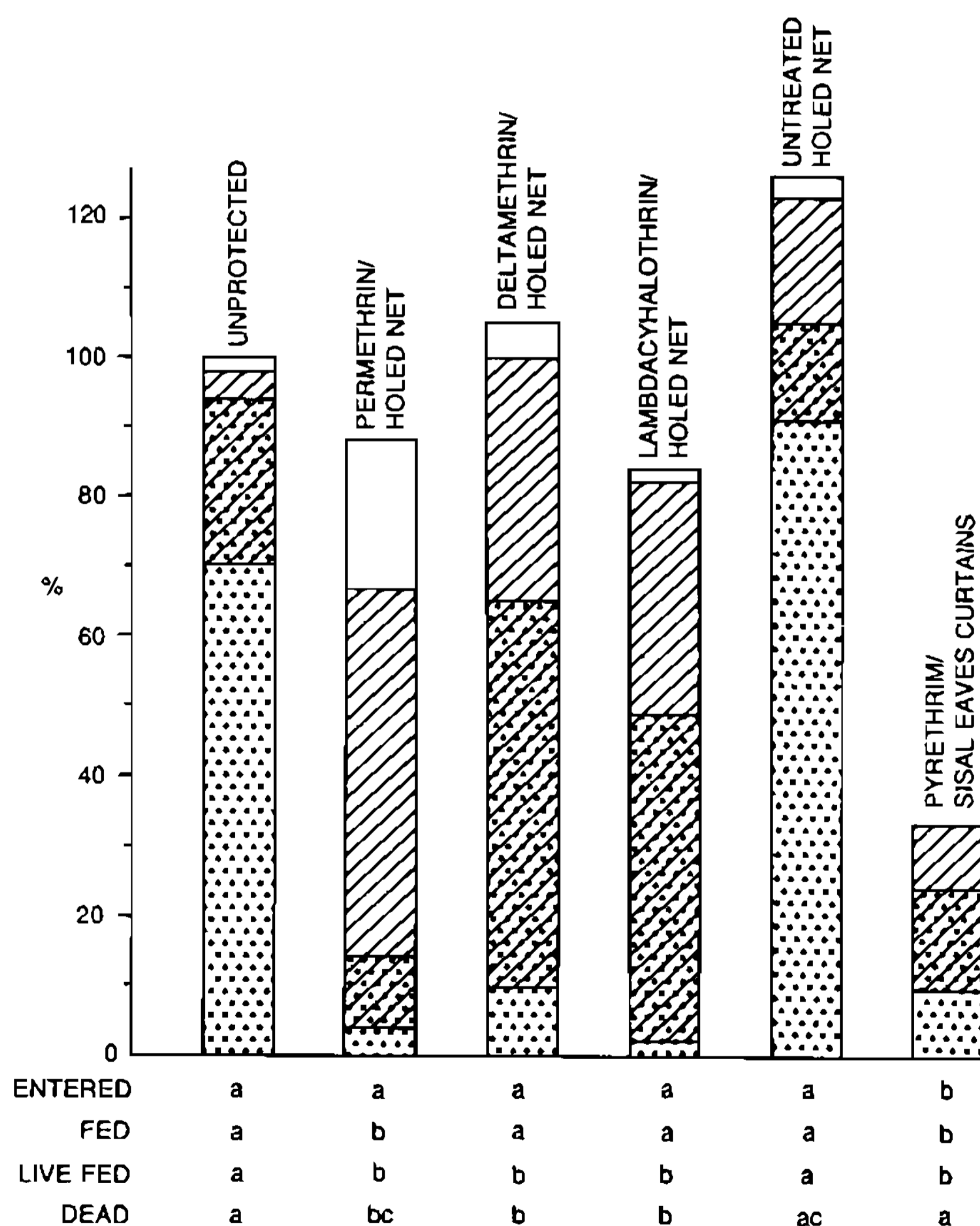


Fig. 3: results of experiment 2 on bednets which had been damaged with 6 holes 5 x 10 cm and on pyrethrum impregnated sisal eaves curtains. Symbols as in Fig. 2.

the alpha-cyano pyrethroids, at least at the low dose (15 mg/sq m) used in this experiment, did not act quickly enough to prevent feeding, but did kill after feeding. This apparently better personal protection of permethrin than lambda-cyhalothrin was not found in an earlier study (Curtis, Wilkes & Myamba, unpubl.) with a dosage of 30 mg/sq m of lambda-cyhalothrin, but that dosage causes appreciable nasal irritation to net users (Njunwa et al., 1991).

Even at the low dosage of 10 mg/sq m the mosquito toxic effect of lambda-cyhalothrin lasts for longer than that of 200 mg permethrin/sq m (Njunwa et al., 1991; Wilkes, unpubl.). In areas where nets are to be provided to the population, it may be the best policy to provide nets which have been industrially treated with an alpha-cyano pyrethroid. After a year or two of usage some damage may become apparent and re-impregnation would be necessary. Permethrin does not cause irritation if splashed on the skin and is therefore more suitable for impregnation by the community and, according to the results in Fig. 3, provides better personal protection on a damaged net. If permethrin were used for re-impregnation, in ar-

eas of perennial transmission this process would have to be repeated about every six months (Njunwa et al., 1991) until the net reached the end of its useful life. Further tests will by necessary to establish whether the use of two different pyrethroids at different stages of the life of a net is indeed the best policy.

TESTS OF BED AND EAVES CURTAINS

Bednets can be bulk-purchased for about \$3 each and a synthetic pyrethroid for impregnation costs much less. However, in some tropical countries foreign exchange is unavailable for such imports. We have therefore tested impregnated curtains made of fibres which are more readily available and natural pyrethrum which is, or could be, grown in many tropical areas. We started to test bed curtains made of sisal, but found that this material is highly inflammable and would not be safe to use in this way. It may, however, be acceptable as fibrous "curtains" over eaves. Untreated curtains of this kind seemed to provide some obstruction to the entry of anophelines, but in the experiment illustrated in Fig. 2, the effect was not statistically significant. With pyrethrum

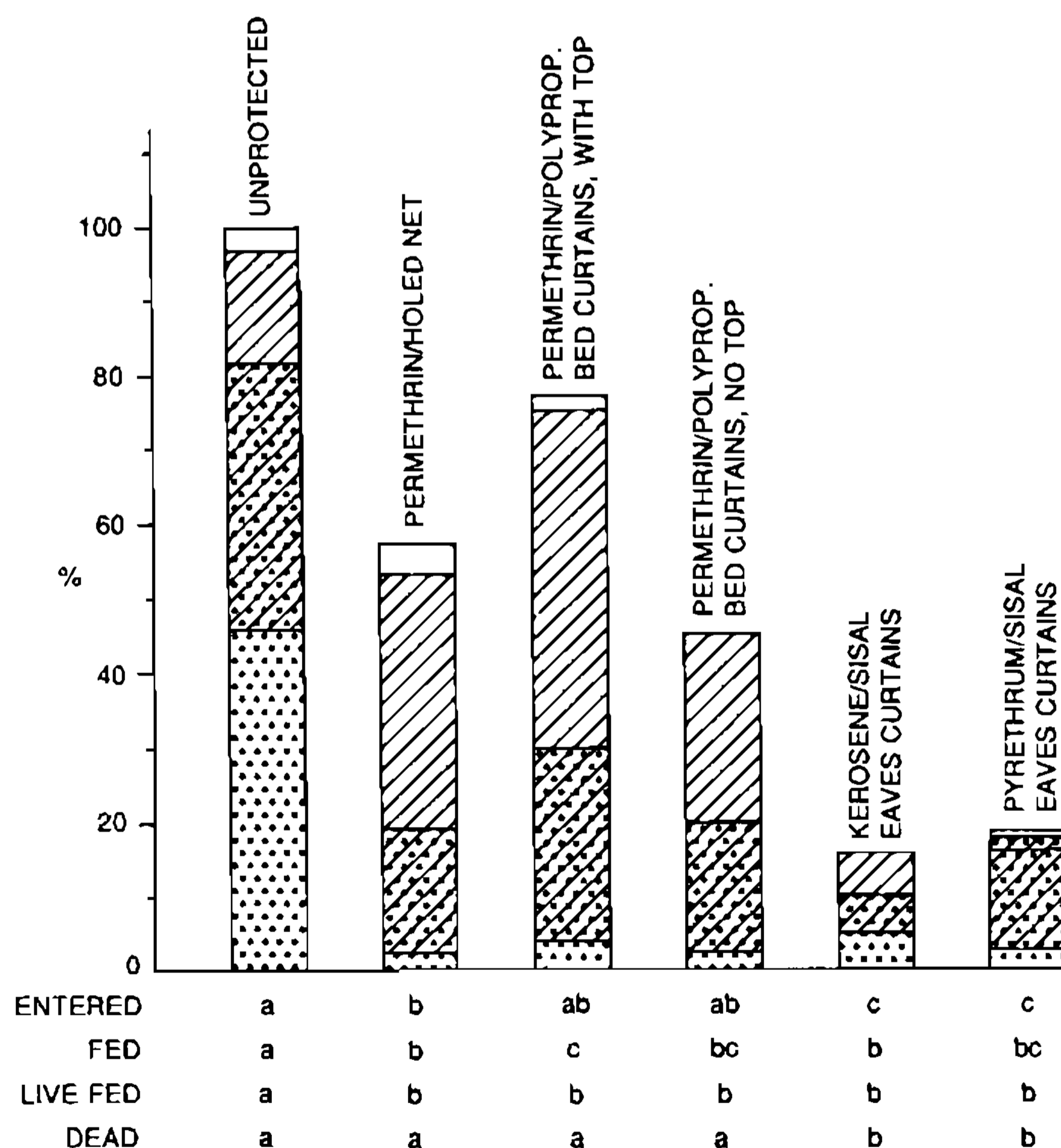


Fig. 4: results of experiment 3 on impregnated polypropylene bed curtains or sisal eaves curtains, in comparison with a bednet. Symbols as in Fig. 2.

dissolved in kerosene, at a dosage of 1 gm/sq m, the obstruction to mosquito entry was statistically significant and the number of mosquitoes available to feed in the huts was therefore also reduced (Figs. 3, 4). However, there was no evidence for any killing of mosquitoes by the pyrethrum. We ran a control of kerosene alone, and to our surprise, this also seemed to be highly effective (Fig. 4) and continued to show this effect long after we could no longer smell kerosene on the fibres. We are re-checking this result.

Polypropylene sacks are widely used and manufactured in many developing countries. A woven piece of sacking may be stripped of the horizontal fibres, except for a band at the top, thus making a "curtain" of vertical fibres, which looks quite attractive around a bed and can be impregnated, for example, with permethrin. As shown in Fig. 4, such a bed curtain reduces biting almost as well as a damaged, permethrin impregnated net, and it does not make much difference if the curtain is open at the top or there is a "roof" to it.

It is clear from the results shown that impregnated damaged nets or curtains reduce biting considerably and in areas of limited malaria transmission would presumably contribute usefully to reduction of disease transmission. However, in areas of saturating, holoendemic transmission, a control method which is "better than nothing" in terms of mosquito biting may have no appreciable impact on prevalence of infection (Rozendaal & Curtis, 1989). It remains to be tested whether such imperfect, but more available, methods may reduce the likelihood of high levels of parasitaemia and hence of severe life-threatening malaria.

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